**ROLE OF ENZYME IMMOBILIZATION IN THE FORMULATION OF ENZYMES FOR SINGLE USE**

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Immobilized enzymes find application in diverse areas of biosciences as biofuels, biosensors and biocatalysis. Development of a immobilization for an enzyme implies a careful selection and optimization of different parameters that have a critical influence on the final properties, e.g. activity, stability [1,2]. Enzyme stabilization via immobilization is a common target and it is achievable when properly performed [2]. There is the general idea that the use of enzyme in free soluble form is associated to single use due to the simplicity and significant lower cost, whereas the use of immobilized preparations is generally associated to facilitate retention, recycling or continuous use. Nevertheless, there are some constraints where immobilization is a fundamental tool to enable the single use of the enzyme under conditions otherwise not-applicable. Among others, these conditions involve the preservation of enzyme activity during extraction, purification, storage and transport, stabilization under condition of use and easy separation from the medium where it is applied. This communication presents some examples of immobilization strategies and benefits for the application of oxidative enzymes.

Glucose oxidase and D-amino acid oxidase were immobilized into porous materials made from natural organic polymers or synthetic organic polymers [3,4]. Under the conditions of use, that involves diluted application and existence of gas-liquid interfaces, the free enzymes have half-lifetime lower than 5 min whereas proper immobilized preparations within porous materials did not show decrease of activity during a time span of observation of 40 h. The storage of the enzyme formulation was also facilitated, immobilized enzymes were stable under simple storage conditions whereas free enzymes required prevention microbiological contamination and tuning of buffer conditions to avoid protein precipitation. Among different strategies, the use of reversible immobilization was evaluated to enable the reuse of support and facilitate an immobilization on/off character. The development reversible one step purification-immobilization simplified the production and application of the immobilizate format compared with the soluble counterpart [3]. Finally, the immobilization was used as a compartmentalization strategy, two enzymes (oxidase-catalase, oxidase-peroxidase) or a pair of oxidase - oxygen probe can be used when they are confined into solid materials compared to incompatible conditions of use in soluble format [5]. Co-joined design of polymeric materials and immobilization strategies is recognized as important for the efficient cost-effective formulation of enzyme immobilizates.

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