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Self-Sufficient Heterogeneous Biocatalysts in continuous flow asymmetric synthesis of β-Hydroxy esters

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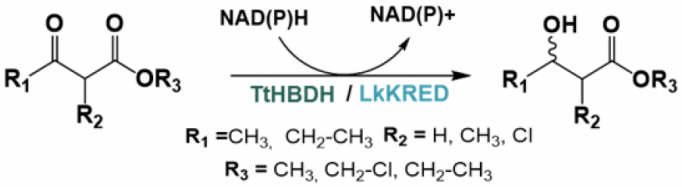
PURPOSE OF THE ABSTRACT
β-Hydroxy esters find applications in the pharmaceutical, food and polymer industries for the synthesis of functional products. While conventional synthetic methods rely on chemical catalysis or whole-cell biosynthesis, the asymmetric reduction of β-ketoesters using cell-free enzymes presents a viable approach for the production of enantiomerically pure β-hydroxy esters. [1] However, the unbearable economic costs associated with enzymes and cofactors calls for the development of approaches to maximize their utilization. Herein, we report the development of two Self-Sufficient Heterogeneous Biocatalyst (SS-HBs) for the enantiodivergent and cost-effective synthesis of β-hydroxy esters from β-keto esters .

Firstly, we carried out an analysis of the reductive activity of two enzymes: a thermophilic (S)-3-hydroxybutyryl-CoA dehydrogenase from Thermus thermophilus HB27 (TtHBDH) [1] and an (R)-Ketoreductase from Lactobacillus kefir (LkKRED) [2] against various β-keto esters. Subsequently, we immobilized them onto macroporous agarose beads and coated them with different cationic polymers to stabilize the quaternary structure of the enzymes and to co-immobilize the required cofactors. As a result, we managed to obtain two SS-HBs that did not require any exogenous supply of NAD(P)H, as both biocatalysts were able to catalyze their own cofactor recycling through 2-propanol oxidation.

We constructed two packed bed reactors and determined the optimal operating conditions by computational simulations and evaluating its productivity at different substrate concentrations (50 - 1000 mM) and residence times. The behavior of the operational stability of the SS-HBs was tested at the optimal conditions, resulting in a Space-time yield (STY) of 55 g L-1 h-1 in the synthesis of enantiopure ethyl 3-(R)-hydroxybutyrate for 6 days with enzyme and NADPH TTN of 350000 and 10300 respectively. In the continuous synthesis of ethyl 3-(S)-hydroxybutyrate we obtained a STY between 49-27 g L-1 h-1 in 2 days with enzyme and cofactor TTN of 146000 and 2980 respectively. A deeper analysis revealed that the loss of activity was caused by thermal decomposition of NADH under operational conditions which was resolved by removing and replacing the cofactor, thus restoring maximum productivity.

In essence, the development of robust and self-sustaining biocatalysts allows for reducing the NAD(P)H costs for the production of β-Hydroxy esters. In this case, from 200 € g-1 to less than 0.07€ g-1 for of ethyl 3-(S)-hydroxybutyrate cell-free production. Consequently, this technology enables the use of these biocatalysts in industrial settings, achieving the necessary cost-effectiveness benchmarks for efficient processes.

FIGURES



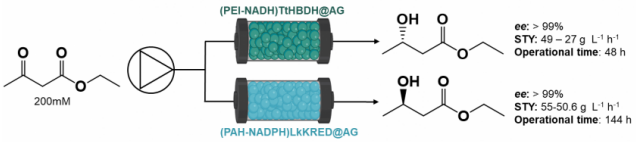


FIGURE 1

Figure 1

Reaction scheme of the asymmetric reduction of different beta-ketoesters by the S-selective NADH-dependent TtHBDH or R-selective NADPH dependent LkKRED

FIGURE 2

Figure 2

Reaction set-up for Self-Sufficient Heterogeneous Biocatalyst packed in Packed Bed Reactors

KEYWORDS

Cofactor Recycling | Industrial Biocatalysis | Beta-Hydroxy esters | Flow biocatalysis

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