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Editorial

Multienzymatic Catalysis and Enzyme Co-Immobilization

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1. Introduction

The evolution of biocatalysis has undergone an unprecedented boost in response to the human demand for sustainable chemistry, which should enable researchers to make the most complex, selective and specific compounds with minimal ecological impact [1–5]. In this context, researchers have tried to mimic living beings' metabolic chains to transform simple and cheap substrates into very complex ones. This involves the use of multi-enzymatic systems to catalyze these multi-cascade reactions [6–10]. Nowadays, mimicking natural evolution mechanisms [11,12], fusion proteins may be generated by coupling two enzymes with their corresponding active centers in a single peptide chain [13–20]. The considerable developments in techniques to generate artificial enzymes have permitted the creation of new active centers on existing enzymes to generate plurizymes, which have been used to catalyze cascade reactions [21–23].

It is in this context of catalyzing cascade reactions that the interest in enzyme co-immobilization has led to considerable development in recent times [24–27], with an important focus in controlling the spatial ordering of the enzymes [28–32]. Enzyme co-immobilization enables the second and further enzymes in the cascade chain can be exposed to a high concentration of their respective substrates (products of the modification catalyzed by the previous enzyme) [33,34]. However, these kinetic gains must compensate for the problems generated by co-immobilization [35].

The co-immobilization of enzymes means the use of the same support and involves a protocol for all related enzymes [24–27,35]. Enzyme immobilization is no longer just a way to recover and reuse enzymes, but also a potent tool to solve many enzyme limitations [36]: enzyme stability can be improved; enzyme activity, selectivity or selectivity may be tuned; inhibitions may be reduced; and resistance to deleterious reagents may be increased [36]. Even enzymes immobilization may be associated with their purification [37]. The standard co-immobilization of enzymes requires that under all co-immobilized enzymes, the surface must be identical. Obviously, only through serendipity can the same supports and immobilization protocols occur be optimal for all involved enzymes. When using a porous support, the size of the largest co-immobilized protein will determine the pore diameter of the support [36]. And usually, a larger pore diameter means a lower loading capacity and weaker mechanical resistance.

One point that is usually ignored is the possibility that one of the co-immobilized enzymes may be much more unstable than the others [35]. This means that after a few operation cycles, some of the enzymes may decrease their activity to a level that makes the previous enzyme ratio optimization inefficient. When using standard co-immobilization strategies, this may involve the necessity to discard immobilized enzymes that are fully active [35]. If, as in some instances, the reuse of a support is interesting from an industrial point of view, the possibility of reusing one or several co-immobilized enzymes to build new combibiocatalysts becomes appealing [35]. This way, new strategies where the most stable enzymes are immobilized following a strategy that is different to the one used for the least stable enzymes (which must be immobilized via a reversible immobilization technique) have been developed [35]. Thus, the most stable co-immobilized enzymes may

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be converted into ionic exchangers via physical or chemical modification, and the least stable enzymes may be then immobilized over them [38]. After their inactivation, the least stable enzymes may be released to the medium and the biocatalyst reused to build a new combibiocatalyst. Heterofunctional supports, which have chemically reactive moieties and adsorbent ones, have been also utilized for this purpose [39]. Similarly, most stable enzymes may be covalently immobilized on supports that, as reaction end point, require a blocking step [40]. This blocking can be used to render the support with physical adsorbent capacity, which enables the reversible immobilization of less stable enzymes [40].

Considering the important role of enzyme immobilization in the final design of industrial biocatalysts, the co-immobilization of several enzymes must be performed after a careful evaluation of the advantages and disadvantages [35].

This Special Issue shows some examples of instances where enzyme co-immobilization offers advantages, and also shows the problems derived from the use of co-immobilized enzymes [41].

Conflicts of Interest: The authors declare no conflict of interest.

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