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Exploring the co-immobilization of multi-enzyme system to scale-up step-wise biotransformations

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PURPOSE OF THE ABSTRACT

The co-immobilization of multi-enzyme systems offers the opportunity to design smart biocatalysts to carry out stepwise biotransformations. However, some aspects related to sequential enzymatic reactions such as the kinetic parameters of the system, the spatial distribution and the orientation of the enzymes on the support surface, must be optimized to maximize the efficiency of the process. In this work, we set out to develop a biocatalyst capable of transforming aliphatic diols into ω-hydroxy acids in the same reactor through a 5-enzyme system. For this purpose, we evaluated the effect of spatial distribution by designing several combinations, testing them in batch, reaching in the first one up to 65% conversion of the final product (1). To immobilize that system, we have previously designed a tri-funtional carrier to assamble a biocatalyst thorugh different chemistries. Two spatial distributions were tested, resulting the one biocatalyst where the 5 enzymes were co-immobilized the most efficient. Despite promising results, no longer than 50% was achieved. We explored the change of localization of ine of the enzyme of the system, resulting an effective increase in the biocatalyst productivity. In addition, we increased the operational stability of the system by a post-immobilization step in which the immobilized enzymes were cross-linked with a polycationic polymer. This more robust biocatalyst was implemented in batch, reaching a conversion up to 80% of the final product. When biocatalyst was applied in flow-through process, presented a espected limitation since regeneration system is a oxygen-dependent reaction. To overcome the oxygen limiting factor, we employed a smart co-substrate for in situ oxygen generation for a successful flow reaction. In a flow rate of 10 µl/min and a 20 mM concentration of main substrate (1,5-Pentanediol), a conversion up to 75% of the final product (5-hydroxypentanoic acid) was achieved, whereas no product was detected by no smart co-substrate application. Overall, this work has allowed us to improve our knowledge in co-immobilized enzyme systems to scale up multi-enzyme transformations. We observed a positive effect by having all enzymes located in the same vessel compared to those separated under flow reaction conditions. Furthermore, we solved an intrinsic problem of employment of oxygen-depedent enzymes in flow. Overall, we demostrated that complex multi-enzymatic reactions can be a reality in the application in flow system through a rational design.

FIGURES

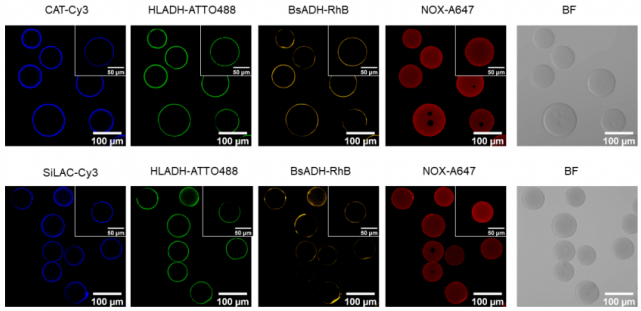
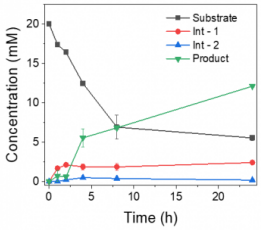


Figure 3: Confocal microscopy images of flurophore-labelled enzymes at 20X (group of particles) and 40X (individual particle).



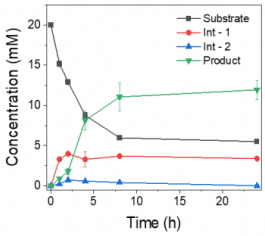


FIGURE 1

Spatial localization of each enzyme involve in the cascade

Confocal microscopy images of flurophore-labelled enzymes at 20X (group of particles) and 40X (individual particle).

FIGURE 2

Study of both distributions under batch conditions

Kinetic at 24 hours of each spatial distribution systems: Distribution 1 (on the left) and Distribution 2 (on the right). Rxn mix: 10 mM 1,5-Pentanediol; 1 mM NAD+, 0.15 mM FAD; T(P) 100 mM pH 8

KEYWORDS

Flow biocatalyst | Multi-enzyme systems | smart co-substrates

BIBLIOGRAPHY