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Immobilized nitrilase in continuous bioreactors: packed bed reactor vs. milli-structured pillar reactor

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Biocatalysis flow context

In order to address the sustainability and energy efficiency issues, biocatalytic synthesis routes have increasingly gained more popularity over the years. This green tool has many benefits to offer such as soft operating conditions (i.e low temperature and pressure), high selectivity, and aqueous medium reducing the demand in petrochemical solvents [1]. To make industrial investments more cost-effective, immobilization strategies are actively studied by the scientific community to propose efficient and stable immobilization techniques. Another profitable aspect of immobilized enzymes is the possible use of continuous processes instead of traditional batch-wise production. However, enzyme immobilization is far from being a mature discipline. Thus, process intensification from batch to continuous production is one of the main point to address for successful deployment of flow biocatalysis at an industrial level [2].

Scope of the study and objectives

A promising primary approach have been published with continuous production of nicotinic acid over 30 days (i.e. \approx 1400 cycles) using a traditional Packed-Bed-Reactor “PBR” with PMMA beads [3]. Considering the ending conversion of 50%, the total turnover “TN” (i.e mass of product per mass of immobilized enzyme) was 8-fold higher with PBR compared to cyclic use of the immobilized enzymes in batch conditions. Even higher performances were obtained changing from a commercial to a thermostable nitrilase from an in-house collection with a fully stable conversion of 100%. However, PBR are known for excessive back-pressure which hampers the scale-up feasibility. Surface coated-reactors may remove this barrier but reported datas in the literature are focused on micro-equipments. For an industrial scale application, both scale-up (i.e. increasing the characteristic dimensions of the channel) and numbering-up (i.e.

increasing the number of reactor) must be considered [4]. For this study, we immobilized the commercial nitrilase in a milli-scale pillar reactor (i.e. channel length of 1mm). The reactor walls were coated with SiO₂ beads for higher loading capacity.

Biocatalytic performances

The experimental results (Figure 1) show a lower stability in the milli-reactor than in the PBR. This may be due to the enzyme immobilization protocol differences (SiO₂ vs. PMMA beads). This aspect will be studied later for the reactor optimization. Compared to the batch reactor using PMMA beads, with similar ending conversion at 50%, the productivity capacity is greatly improved with the continuous mode: 14 $\mu\text{mol/h}$ instead of 1 $\mu\text{mol/h}$. The TN is also 2-fold higher with milli-structured reactor compared to the batch mode.

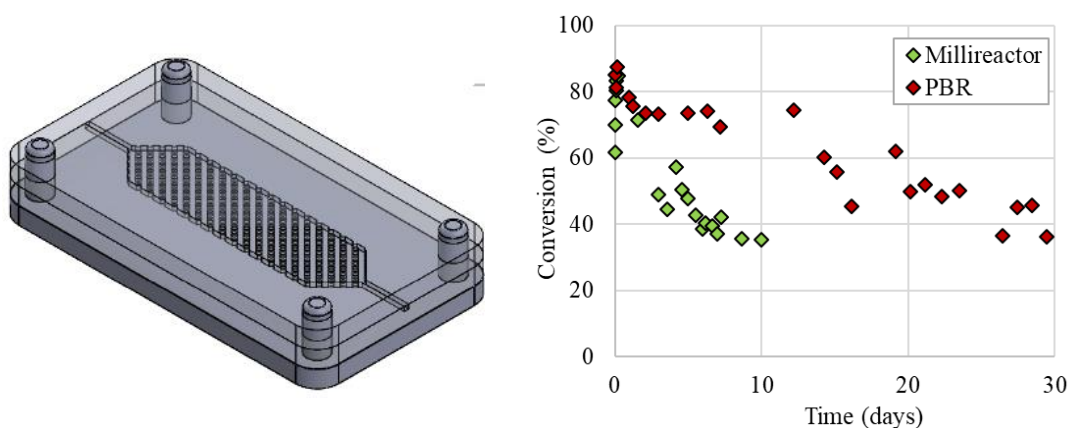


Figure 1: (Left) 3D CAO-view of the milli-pillar reactor; (Right) Biocatalytic conversion of the PBR and millireactor - Both reactors have an internal volume of 0.5 ml and the liquid flowrate is 0.03 ml/min

Conclusion and perspectives

Continuous biocatalytic synthesis with a milli-pillar reactor gives greater TN and productivity compared to a batch mode production. The process scalability potential is enhanced changing from PBR to a milli-structured reactor by a decrease in the pumping power (i.e theoretical pressure drop at least 50% higher for PBR than the one measured with millireactor). Multiple scale-up stages are planned for a final aiming flow of 1L/h by combining CFD modeling and experimental tests. Also, the reactor structure influence will be considered with comparison with a zigzag meandering milli-channel.

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[2] J.M. Bolivar et al., *Chem. Soc. Rev.*, 51, 2022, 6251-6290.

[3] C. Teepakorn et al., *Biotechnol.J.*, 2021;16 :2100010.

[4] S.G. Newman and K.F. Jensen, *Green Chem.*,15, 2013, 1456-1472.