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An intimate look at immobilized enzymes toward the design of robust and efficient heterogeneous biocatalysts

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PURPOSE OF THE ABSTRACT
In applied biocatalysis, protein immobilization is a key technology to enhance biotransformations. In the last decade, single- and multi-enzyme systems together with their cofactors have been co-immobilized on solid materials with the aim of boosting the throughput of enzymatic cascades.[1] In one of the most striking advances for the design and assembly of multienzyme cascades reported in recent years, Merck and Codexis in a joint effort have brought together 5 enzymes, co-immobilizing two of them, for the synthesis of the antiviral Islatravir.[2]
The heterogenization of enzymes and their corresponding cofactors to carry out chemical reactions without the exogenous supply of the latter gives rise to a new generation of self-sufficient heterogeneous biocatalysts that facilitate the separation and recycling of all the elements that form the biocatalysts. However, the immobilization process in biocatalysis is still too empirical and enzyme behavior (kinetics and stability) inside solid materials remains as a black box that we barely understand.
In this work, we advance the characterization of the spatiotemporal behavior of heterogeneous biocatalysts to better understand the stability and kinetic properties of enzymes when confined within the solid surface of porous materials. We have exploited image processing derived from time-lapse fluorescence microscopy experiments to determine the intraparticle kinetics of self-sufficient heterogeneous biocatalysts composed of different enzymes (oxidoreductases, hydrolases and transaminases) and their corresponding cofactors (NAD(P)H, PLP or FAD).[3] The resulting self-sufficient heterogeneous biocatalysts were analyzed under static and in operando conditions to investigate the thermodynamics of cofactor binding, enzyme density and apparent Michaelis-Menten (MM) kinetics of the enzyme, both at single particle and intraparticle level. Furthermore, we revealed that unexpected migration of immobilized enzymes across the solid surface occurs when the enzymes are reversibly bound, and that this process takes place both during storage of the biocatalysts and during their operational performance. Our studies also revealed that enzyme concentration and spatial organization are the main sources of functional variability in these self-sufficient systems and have a significant impact on their catalytic efficiency. This knowledge, which could only be revealed by single particle microscopic studies, led us to optimize highly efficient heterogeneous biocatalysts in which both enzyme(s) and cofactor(s) are reused either discontinuously (batch reactors)[1] or continuously (flow reactors),[4] and can reach total turnover numbers as high as 105 and 104, respectively, exhibiting industrially relevant productivities (50 g L-1 h-1).

FIGURES

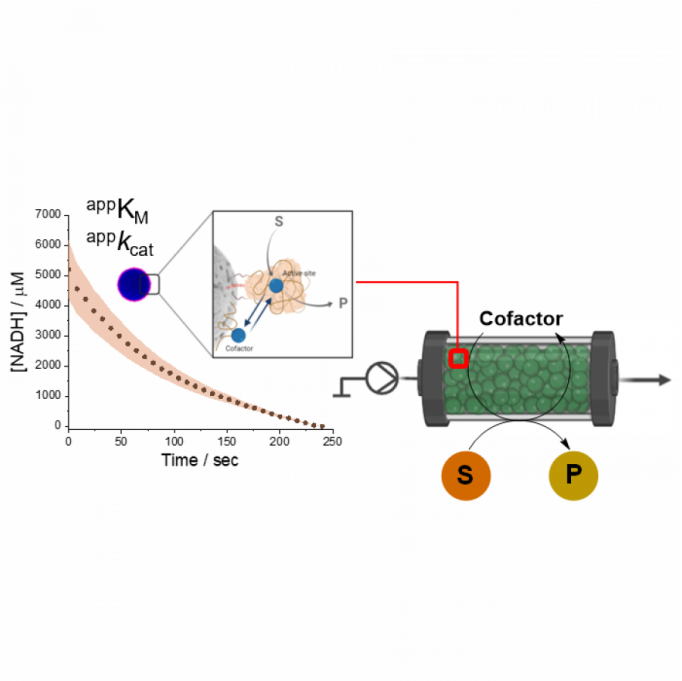

FIGURE 1

Intraparticle kinetics of self-sufficient heterogeneous biocatalysts to be applied in flow-reactors

The heterogeneous biocatalyst packed into a plug-flow column can be analyzed in operando through fluorescence microscopy to determine the enzyme kinetics

FIGURE 2

KEYWORDS

enzyme immobilization | ketoreductases | transamianses | enzyme kinetics

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