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Application of Self-Immobilizing Decarboxylases: From Biogenic Nanoparticles to Monolithic All-Enzyme Hydrogels in Flow

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PURPOSE OF THE ABSTRACT

Introduction and Research Concept: The beneficiation of lignin as the largest regenerative source for aromatic compounds is in the focus of scientific research. For this matter, the application of biocatalysts such as phenolic acid decarboxylases (PADs) are required to valorize lignin product streams. Biocatalytic flow reactor systems enable the synthesis of industrially relevant styrenes. However, high and stable conversion is limited by inefficient immobilization of enzymes. Hence, new strategies providing precise temporal and spatial reaction control in flow reactors are needed.[1] Given the tremendous impact of continuous reactor formats, we developed decarboxylative flow reactors based on self-immobilizing PADs and evaluated their performance to boost productivity.

Methods and Results: Carrier-free enzyme immobilization technologies are a major development in the field of streamlined continuous processes. These were expanded to PADs in the form of monolithic, self-assembling all-enzyme hydrogels, generating highly active materials with tunable rheological properties. Arranged in microfluidic reactors, the biocatalytic gels reached near quantitative conversion (> 90%) along with high robustness under process and storage conditions.[2]
Complementary, isolated biogenic magnetosomes displaying SpyCatcher moieties enable a flexible magnetic reactor design.[3] Following in vivo expression of such constructs, Spy-Tag-equipped PAD could be immobilized on the nanoparticles in vitro, demonstrating the simplicity and versatility of the application in biocatalytic processes. Commercially available particles with similar functionalizations were surpassed by the stable substrate conversion for prolonged times by our nanobiocatalysts.
In a parallel approach we demonstrate the utility of thermostable enzymes in the generation of biocatalytic agarose-based inks for a simple temperature-controlled 3D printing process for a two-step sequential biotransformation in a fluidic setup.[4]

Conclusion: Genetically encodable immobilization tags enable self-assembling biocatalytic reactor concepts with unprecedented elegant simplicity and efficiency. Especially in combination with magnetosomes, they enable a completely biologically produced alternative to commercial solutions.

FIGURES

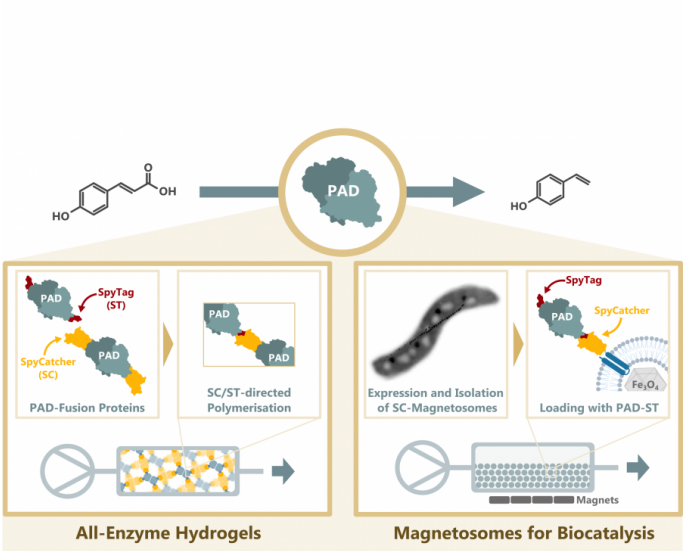

FIGURE 1

Immobilizing Strategies for PAD in Flow Reactors

Continuous flow production of p-hydroxystyrene from p-coumaric acid by a phenolic acid decarboxylase (PAD) immobilized as an all-enzyme hydrogel (left) or on functionalized magnetosomes using the SpyCatcher-SpyTag system.

FIGURE 2

KEYWORDS

biocatalysis | enzyme immobilization | phenolic acid decarboxylase | genetic engineering

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