DESIGN OF NANOSTRUCTURED MATERIALS FOR ENZYME IMMOBILIZATION

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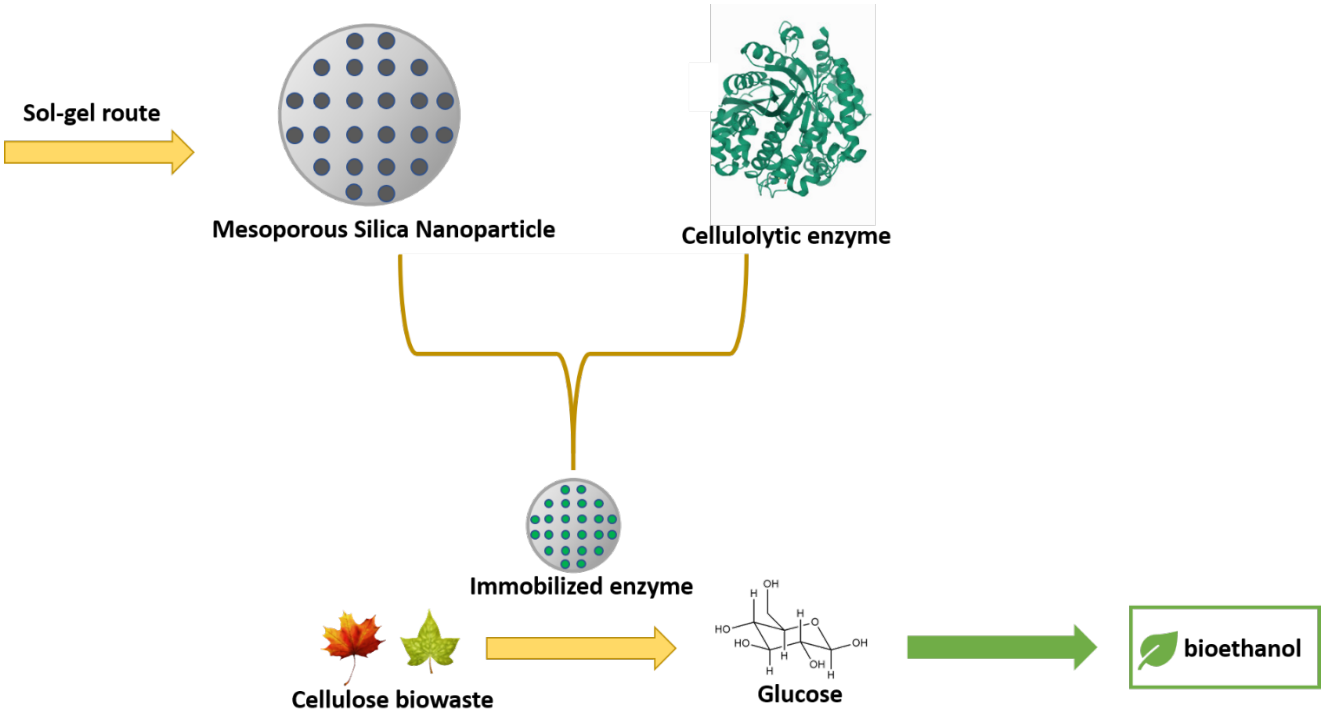
Curriculum: Ingegneria dei Materiali e delle Strutture

Enzymes are an interesting class of proteins involved in the catalysis of a wide range of physiological reactions which constantly take place in living organisms. Moreover, in the last decades enzyme catalysis gained attention among the industrial community due to the number of advantages that the use of this class of biocatalysts implies, such as milder reaction conditions with respect to traditional catalysts, exceptional product selectivity, lower environmental and physiological toxicity. These outstanding features led pharmaceutical, food and beverage, detergent and biofuel industries to consider the use of enzymes for commercial-scale applications. However, the high production costs, the considerable instability to temperature, solvent, pH as well as the difficult separation from the reaction products have undermined the further diffusion of enzymes as catalysts for industrial processes so far. Enzyme immobilization stands as a suitable strategy to overcome these drawbacks. As a matter of fact, anchoring the protein onto a solid support improves its stability in a broader range of reaction conditions, facilitates the separation from the reactor, ensures its reusability and functionality for use in continuous processes. Enzyme immobilization can be achieved through either chemical or physical binding. Covalent binding ensures high enzyme loading on the support and prevents protein leakage, although it could undermine the substrate-enzyme affinity. On the other hand, physical immobilization leads to less strong protein-support interaction but preserves most of the activity of the enzyme in its free form. There is no perfect support for all the enzymes. The challenge is to choose the matrix that creates the optimal microenvironment for the protein, preserving most of its secondary structure and allowing for an efficient interaction between the active site and the substrate to be converted.

This PhD project focuses on the design of nanostructured materials for enzyme immobilization, with particular attention to the correlation between the physico-chemical features and the catalytic performances of the prepared systems. The research activity aims at shedding new light on the dynamics of the interaction between support and protein, with the purpose to provide a better understanding of the protein organization onto the matrix and optimize the process of immobilization.

During my first year of PhD, most of the work was focused on the synthesis of ceramic nanoparticles through sol-gel route and the subsequent physical adsorption of cellulolytic enzymes onto them. The nanostructures underwent physico-chemical characterization through Fourier-Transform Infrared Spectroscopy (FTIR), Dynamic Light Scattering (DLS), N_2 physisorption, Thermogravimetric analysis (TGA). The organo/inorganic hybrid biocatalysts were tested in the hydrolysis of lignocellulosic biomass to glucose, whose further fermentation produces bioethanol, a carbon-neutral biofuel. Preliminary results have identified mesoporous silica nanoparticles as a suitable support. Indeed, the high surface area and the open pore structure exhibited by the inorganic silica skeleton ensure enough enzyme accessibility and reduce mass transfer limitation for both substrate and reaction product. Catalytic assays showed that the prepared biocatalysts performed remarkably well in the conversion of cellulose derivates, providing high glucose yields.



Giulio Pota, PhD student XXXVI cycle, May 2021