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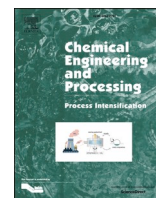
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New type of basket stationary bed reactor for heterogeneous biocatalysis

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ABSTRACT

Effectiveness of catalytic processes using heterogeneous biocatalysts depends not only on the activity of the enzyme, but also on the efficiency of the used reactor. In this paper, we present a novel design of a basket reactor with a stationary catalyst bed (StatBioChem). The developed design was compared to a commercially available rotating bed reactor (SpinChem®). The biocatalysts used were invertase and acyltransferase from *Mycobacterium smegmatis* (MsAct) immobilised on macroporous silica supports. The obtained values of initial reaction rate, both in the reaction of saccharose hydrolysis and 2,2-dimethyl-1,3-propanediol (NPG) transesterification, were twice higher for the StatBioChem reactor. A similar relationship was also observed regarding the process efficiency expressed as STY.

1. Introduction

Heterogeneous biocatalysts, especially immobilised enzymes, are currently in great demand [1–6]. As enzymes by nature are very active catalysts their process utilisation requires effective mass and heat transfer. For this suitable reactors need to be devised. In addition to the effective mass and heat transfer, these reactors should ensure protection of the grains of immobilised biocatalyst from mechanical damage [5,7]. In the recent years, one of the most commonly used apparatus has been the SpinChem® (Fig. 1) rotating bed reactor that allows for a simultaneous mixing and percolation of reactants through the bed of heterogeneous catalysts placed in a cylindrical basket/cell, mounted at the end of the shaft [6,8–17]. The SpinChem® [18] is a modification of a standard basket and annular spinning basket-reactor concept which combines the advantages of both fixed bed (FBRs) and stirred tank (STRs) reactors. The reactants inside the rotating annular flow cell are pushed away by centrifugal forces and their new portions are drawn into the cell from the bottom, thus enabling their recycling inside the vessel [19,20]. In this type of design radial pattern of flow is predominant as the reactants are transported radially through the bed of the catalyst. It may lead to a non-uniform distribution of the velocity profile and formation of dead zones. Due to the necessity of mounting the catalyst basket at the end of the agitator shaft, the shaft is exposed to increased transverse

vibration, which may result in adverse mechanical wear of the reactor internals and the shaft [21,22]. Careful design is required in the assembly. Uniform distribution of the catalyst in the bed must be aimed at. Additionally, the manufacturer recommends [18] a relatively low range of rotational speed, typically not exceeding 1000 rpm (or 500 rpm when a shaft guide is used). The rpm values given apply to the smallest of the proposed reactor solutions (vessel volume range 100–500 mL); for larger reactors/tanks the permitted maximum rpm values are even lower [18]. Therefore efficient mass and heat transfer may be limited. Within this short communication, we would like to propose a new technical solution for a stationary bed reactor (called here StatBioChem, Figs. 1 and 2A). This reactor consists of a tank, a straight blade paddle agitator, and an annular basket with a fixed/stationary catalytic bed, located along the vertical axis of the tank (Figs. 1 and 2A). The agitator shaft passes through the cylindrical opening inside the basket. The movement of the agitator mounted at the end of the shaft causes the liquid flow through the basket. The entire basket is made of openwork material (Fig. 2A), such as a metal net. It is important that the size of the holes is adjusted so as to prevent the catalyst grains from escaping the basket. There are four vertical perforated partitions, baffles inside the basket, preventing the vortexing of both the liquid and the catalyst particles and allowing the circumferential flow of reagents. The bottom and top covers of the basket are also made of openwork material to allow vertical movement

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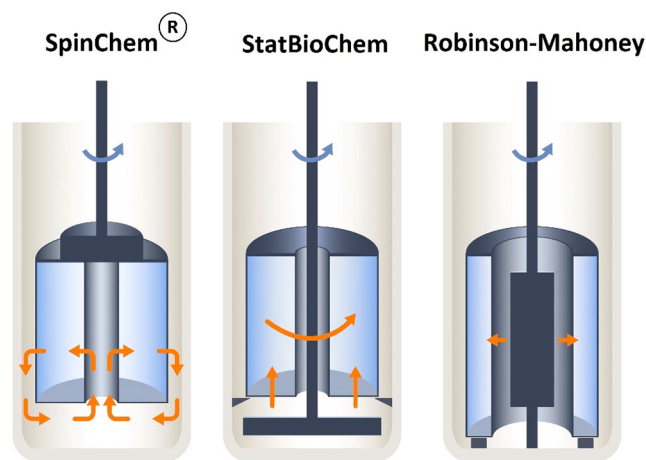


Fig. 1. Scheme of rotating bed reactor (SpinChem®) and stationary bed reactors (StatBioChem) and Robinson-Mahoney.

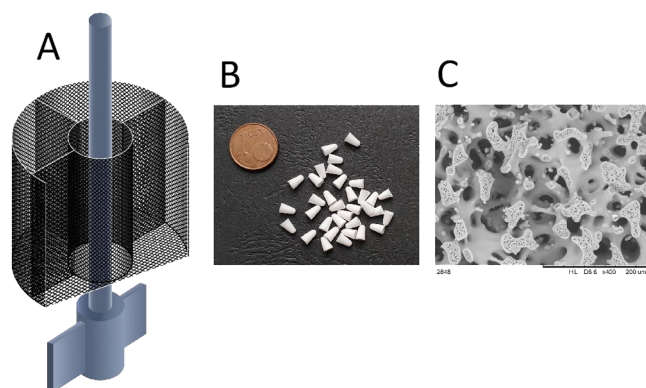


Fig. 2. Scheme of perforated basket of StatBioChem reactor (A), photo (B) and SEM image (C) of biocatalyst grain.

of reagents through the catalytic bed and create an axial pattern of flow. Probably synergy of circumferential and vertical type of flow could create a mixed type of flow and this in turn results in a more uniform velocity distribution within the catalytic bed, virtually eliminating the occurrence of dead zones. In addition, the use of a StatBioChem, where the basket is separated from the rotating agitator, does not load the mixer shaft allowing for higher mixing speeds and in this way enhances mass and heat transfer, when required. When compared to SpinChem®, the novel apparatus can be found as less challenging with regard to mechanical design, in particular, transversal vibrations are suppressed. Our proposed solution is somewhat similar to the stationary catalytic basket stirred tank reactor called Robinson-Mahoney (Fig. 1), however, in the latter, the mixing element is placed inside the annular basket, in the center of the cylindrical hollow space [19,20,23]. The agitator operation forces draft of the liquid from either the top and bottom of the basket. Such an arrangement of the mixing element causes the reactants to flow through the catalytic bed in the horizontal direction only. Again, the predominant radial pattern of flow is observed [21,22]. As in the case of SpinChem®, a maldistribution of velocity profile can be an issue and could cause the occurrence of the dead zones limiting efficient use of the catalyst bed.

As part of this short communication, we would like to present a comparison of the performance of our proposed reactor solution (StatBioChem) with the SpinChem® reactor. Both reactors were tested in the hydrolysis reaction of sucrose using immobilised invertase in an aqueous mixture of reactants and in the transesterification reaction of 2,2-

dimethyl-1,3-propanediol (NPG) using immobilised acyltransferase from *Mycobacterium smegmatis* (MsAcT) in a biphasic mixture of reactants (organic solvent/buffer). This demonstrates the applicability of the StatBioChem reactor in heterogeneous biocatalysis.

2. Materials and methods

2.1. General

Unless otherwise stated, all chemicals were purchased in the highest available purity from commercial sources. Invertase was obtained from Novozymes and *Mycobacterium smegmatis* acetyltransferase was produced as described in [24–26]. Glucose OXY DST test for invertase activity assay was purchased from Alpha Diagnostics. Polyethylene glycol 35,000 (PEG), tetraethoxysilane (TEOS), 3-aminopropyltrimethoxysilane and ethyl acetate were from Sigma-Aldrich. 2,2-Dimethyl-1,3-propanediol (NPG), cetyltrimethylammonium bromide (CTAB) and glutaric dialdehyde (25 wt.% in water) were from Acros Organics. The other chemicals used for synthesis and immobilisation were obtained from Avantor Poland.

2.2. Reactors

The study compared two reactors: a SpinChem® RBR S2 (outer diameter of the basket = 45 mm, height=30 mm); and our original solution - StatBioChem (outer diameter of the basket = 55 mm, height = 40 mm, diameter of the inner shaft cover = 20 mm; construction details are available upon request) (Fig. 2A). Both baskets were placed in glass reactor (diameter = 58 mm, height = 120 mm)

2.3. Synthesis of silica pellets and their functionalisation

Polyethylene glycol 35,000 (8.67 g) was dissolved in 1 M HNO₃ (100 mL) and then tetraethoxysilane (82.8 mL) was added dropwise under stirring (500 rpm) in an ice bath. Cetyltrimethylammonium bromide (3.8 g) was added next and after mixing the solution was pipetted into small conical vessels (50 µL) and left to gel at 40 °C and aged for 7 days. The received alcogels were impregnated with 1 M ammonia water solution for 9 h at 90 °C, rinsed with water, dried, and calcinated at 550 °C for 10 h (ramp of 1 °C min⁻¹) to obtain silica pellets. The silica pellets were modified for non-specific enzyme attachment by amino groups. Before functionalisation, siliceous particles were dried at 200 °C for 2 h. Then amino groups were grafted onto the surface of the pellets by reacting with 3-aminopropyltrimethoxysilane (1.5 mmol per g of silica) in dry toluene (40 mL per g of silica) for 72 h at 85 °C. After that modified particles were washed with ethanol at 50 °C for 5 h and air-dried.

2.4. Enzymes immobilisation

Before the attachment of invertase (or acetyltransferase) to the amino-functionalised silica pellets, the siliceous particles were washed with ethanol, then with distilled water (3 × 30 min), and 0.1 M phosphate buffer pH 7.0 (Na₂HPO₄/KH₂PO₄, 3 × 30 min). For amino groups activation, 2.5 vol.% glutaraldehyde solution in 0.1 M phosphate buffer pH 7.0 was used. To remove glutaraldehyde excess, the pellets were rinsed with distilled water and then with 0.1 M phosphate buffer (pH 6.0 for invertase and pH 7.5 for acetyltransferase, 3 × 20 min). Activated pellets were immersed in enzyme solution in phosphate buffer (pH 6.0, 3.0 mgprotein/mL for invertase and pH 7.5, 3.2 mgprotein/mL for acetyltransferase) for 5 h at 25 °C and then overnight at 6 °C. Excess protein was washed off with proper buffers: 0.1 M phosphate buffer (pH 6.0 for invertase and pH 7.5 for acetyltransferase); 0.1 M phosphate buffer with 0.5 M NaCl, 0.1 M acetate buffer pH 4.5, and finally with distilled water. All the eluates/supernatants were collected and analyzed for the presence of protein. The amount of immobilised

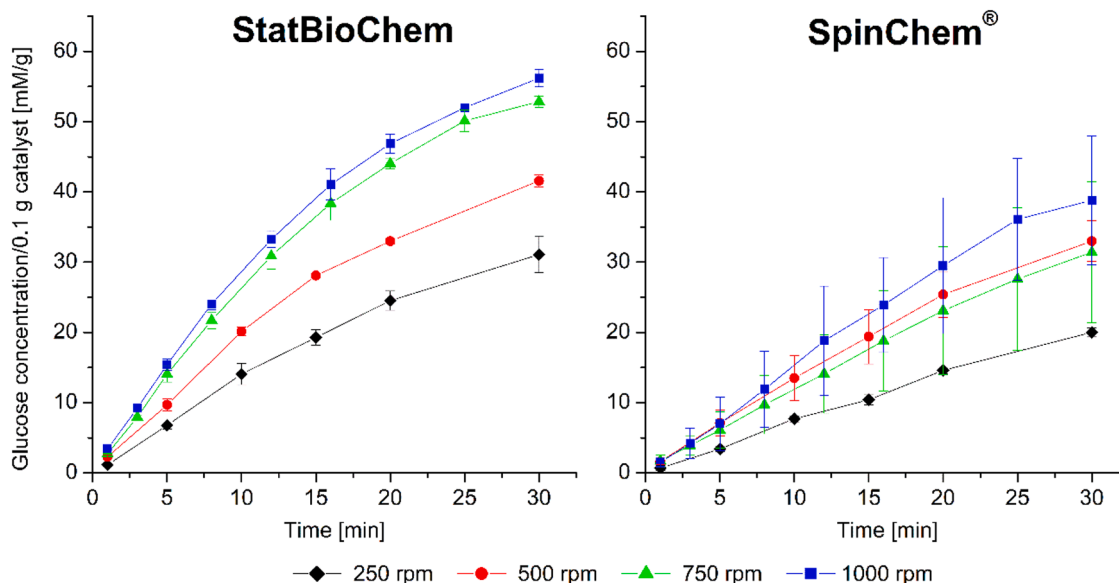


Fig. 3. Saccharose hydrolysis by immobilised invertase in basket stationary bed reactor (StatBioChem) and in rotating bed reactor (SpinChem®) at different stirring speed. Each experiment was carried out in triplicate.

enzyme was determined spectrophotometrically by the Lowry Assay. To block unreacted active groups, the pellets with immobilised enzyme were rinsed with 0.5 M Tris-HCl buffer pH 7.8 and then stored at 4 °C. 1 g of carrier contained 10.3 mg of invertase or 6.5 mg of MsAcT.

2.5. Hydrolysis of saccharose

The immobilised invertase (0.33 or 0.24 g corresponding to 3.4 or 2.5 mg of invertase) was placed into the StatBioChem or SpinChem® reactors and 125 mL of preheated (40 °C) saccharose solution (175 mM in sodium acetate buffer pH 4.5) was added. To control reaction progress 10 µL of samples were taken during regular intervals and glucose concentration was determined. To control the progress of the saccharose hydrolysis 10 µL of samples were taken off, diluted in 1 mL of Glucose OXY DST test, and incubated at 37 °C for 5 min. Glucose concentration was then determined spectrophotometrically at 500 nm using HITACHI U-2800A and recalculated by the glucose standard absorbancies (15 mmol).

2.6. Transesterification of 2,2-dimethyl-1,3-propanediol

The immobilised acetyltransferase (0.22 g corresponding to 1.4 mg of MsAcT) was placed into the StatBioChem or SpinChem® reactors and 125 mL of biphasic solution (1:1, v/v) of 0.05 M phosphate buffer pH 7.5 and 2,2-dimethyl-1,3-propanediol (NPG) solution (96 mM in ethyl acetate) was added. The reaction was carried out at room temperature and controlled by gas chromatography in accordance with [24]. The concentrations of the 2,2-dimethyl-1,3-propanediol (NPG) and its esters were determined on an Agilent Technologies 6890 N Network GC System equipped with a flame ionisation detector (FID) and an Agilent Technologies HP-5 capillary column (30 m × 0.32 mm × 0.25 µm). The following temperature program was applied: initial oven temperature 70 °C held for 9 min, next to 135 °C (45 °C min⁻¹) and held for 1 min, then from 135 °C to 140 °C (45 °C min⁻¹) and held at 140 °C for 1 min and finally to 300 °C (50 °C min⁻¹). The injector and detector temperatures were 320 °C and 350 °C, respectively. The carrier gas was helium with a flow rate of 2.5 mL min⁻¹. The retention times of the NPG and its esters under these conditions were: ~4.4 min (NPG), ~7.9 min (monoester), and ~11.4 min (diester). The concentrations were calculated using calibration curves.

Table 1

Initial reaction rate of saccharose hydrolysis and STY in basket stationary bed reactor (StatBioChem) and in rotating bed reactor (SpinChem®).

		250 rpm	500 rpm	750 rpm	1000 rpm
reaction rate [mM/min . g _{catalyst}]	SpinChem®	7.12 ± 0.25	13.18 ± 2.82	12.27 ± 5.07	14.58 ± 7.04
	StatBioChem	13.24 ± 0.92	19.19 ± 0.21	27.33 ± 1.44	30.35 ± 1.09
STY* [g/L . h]	SpinChem®	7.21 ± 0.23	11.87 ± 1.04	11.28 ± 3.61	13.95 ± 3.30
	StatBioChem	11.18 ± 0.92	14.96 ± 0.31	19.02 ± 0.28	20.23 ± 0.46

* STY calculated after 30 min of reaction for 0.1 g of catalyst.

3. Results and discussion

The selected enzymes were immobilised on silica pellets with a hierarchical pore structure and macropore size of 20–50 µm (Fig. 2B-C). These carriers have already been successfully used for enzyme immobilisation and are well-suited for reactions carried out in aqueous and organic solvents [2,3]. To obtain stable biocatalysts, the silica matrix was functionalised with amino groups, which were subsequently activated with glutaraldehyde in order to achieve covalent binding of enzyme and carrier. Two enzymes were selected for the tests: invertase catalysing the hydrolysis of saccharose into glucose and fructose (process carried out in aqueous solvent) and acyltransferase from *Mycobacterium smegmatis* (MsAcT) catalysing the transesterification of NPG. In the latter process, the reactants are dissolved in an organic solvent, and a buffer is necessary to ensure the stable operation of the enzyme [24]. This selection of immobilised enzymes and catalysed processes probes the new reactor solution in a biphasic (solid catalyst/water) and a triphasic system (solid catalyst/water/organic solvent). The hydrolysis of saccharose in both reactors (SpinChem® and StatBioChem) was conducted at rotation speeds of 250, 500, 750, and 1000 rpm and the results are shown in Fig. 3. As expected, an increase in reaction rate/product concentration was observed with increasing rotation speed for both types of reactors – a clear indication that the reaction kinetics are controlled by external diffusion. However, the reaction rates determined under initial conditions for the StatBioChem were about twice as high as those for the commercial solution (SpinChem®, Table 1). Significant

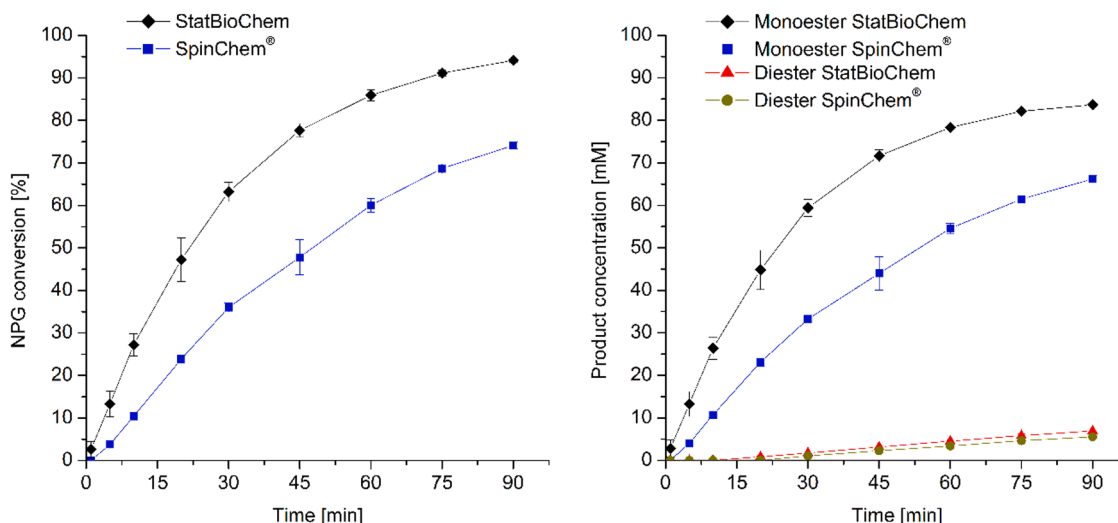


Fig. 4. Transesterification of NPG by immobilised MsAcT in basket stationary bed reactor (StatBioChem) and in rotating bed reactor (SpinChem®) at 500 rpm. Each experiment was carried out in triplicate.

differences in favour of our proposed solution were also observed in the process efficiency determined by space-time yield after 0.5 h of reaction (STY, Table 1). Moreover, for the SpinChem® reactor, the use of a rotation speed higher than 500 rpm resulted in the formation of a liquid vortex and may have interfered with the mass transport of reactants, which is reflected in very poor reproducibility of the obtained results (Fig. 3, Table 1). In the case of a StatBioChem, the openwork construction of the reactor, and in particular the radially distributed openwork partitions, prevent the formation of a liquid vortex completely, guaranteeing stable operation of the reactor. Both reactors were also tested in the NPG transesterification reaction at rotation speed of 500 rpm (Fig. 4). In this process, it is possible to obtain mono- and diester, the latter only appearing when a significant part of the NPG is converted to monoester [24]. In a three-phase system, we have also demonstrated the superiority of StatBioChem over the commercial solution. The determined value of the reaction rate (regarding the substrate) for the stationary bed reactor was $11.9 \text{ mM/min} \cdot g_{\text{catalyst}}$, whereas for the SpinChem® reactor it was two times lower ($5.6 \text{ mM/min} \cdot g_{\text{catalyst}}$). Similar differences were observed when comparing the process efficiency (regarding monoester) after 30 min; the STY for the SpinChem® reactor and the StatBioChem were 9.72 and $17.37 \text{ g/L} \cdot \text{h}$, respectively.

4. Conclusions

The presented results indicate that the proposed reactor design allows to considerably intensify the process by enhanced transport of reagents, and uniform use of the catalyst bed volume. This translates into an increased reaction rate and allows to reduce the duration of the process. Additionally the comparison evidences that the proposed new design leads to less influence of transport limitations. It is well known that those limitations should be negligible to get reliable kinetic information from experiments. As it can be seen reaction rates and STY were about twice as high for the StatBioChem reactor compared to those determined for SpinChem®. Moreover, the proposed assembly is a very simple design, which will undoubtedly be of key importance in the attempts to increase the reactor size, a scale-up to technical capacities. The separation of the basket (in which the catalyst is placed) from the mixing element significantly reduces the load on the agitator shaft and allows to increase rotation speed. The developed reactor design might further be adjusted by determining the shape, size, location and type of the agitator, and the distance of the basket from the tank to name a few possibilities. This opens a broad domain of successive, ongoing research

where CFD modelling could also be employed for geometry optimisation as well as scale-up of the apparatus towards larger scale industrial applications. At that moment StatBioChem reactor could be successfully applied for lab-scale units aimed at getting kinetic and activity data, which can be obtained by higher agitation velocities thus allowing to limit influence of external mass transport limitations.

CRediT authorship contribution statement

Dominika Stradomska: Methodology, Investigation, Visualization. **Daria Świętochowska:** Methodology, Investigation. **Robert Kubica:** Formal analysis, Writing – review & editing. **Ulf Hanefeld:** Writing – review & editing. **Katarzyna Szymańska:** Conceptualization, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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