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Flow mode enantioselective transamination using transaminase enzymes immobilized in a macroporous silica monolith

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PURPOSE OF THE ABSTRACT

Transaminases (TA) are currently attracting a great deal of attention for the greener production of chiral amines, which are precursors of important drugs.¹ Enzymes are extremely appealing in the chemical sector but their application as catalysts in industrially relevant processes is not straightforward. The use of free enzymes in homogeneous batch processes suffers from severe limitations, some which can be tackled using immobilization strategies. To go even further towards green processes, the design of continuous flow production processes is a relevant strategy.² In this context, beside enzyme immobilization, catalyst shaping is a crucial aspects.

Here, we will present the immobilization of TA on cylindrical silica monoliths that are particularly appropriate for the design of continuous flow reactors (Fig. 1A). The monoliths (Fig. 1B-E) are prepared by a bottom-up sol-gel method based on emulsion templating.³ They minimize pressure drop, ensure a plug flow regime, and are easy to manipulate. Their surface is functionalized to bring epoxy or amino groups on the surface, allowing to anchor the enzyme.

A simple flow reactor featuring covalently immobilized TA (ATA-117 from Codexis) is presented as a proof-of-concept.⁴ The transamination of pyruvate with racemic 4-bromo- α -methylbenzylamine (BMBA) to produce bromoacetophenone (BAP) was used as a model reaction (kinetic resolution). We show how the immobilization of the enzyme α and therefore the catalyst performance α can be optimized by tuning parameters of the monolith surface functionalization. The biocatalysts obtained through covalent grafting of the commercial enzyme with APTES showed good performance and high repeatability. By adjusting the contact time, full conversion can be obtained. The solid biocatalyst was fully enantioselective, stable, recyclable, and showed no diffusional limitations.

We then present decisive improvements of the system. In fact, a poor APTES dispersion in the monolith was macroscopically observed (Fig. 1C) and confirmed by TGA and IR analyses. This was assigned to the functionalization mode in which excess APTES in the solution accumulates at the outer layers of the monolith, causing an egg-shell dispersion. Parameters known to affect the silanization have been controlled in order to obtain a better dispersion of the amino groups. It turned out that switching to a dry impregnation mode and controlling the water content during the functionalization step allows obtaining a homogeneous APTES dispersion (Fig. 1 D). This was correlated with a 3-fold increase in activity. Also, the temperature at which the enzyme grafting is performed is shown to have a great impact on the final performance.

As the purity of the commercial enzyme was questioned, a TA was produced in the lab from the plasmid (GeneScript) via expression in *E. coli* and HIS-tag purification. This enzyme showed much higher purity and intrinsic activity. When immobilized, conversion was increased by a factor 4 as compared to the commercial enzyme.

Finally, the enantioselective synthesis of R-BMBA from BAP and alanine was studied, using TA, together with glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH). This multi-enzyme system was proposed by

Turner et al.,⁵ but is here used in the flow mode to run a cascade biocatalytic process which shifts the thermodynamic equilibrium towards the desired chiral amine.

The effective immobilization of enzymes on macrocellular supports that can withstand flow conditions opens up new perspectives for the design of efficient biocatalytic processes, especially relevant for the synthesis of high added value chemicals.

FIGURES

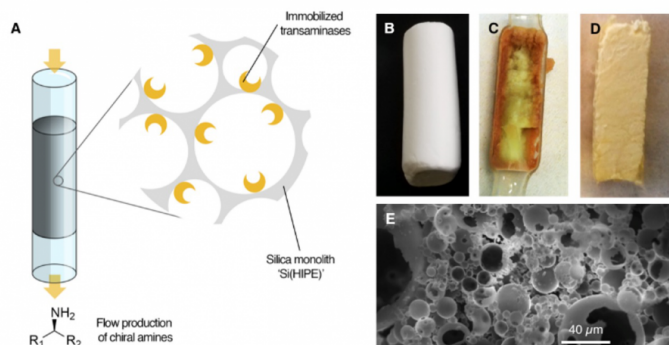


FIGURE 1

Figure 1

(A) Flow strategy for the synthesis of chiral amine using TA immobilized in a macrocellular silica monolith. (B) Picture of the silica monolith. Longitudinal cut in the monolith showing poor (C) and excellent (D) APTES dispersion. (E) SEM micrograph of th

FIGURE 2

KEYWORDS

biocatalysis | Flow chemistry | transaminase | enantioselective synthesis

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