Enzymatic Tandem Reactions in UF Membrane: the role of proximity and spatial organization.

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In recent years, enzymatic cascade reactions have seen increased scientific and industrial applications, particularly in the synthesis of pharmaceuticals [1, 2] and the development of biosensors [3]. This is due to the benefit of multistep chemical reactions occurring in a one-pot set-up. An important factor for efficient cascade reactions is the spatial organization of the multi-enzyme component. Different approaches have been developed to arrange/orient the multi-enzyme constructs, namely (i) enzyme scaffolds [4], (ii) recombinantly-linked multi-enzyme as a fusion protein [5] and (iii) immobilization on a matrix [6].

Recent advances in materials science have driven the synthesis of different supporting matrices suitable for enzyme co-immobilization [7] or compartmentalization [8]. The major advantage of these enzyme-matrix structures is the direct transfer (defined as substrate channeling) of a substrate from one enzyme to another without equilibration, for a rapid conversion of unstable intermediates, avoidance of substrate competition, and regulation of unfavorable equilibria [9]. Concentration gradient diffusion is the predominant mass-transfer mechanism for substrates, intermediates and products around an immobilized enzyme and the high diffusion resistance greatly hindering the catalytic efficiency of these structures, limiting their applications [10].

The use of porous membranes to support enzyme cascade reactions, despite being overlooked, may overcome these diffusional limitations and increase reaction efficiency, owing to co-enhancement of diffusion and convection [11]. Moreover, the enzyme microenvironment, which plays a crucial role in multi-enzyme catalysis, affects enzyme activity and stability and can be tuned to regulate the system when using porous membranes [12].

Herein, glucose oxidase (GOx) and horseradish peroxidase (HRP) were immobilized in asymmetric ultrafiltration (UF) membrane (i.e., underneath the separation layer) in a sandwich-like assembly. A commercial polyethersulfone (GR80PP, Alfa Laval) and a regenerated cellulose (RC70PP, Alfa Laval) membrane with MWCO of 10kDa were used. The role of microenvironment, enzyme proximity and substrate competition were evaluated and demonstrated complex dynamic responses when varying substrate concentration and reaction conditions. The hierarchical enzyme distribution facilitates the ordered substrates

transport: glucose and molecular oxygen first migrate to GOx, located under the membrane separation layer, while the product of the GOx-catalyzed reaction is directed to HRP, in the core of the UF membrane. The selective separation function of UF membrane, coupled with the tandem reaction of GOx-HRP, provide a robust model system to harness the synergism of membrane separation and enzymatic catalysis at the liquid/solid interface, with potential application in chemicals and pharmaceuticals synthesis.

Keywords: cascade reaction, nanofiltration, compartmentalization, reverse filtration, bio-catalysis

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Topic name / number: Applications of Membrane/1
Category preferred: Poster/Oral Presentation
Would you like to be considered for a presentation award (Student and ECR only, please check eligibility on www.imstec2022.org)?: Yes

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