Chemical Engineering Journal 315 (2017) 18–24

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

A novel hierarchically structured siliceous packing to boost the performance of rotating bed enzymatic reactors

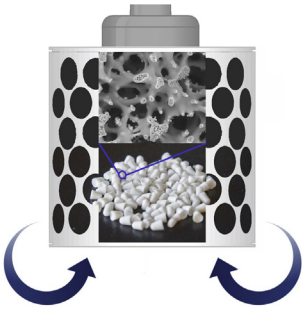
Katarzyna Szymańska [a,*], Klaudia Odrozek [a], Aurelia Zniszczoł [a], Wojciech Pudło [a], Andrzej B. Jarzębski [a,b]

[a] Department of Chemical Engineering and Process Design, Silesian University of Technology, 44-100 Gliwice, Ks. M. Strzody 7, Poland
[b] Institute of Chemical Engineering, Polish Academy of Sciences, 44-100 Gliwice, Bałtycka 5, Poland

HIGHLIGHTS

- Engineering of silica pellets with hierarchical pore structure devised and tested.
- Effective immobilization of model enzyme to obtain structured biocatalysts.
- Rotating bed (SpinChem) reactor performance boosted ten-fold against benchmark.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 October 2016
Received in revised form 21 December 2016
Accepted 31 December 2016
Available online 3 January 2017

Keywords:
SpinChem® rotating bed reactor
Macro/mesoporous silica pellets
Biocatalysts

ABSTRACT

In this paper we demonstrate exceptional performance of a new type of packing made of the siliceous pellets with hierarchical pore structure in micrometric and nanometric scales, devised for the application in SpinChem® reactors. Rotating bed (enzymatic) reactors of SpinChem® type emerge as a new type of very effective, small catalytic reactors designed for the synthesis of fine chemicals. The pellets/catalysts of two sizes and two hierarchical pore structures were fabricated using sol-gel method combined with phase separation and pore templating, and after covalent attachment of a model enzyme (invertase) tested in a sucrose hydrolysis reaction. For the same enzyme load, the pellets/catalysts of ca. 3.5–5.5 mm in size with pore structure predominated by flow-through macropores of 20–50 μm (pore volume ca. 2.5–3 cm^3/g) and mesopores of about 30 nm (pore volume > 1 cm^3/g) appeared to be more active than somewhat smaller ones with the same pore structure, and 6–7 times more active than those of the same size but with macropores of 10–12 μm, and nearly ten times more active than prepared using mesopores predominated Kieselgel 60 (considered as benchmark). Nearly full (99%) conversion was obtained in 75 min reaction time using the proposed structured catalysts/pellets, whereas for the benchmark it was 26%. The catalysts were shown to be stable in ten reaction runs and the pellets of used catalysts could be regenerated by calcinations. The regenerated pellets could also be reused at least 4–5 times as supports for the new/fresh enzyme.

1. Introduction

Nowadays, enzyme immobilization is the well established approach leading to improvement in the biocatalysts' stability, facilitating recovery, repeated utilization and application in continuous processes. The enzymes are usually attached to the solid,

* Corresponding author.
E-mail address: Katarzyna.Szymanska@polsl.pl (K. Szymańska).

http://dx.doi.org/10.1016/j.cej.2016.12.131
1385-8947/

preferably porous carriers by covalent binding, adsorption or entrapment within a matrix [1–3]. But immobilization of the enzyme – and engineering of the biocatalyst in particular – should make full use of specific properties of the reactor setup. In simple stirred-tank reactors (STRs) the (bio)catalysts encounter serious mechanical challenges. A vigorous agitation results in shearing and tensile forces, which stimulate collisions of catalysts, their abrasion and even disintegration, thus enhancing enzyme leaching from the supports. In simple fixed-bed reactors (FBRs) an excessive pressure drop and limited mass transfer are a serious problem, and swelling of polymer catalysts particles in organic solvents precludes their applications. All these shortcomings could be overcome, or significantly reduced, in the continuous-flow monolithic siliceous microreactors with bi- and even tri-modal hierarchical pore structure, which enabled exceedingly high rates of the reactions at much reduced pressure drop [4–10]. However, in the cases of longer reaction times (slow kinetics) an external recycle of reactants still had to be applied, and this increased both costs and technical complexity.

The rotating bed reactor of SpinChem® (SCR) type [11] is the most recent modification of a standard basket- and annular spinning basket-reactor concept which combines the advantages of both FBRs and STRs. Its four compartment rotating annular flow cell filled with a solid-phase catalysts/immobilized enzyme enables the simultaneous stirring and unhindered percolation of reactants through the catalysts' bed. The reactants inside the rotating cell are pushed away by centrifugal forces and their new portions drawn into the cell from both the top and the bottom, thus enabling their recycling inside the vessel [12,13]. Moreover, the flow cell can easily be separated from the reaction solution, washed if necessary, and reused without the (bio)catalysts filtration. That was more recently shown to prevent the biocatalysts' activity decay in repeated applications [14]. But more importantly perhaps, the same studies showed that the biocatalyst (Novozyme 435, immobilized CAL-B lipase) was as highly active in the SCR as in the STR and outperformed that packed into the FBR [14].

The extensive studies carried out over the last two decades showed that functionalized mesoporous siliceous materials (MPS) of mesocellular foam (MCF) or Santa Barbara amorphous (SBA) families can be excellent enzyme supports [15–23]. In addition to the environmental acceptability MPS are structurally more stable in organic solvents and more resistant to microbial attacks than the polymeric enzyme carriers, and can also be regenerated and reused. Moreover, their large surface area can be densely covered with various groups to anchor even larger enzyme molecules and a restricted space of mesopores enables the exploitation of the positive effects of proteins' confinement to boost their activity [6,16,19]. Also an open structure of the MPSs and uniformly sized pores hamper their blocking and hence improve biocatalysts' stability. However, fine particles of most MPS, typically less than 50 μm in size, while being perfectly suited for the STR-slurry systems, are not suitable for the SCR. In view of the growing industrial interest in using various small/milli- and micro-scale technologies for the fine chemicals synthesis [24,25], and significant application potentials of the SCRs, we deemed it important to develop a new type of the high performance packing aimed at the SCRs. For that we decided to study the siliceous monolithic pellets with a hierarchical macro- and mesopore structure, identical to that of the continuous-flow microreactors, but much smaller, ca. 2.5–5 mm in size. It was expected that an important advantage of such supports would be facilitated convective penetration/infiltration and very low diffusion limitation of the reactants transport to and from active sites, originating from a unique structure of interconnected (super-large) macropores and (large) mesopores [26]. To obtain a broader view we decided to investigate both the monoliths possessing macropore channels of about 10 μm, prepared using the procedure devised by Montpellier group [4,5], and also those with even larger (20–40 μm) macropores, successfully applied as the immobilized enzyme reactors by our group [6,9,10]. Their performance has been investigated in the reaction of sucrose hydrolysis and compared with that of the biocatalysts prepared in the same way but using a typical mesoporous siliceous enzyme support (Kieselgel 60) applied as benchmark [20]. While the performed studies primarily aimed at the development of a very effective packing to boost the SCRs' performance, the reaction of sucrose hydrolysis catalysed by invertase is itself of major practical significance and the data reported previously could be used in the evaluation of the SCR-(bio)catalyst system performance [6,17,19].

2. Experimental

2.1. Materials

Hexadecyltrimethylammonium bromide (CTAB) and Tetraethoxysilane (98%, TEOS) were from Acros Organics. Polyethylene glycol 35000 (PEG) was purchased from Sigma-Aldrich, so as was 3-aminopropyl-trimethoxysilane (APTS) applied as donor of amino groups. Glutaraldehyde (GLA), trihydroxymethylaminomethane hydrochloride (Tris-HCl), sucrose and other chemicals were purchased from Avantor Performance Materials (Poland) and used without further purification.

2.2. Synthesis of silica monoliths and modification of surface

The monoliths were prepared using a modified Nakanishi [27–29] method. However, the application of two different sol-gel synthetic protocols, described in more detail in Refs. [4–6,9], and of small conical moulds of two sizes, resulted in the fabrication of four different pellets.

The silica pellets labelled M1 were synthesized using a protocol described in detail in Refs. [6,9]. First polyethylene glycol 35000 (PEG, 9.09 g) was dissolved in 1 M HNO_3 (104.6 mL), after which tetraethoxysilane (TEOS, 87.1 mL) was added slowly under stirring to the PEG solution in an ice bath followed by the addition of cetyltrimethylammonium bromide (CTAB, 4.016 g). The solution was mixed, pipetted into small conical vessels of two volumes: 50 μL (M1-50) and 30 μL (M1-30), then left to gel at 40 °C and aged for 7 days. Next the alcogels obtained were impregnated with ammonia solutions: 1 M for 9 h at 90 °C. The samples were washed with water, dried at room temperature and then calcined at 550 °C for 8 h (ramp of 1 °C/min) to obtain uniform silica monoliths of conical shape with average dimensions: 5.1 mm height and 3.3 mm dia. (M1-50) and 3.7 mm height and 2.9 mm dia. (M1-30) (Fig. 1).

A general synthetic procedure of the monoliths labelled M2 was proposed in Refs. [4,5] and the applied protocol was as follows. At first PEG (2.597 g) was dissolved in aqueous HNO_3 (1.86 mL 65% HNO_3 and 25 mL H_2O) after which TEOS (21.639 mL) was added slowly to the PEG solution in an ice bath. The solution was mixed, then left to gel at 40 °C in identical conical vessels as M1, and then aged for 3 days at the same temperature. Next, the alcogels obtained were impregnated with ammonia solutions: 0.1 M for 20 h at 40 °C. Before drying (3 days, 40 °C) the samples were washed with water and then calcined at 550 °C for 8 h (ramp of 1 °C/min) to obtain small monoliths/pellets of dimensions: 3.4 mm height and 2.5 mm dia. (M2-30) or 4.6 mm height and 2.9 mm dia. (M2-50). Noteworthy are differences in external dimensions of the corresponding samples: M1-x and M2-x caused by differences in shrinkage of the corresponding wet gels during drying and thermal treatment. They appeared to be much larger