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Immobilisation of oleate hydratase on solid supports

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PURPOSE OF THE ABSTRACT
Oleate hydratase (Ohy) is an FAD dependent enzyme that catalyzes the hydration of unsaturated fatty acids forming hydroxy-fatty acids.[1] This catalytic activity makes this enzyme a potential catalyst for valorisation of biorenewable fatty acids into products for the chemical and polymer industry. However, the solubility of fatty acids in aqueous media is strongly limited and high amounts of co-solvents are necessary for solubilisation, which are reported to deactivate the enzyme.[2]

In this regard, we studied the immobilisation of the Ohy from Rhodococcus erythropolis on commercially available solid supports. Immobilisation is a powerful technique to improve the performance of enzymes in biotechnological processes. The carrier binding separates the enzyme from the reaction medium and prevents protein aggregation, thus, often leading to higher stability.[3] However, there is only one report of a successful covalent immobilisation of Ohy on chitosan.[4] We used a screening kit[5] to test carriers (spherical polymeric beads) with different interaction types: a) covalent, b) adsorption, c) cationic, d) anionic and e) His Ni^{2+}. The immobilisation process was evaluated by determination of the protein concentration in the supernatant before and after immobilisation as well as with activity measurements.

We screened 18 different carriers from ChiralVision[5] and achieved a maximum activity recovery of approximately 30 % compared to the free enzyme, which is a good value taking into account that enzymes are often (partly) deactivated during the immobilisation process. Subsequently, we characterised the immobilised Ohy and studied its stability against different co-solvents and higher temperature. Leaching of the immobilised enzyme into the supernatant and storage stability were investigated as well. Lastly, we conducted recycling experiments with the immobilised enzyme. In conclusion, we report the first successful immobilisation of Ohy on solid carriers by ionic interaction.

FIGURES

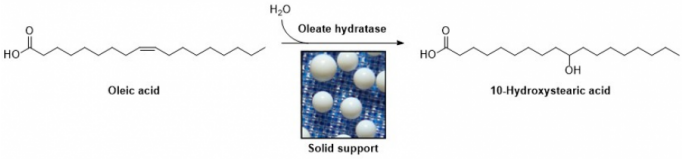

FIGURE 1

Figure 1

Hydration of oleic acid catalysed by immobilised oleate hydratase.

FIGURE 2

KEYWORDS

Immobilisation | Water addition | Fatty acid hydratase | Biorenewable

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