

## Editorial

# Enzyme Immobilization, an Old-Fashioned Tool or a Modern Strategy to Improve Enzyme Properties?

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## Editorial

Enzymes are very promising biocatalysts, due to their high activity under mild conditions, high specificity and selectivity [1]. However, due to their biological origin, enzymes have some drawbacks when used as industrial biocatalyst. They are water soluble molecules difficult to separate and recover in many instances, unstable under conditions far from the physiological ones, the high activity, selectivity and specificity refers to their physiological substrate, etc.

The solubility of enzymes may be solved by immobilization techniques. Thus, immobilization is a general requirement for the use of enzymes as industrial biocatalysts in most cases [1-3]. Considering this need, many efforts have been paid to joint immobilization to the solution of other enzyme limitations [4,5].

A proper immobilization may improve enzyme stability if a multipoint or a multisubunit enzyme immobilization is achieved [6,7]. Multipoint covalent attachment may prevent the enzyme distortion caused by any adverse condition, increasing enzyme rigidity, this may even improve enzyme activity under unfavorable conditions [8]. Multi-subunit immobilization may prevent multimeric enzyme dissociation, enabling the use of multimeric enzymes under potentially dissociation conditions [7]. To get this, it is precise to select a proper immobilization system, not only a suitable support is necessary, also an adequate support reactive group, proper immobilization conditions and protocol is required to take full advantages of the potential of enzyme immobilization [4].

Moreover, if an adequate microenvironment of the enzyme environment is generated around the enzyme, hydrophobic (e.g., organic solvents, oxygen) or hydrophilic (e.g., hydrogen peroxide) compounds may be partitioned out of the enzyme environment, improving the enzyme stability/activity in the presence of this delirious reagents [4,8]. Furthermore, immobilization has proved to solve other enzyme limitations. For example, enzyme inhibition may be decreased via immobilization (e.g., blocking the access of the inhibitor to the inhibition site) [4,8].

However, one point where immobilization has proved to be unexpectedly powerful is in the modulation of enzyme selectivity and specificity [8]. This occurs mainly when the enzyme has a flexible active center, like lipases, penicillin G acylase or multimeric

enzymes. The partial distortion of some areas of the protein or the rigidification of other that avoid certain conformational changes, produce severe alterations of the enzyme properties [9]. This is a random modification, but it should be considered that will occur even if the researcher did not desire that this modulation may be produced. The experience shows that to have a biocatalyst of these enzymes with good activity/selectivity features, the best way is to have a very wide library of immobilized biocatalysts, as different as possible [8].

For almost all applications of immobilization, it is necessary to achieve a deeper knowledge of the immobilization processes, the exact mechanism of even very old immobilization protocols may give us clues on the control of the immobilization process [10]. Moreover, the development of new immobilization protocols (new reactive groups, new supports, new strategies, etc) may open the opportunity for new applications of immobilization to improve enzyme properties [4].

One of the most interesting tendencies is the coupled use of different strategies to improve enzyme properties [7,11-14]. Immobilization is compatible with any other enzyme tuning strategy, and in certain cases a genetic [11,14] or chemical modification [11-13] designed for an improved immobilization has proved that may be a very powerful tool to further enhance the enzyme features. The modified enzymes will be in any case immobilized for their use, thus the design of modifications to improve the immobilization seem to be a very interesting strategy. On the other hand, the solid phase modification of enzymes is clearly advantageous compared to the modification of the free enzyme [13].

Regarding the immobilization strategies, new ones like the production of enzyme cross linked aggregates have open new opportunities for the immobilization of complex enzymes [15]. This methodology has advantages and drawbacks, but its interest is indubitable [4].

However, the use of preexisting solids is still the most used, and also is the most versatile, method for enzyme immobilization. Future trend intend the design of hetero functional supports for a more controlled and directed immobilization, mixing moieties with different function [16]. One point or multipoint immobilization are critical concepts on these strategies, a final support with a surface as inert as possible is also required (in general, but more in these cases). Moreover, immobilization (the incorporation of the enzyme to the support) and enzyme-support multi-interaction need to be clearly secluded, as may be two different steps in most cases.

The advances of nanotechnologies have open new opportunities for immobilized enzymes applications, for example for the use of immobilized enzymes in medicine, but also in biocatalysis [17-19]. The control at the nanoscale of the support morphology and of its activation may permit to solve many of the problems of enzyme immobilization.

Thus, this apparently old-fashioned technology is really starting to be aware of its huge potential for enzyme properties improvements.

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