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# Milli-structured bioreactor with immobilized commercial nitrilase for continuous production of nicotinic acid

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- Higher enzyme rentability with a continuous mode
- A 10-fold increase in the productivity with the continuous reactor compared to a batch one
- Improvement of the process scalability compared to a conventional packed-bed-reactor

## Introduction

In order to address the sustainability and energy efficiency issues, biocatalysis 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 chemical solvents [1]. To make industrial equipments more cost-effective, immobilization strategies are actively studied by the scientific community to propose efficient and stable immobilization protocols. 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. Indeed, researches are constantly challenged by high purchase cost of enzymes, few uniform reported datas and experiments not always well-designed to evaluate immobilization [2]. As a consequence, the pharmaceutical industry is still traditionally operating with a batch approach [3]. Thus, going from batch to continuous processes is one of the main point to address for successful deployment at an industrial level [2].

In this study, promising results have been published with continuous production of nicotinic acid over 30 days (i.e.  $\approx 1400$  cycles) using a traditional Packed-Bed-Reactor "PBR" [4]. 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 mainly focused on micro-scale 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 reactors) must be considered [5].

## Methods

For this study, the commercial nitrilase was immobilized in a milli-scale reactor (i.e. channel height of 1mm) with a pillar geometry (Figure 1). For fair comparison with the PBR the volume was kept the same, i.e. 0.5 ml. The reactor walls were coated with SiO<sub>2</sub> beads for higher enzyme loading capacity. The bioreactor performances were measured continuously on long term experiments (i.e. several days), three time replicated, and stopped once the conversion dropped below half of the initial value.

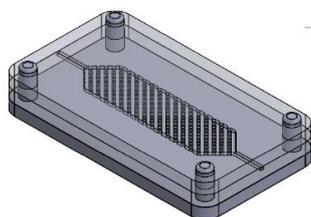


Figure 1. A 3D CAO representation of the milli-pillar reactor

## Results and discussion

According to the experimental results obtained with an ending conversion at 50% (Table 1), the TN in the milli-reactor is increased by 2-fold compared to the batch mode. The productivity capacity is also greatly improved with the continuous mode: 14  $\mu\text{mol/h}$  instead of 1  $\mu\text{mol/h}$  with the batch process.

	Batch	PBR	Milli-pillar
Liquid flowrate (ml.min <sup>-1</sup> )	-	0.03	0.04
TN (g. g enz <sup>-1</sup> )	51	405	128
Productivity ( $\mu\text{mol/h}$ )	1 <sup>(a)</sup>	12	14
Time of use or n <sup>o</sup> of cycles	7x3h	29 days	3 days

<sup>(a)</sup>  $(X_{mean} \times [S] \times V_{liquid}) / \text{length of time of one batch}$

Table 1. Biocatalytic performances with an ending conversion  $X_f$  fixed at 50%. 10 mM substrat concentration ([S]) - 40°C

In comparison to the PBR performance overtime (Figure 2), the lower stability with the milli-reactor can be due to the enzyme immobilization protocol modification (SiO<sub>2</sub> beads vs. PMMA beads). This particular point will be studied later for the reactor performances optimization.

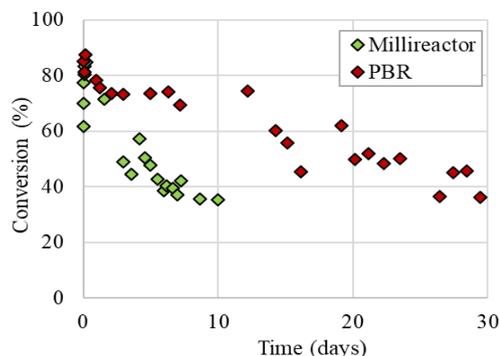


Figure 2. Biocatalytic conversion of the PBR and millireactor over time

## Conclusions and perspectives

Continuous biocatalytic synthesis with a milli-pillar reactor shows greater TN and productivity compared to a batch mode production. The influence of the immobilization protocol on the milli-reactor performance needs deeper study for longer stability as obtained with the PBR. However, changing from PBR to a milli-structured reactor allows considering the process scaling-up thanks to a decrease of the pumping power (i.e. theoretical pressure drop at least 50% higher for PBR than those measured with millireactor). Multiple scale-up stages are planned to get a final pilot-scale throughput of 1 L/h. This will be done by combining CFD modeling and experimental tests. The influence of the reactor design will also be considered by comparing the pillar reactor with a zigzag meandering channels.

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