

# Continuous production of pseudo-ceramides catalyzed by immobilized *Candida antarctica* lipase B (Novozym® 435) in a packed-bed bioreactor

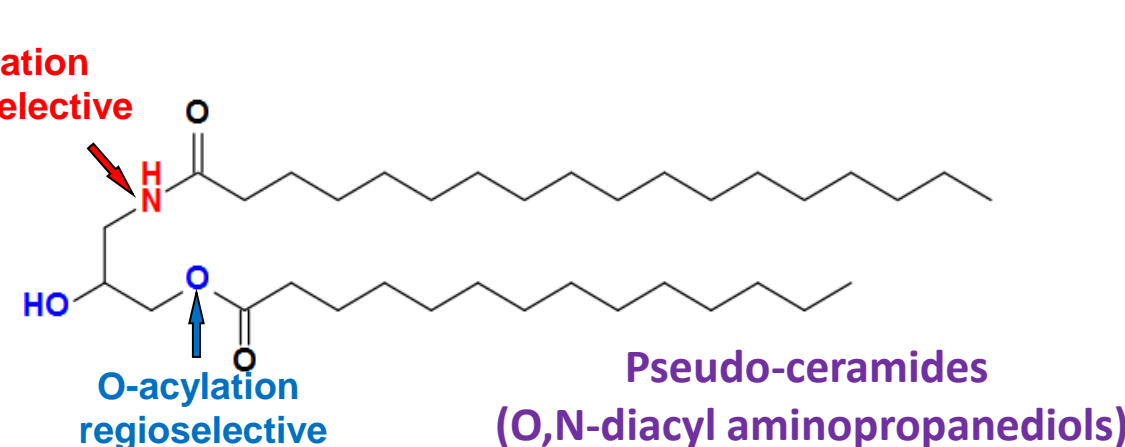
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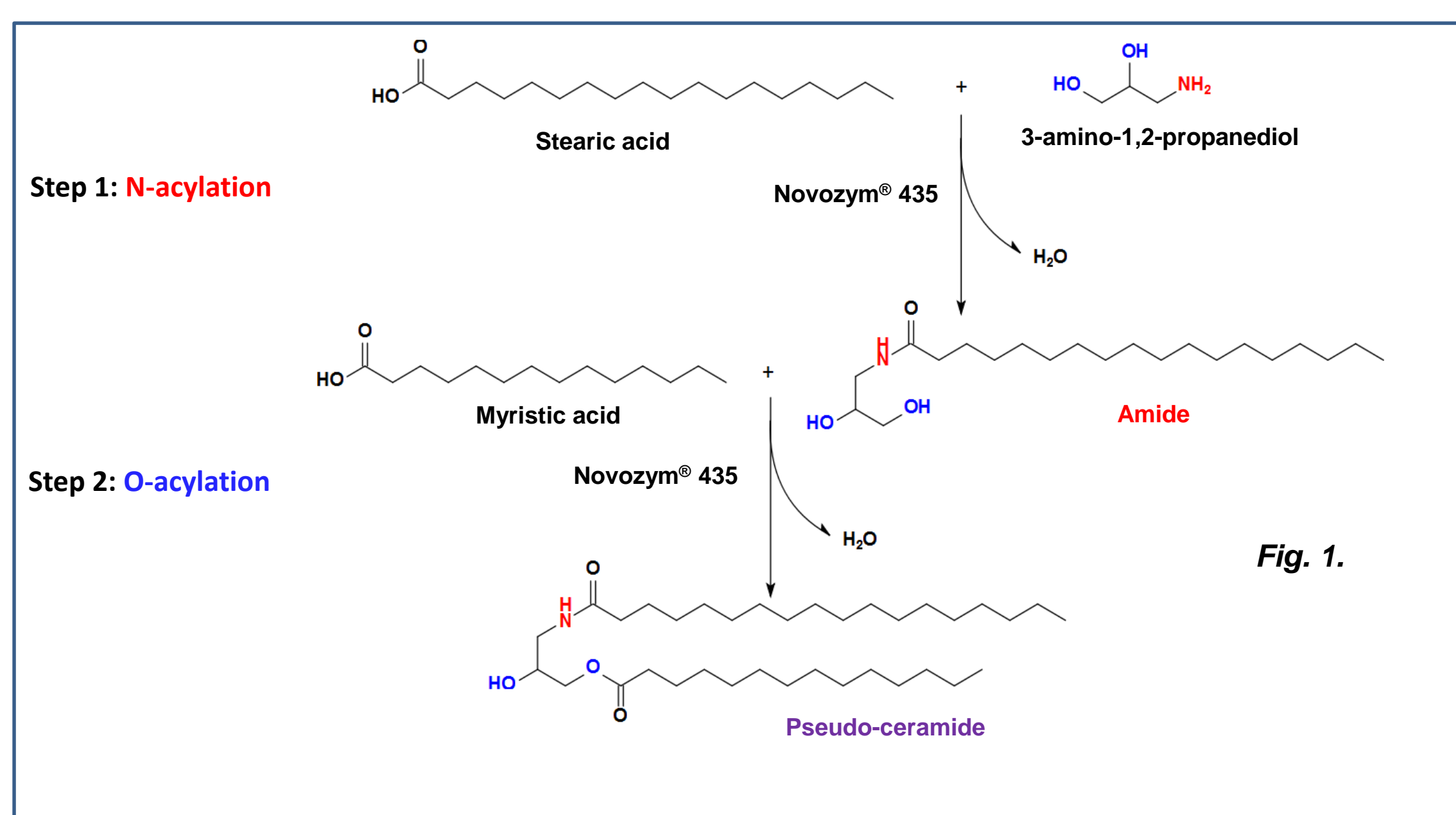
**CONTEXT AND OBJECTIVES:** Ceramides are spingolipid compounds that are very attractive as active components in both the pharmaceutical and the cosmetic industries. In this work, the synthesis of ceramide analogs, the so-called pseudo-ceramides, was carried out using for the first time a two-step continuous enzymatic process with immobilized *Candida antarctica* lipase B (Novozym® 435) in a packed-bed bioreactor [1]. The first step involved the N-acylation of 3-amino-1,2-propanediol using stearic acid as the first acyl donor (Fig. 1; step 1). This was followed by the selective O-acylation of the product synthesized in the first step, with myristic acid, to produce a N,O-diacyl 3-amino-1,2-propanediol-type pseudo-ceramide (Fig. 1; step 2).

## Optimization of the process in a laboratory scale bioreactor:

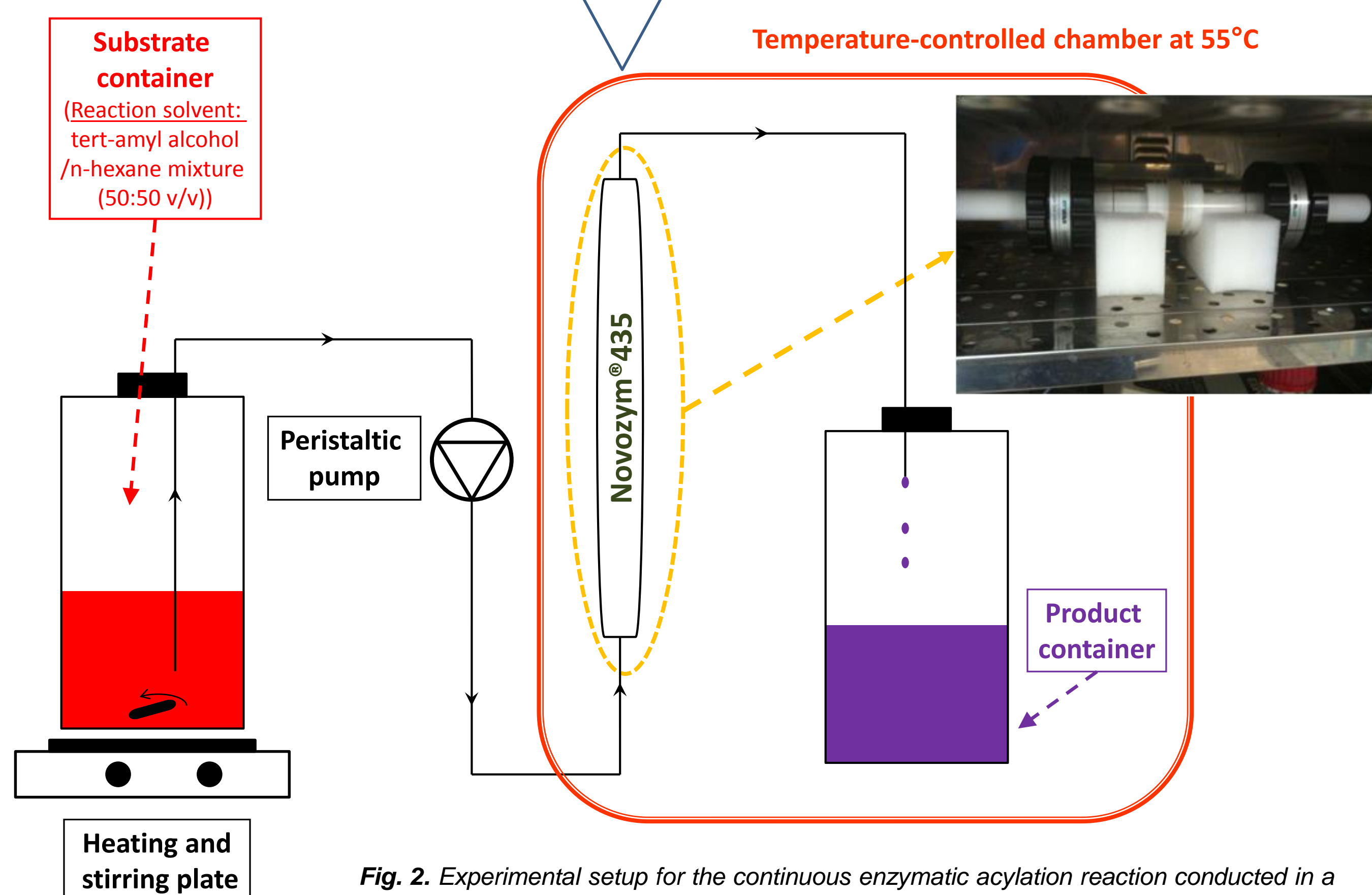
Example of pseudo-ceramide structure:



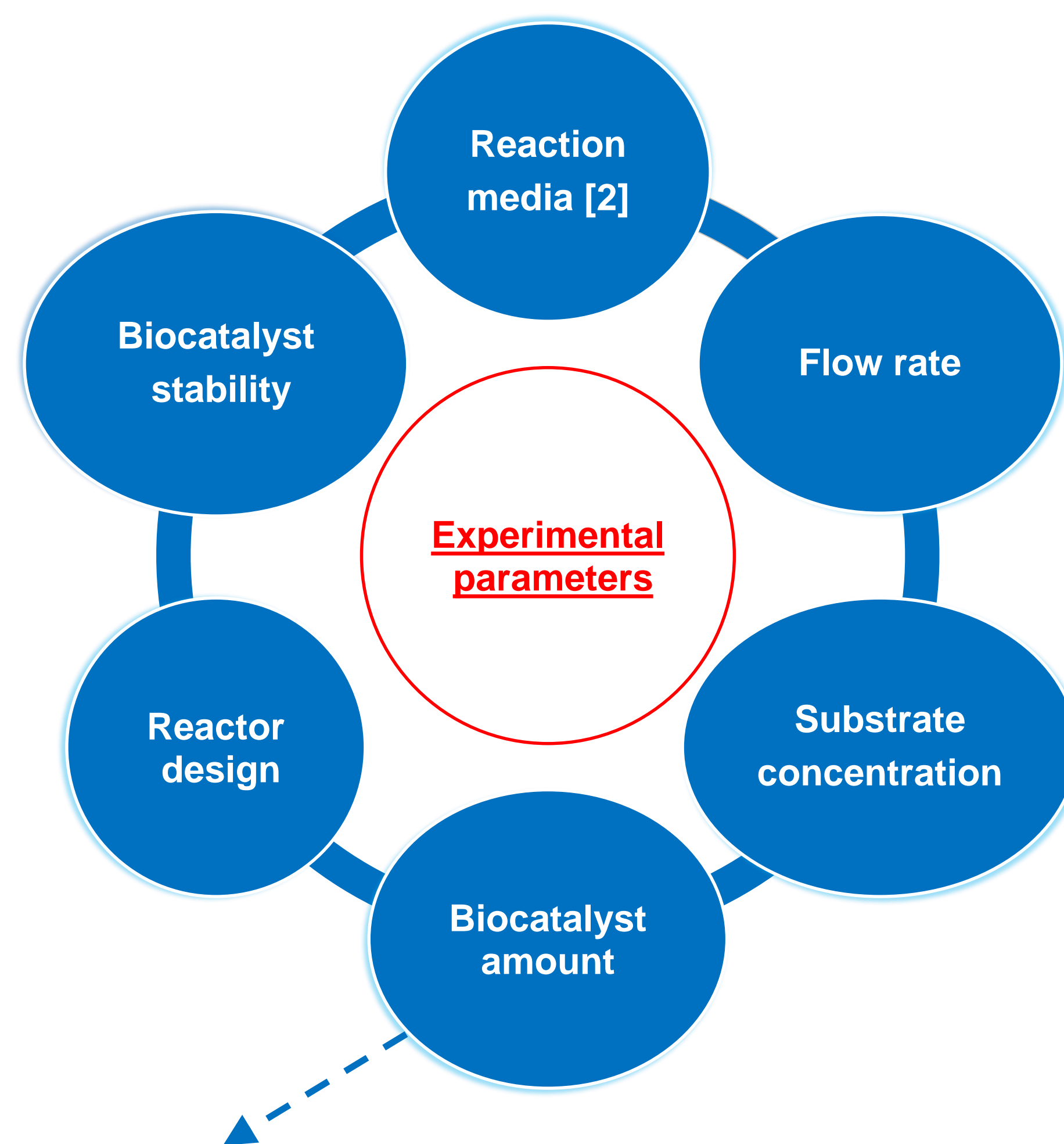
**Two-step process for the selective enzymatic synthesis of 1-O,3-N-diacyl 3-amino-1,2-propanediol-type pseudo-ceramides catalyzed by Novozym® 435 in a packed-bed bioreactor (Fig 1):**



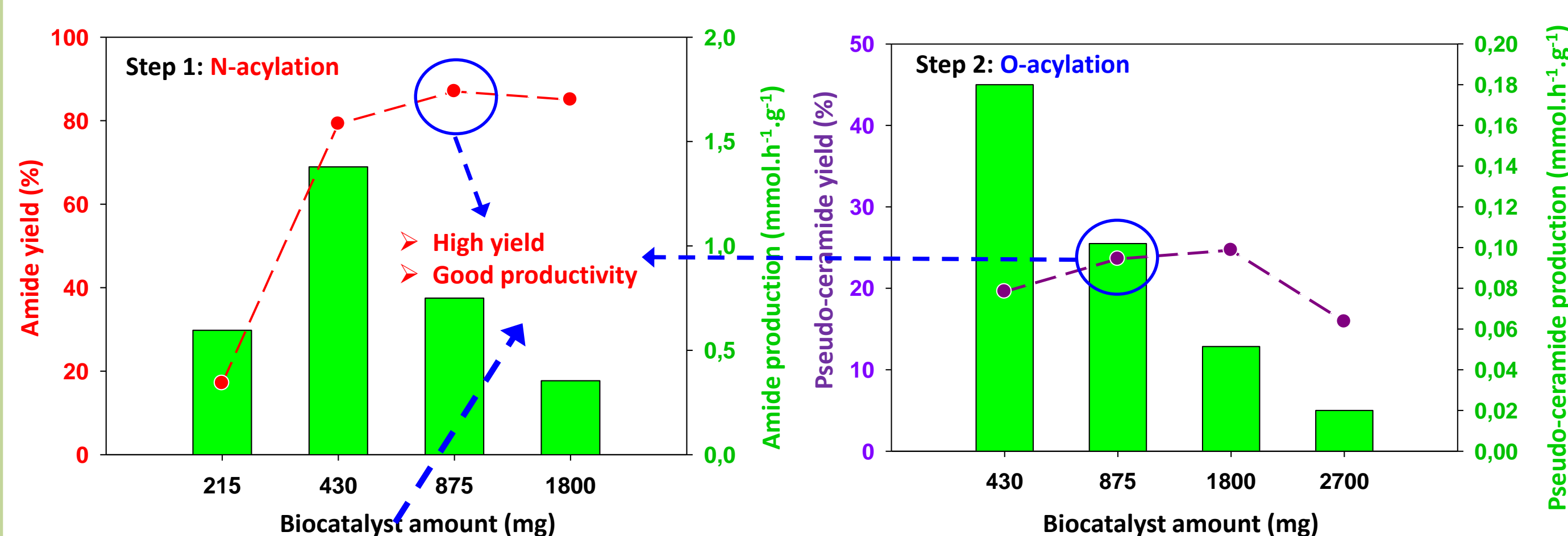
The two steps are performed independently in a packed-bed bioreactor (Fig. 2)



## Determination of optimal experimental conditions:



Example of parameter optimization: biocatalyst amount (Fig. 3):



Best compromise between yield and productivity

Optimal biocatalyst amount (Novozym®435) : 875 mg

Finally, productions were performed on the two steps under optimal experimental conditions (Table 1):

Table 1: Yields and Production of the two steps under optimal experimental parameters

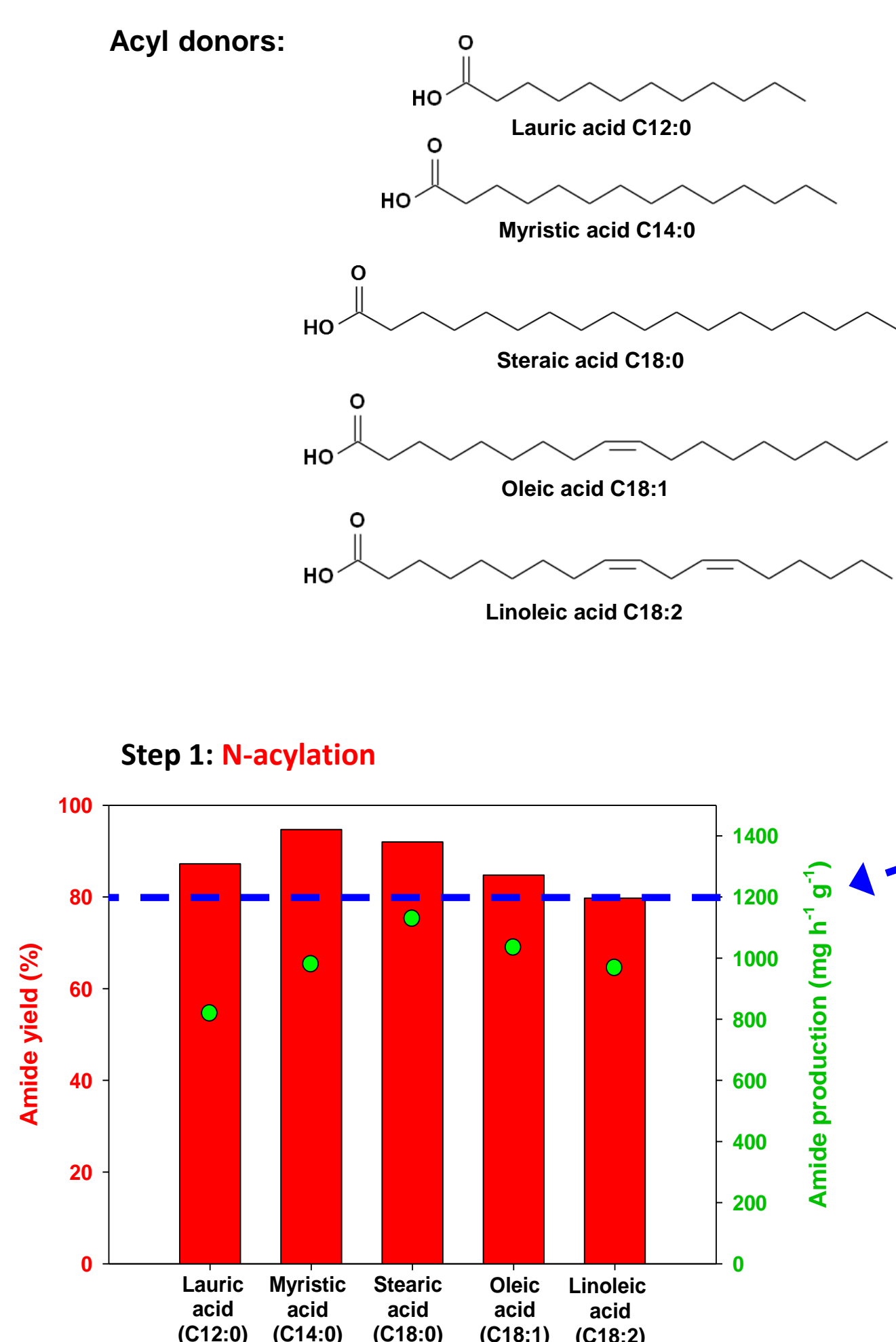
Parameters	feed flow rate (µL.min⁻¹)	Novozym® 435 (mg)	Acyl donor (mM)	Acyl acceptor (mM)	Column (Lxd, mm x mm)	Yield (%)	Production (g.h⁻¹ · g <sub>biocatalyst</sub> ⁻¹)
Step 1	500	875	100	100	145x5	92	1,1
Step 2	250	875	150	50	145x5	54	0,3

Final results of the continuous production of pseudo-ceramide after the two steps process:

- Good performance: 50%
- Good productivity:  $\approx 0,3 \text{ g.h}^{-1} \cdot \text{g}_{\text{biocatalyst}}^{-1}$

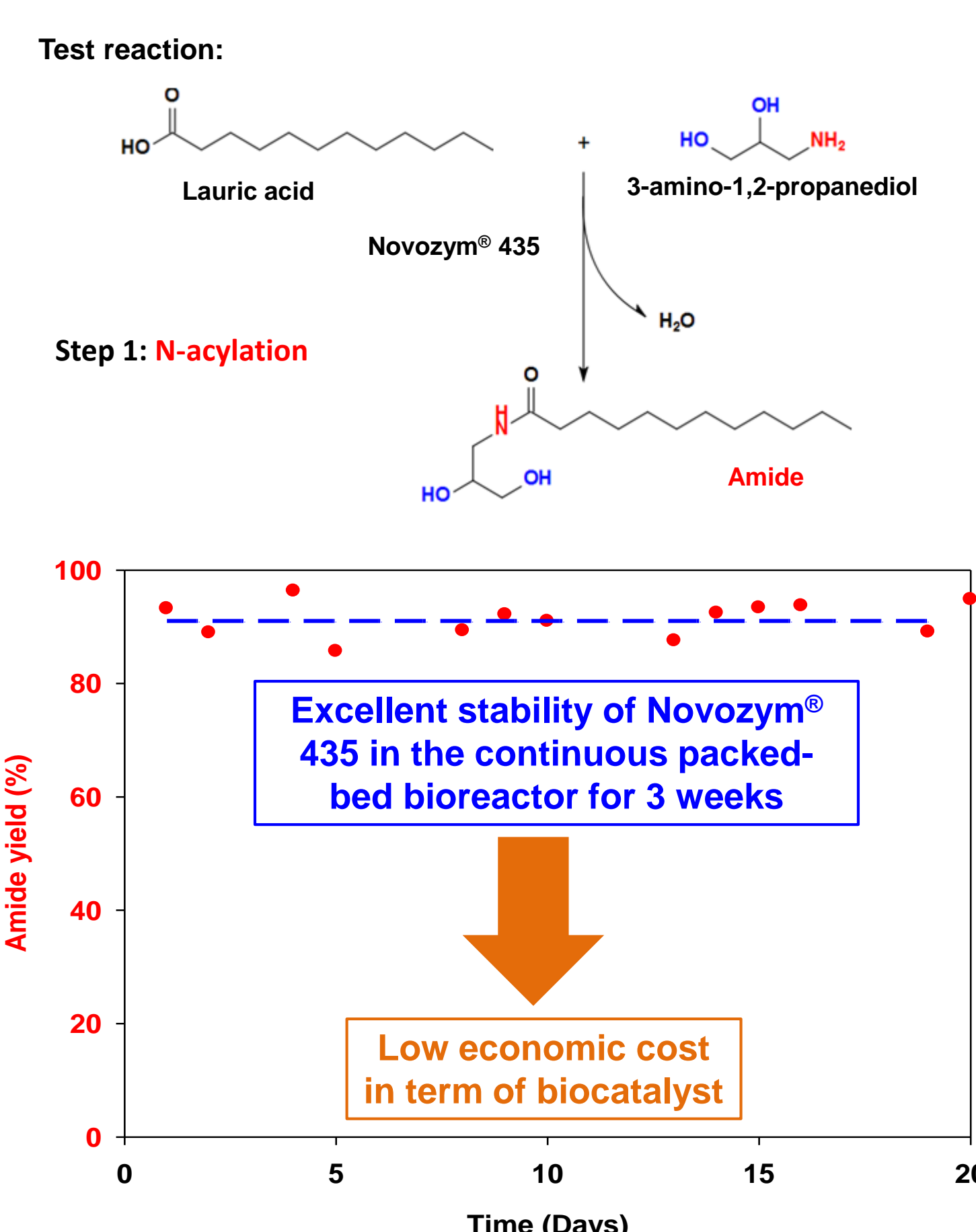
## Evaluation of parameters for scale up of the process:

### 1/Effect of the nature of the fatty acid (Fig 4):



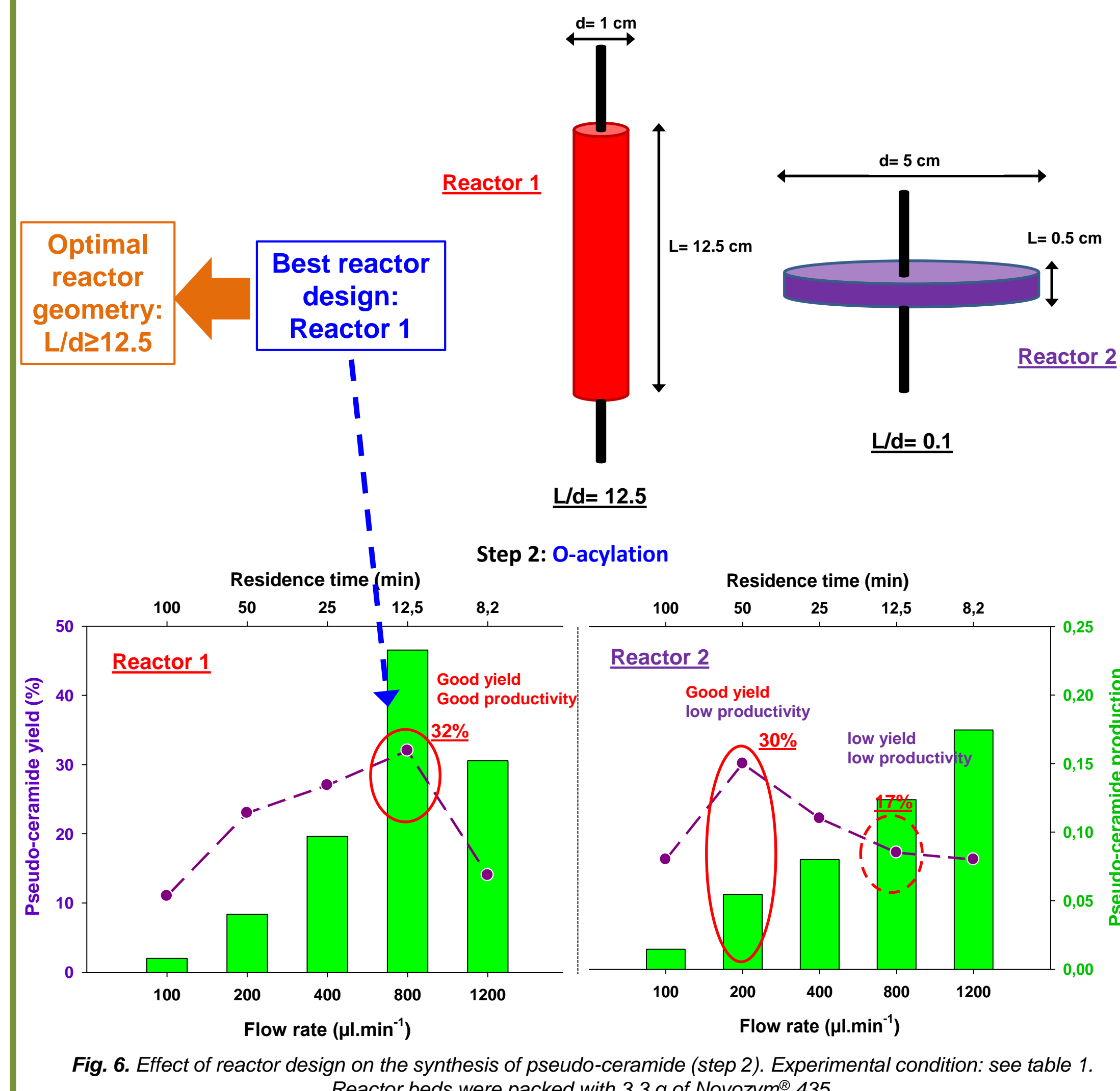
An amide yield superior or equal to 80% was obtained with all fatty acid used  
 Production of pseudo-ceramides with various carbon chains

### 2/Biocatalyst stability in the continuous synthesis process (Fig. 5):



Excellent stability of Novozym® 435 in the continuous packed-bed bioreactor for 3 weeks  
 Low economic cost in term of biocatalyst

### 3/Effect of reactor design (Fig. 6):



Optimal reactor geometry: L/d ≥ 12.5  
 Best reactor design: Reactor 1

**Conclusion:** In this work, we developed a new efficient continuous process for the selective Novozym® 435-catalyzed synthesis of pseudo-ceramides, conducted in a packed-bed bioreactor. The pseudo-ceramide was produced at a satisfying yield of 50% and a production rate of 0,3 g h<sup>-1</sup> · g<sub>biocatalyst</sub><sup>-1</sup> (120 g in 3 weeks under our optimal experimental conditions). Moreover, our results are encouraging in terms of the future development of this process for production of various pseudo-ceramides on an industrial scale: low cost in terms of biocatalyst, similar synthesis yields with various fatty acids used as acyl donors and optimization of the reactor design.

[1] F. Le Joubiou, N. Bridiau, M. Sanekli, M. Graber, T. Maugard, J. Mol. Catal. B Enzym. (2014) 109;143–153.

[2] F. Le Joubiou, N. Bridiau, Y. Ben Henda, O. Achour, M. Graber, T. Maugard, J. Mol. Catal. B Enzym. (2013) 95;99–110.