Characterization of different biocatalyst formats for BVMO-catalyzed cyclohexanone oxidation

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Abstract

Cyclohexanone monooxygenase (CHMO), a member of the Baeyer-Villiger monooxygenase family, is a versatile biocatalyst that efficiently catalyzes the conversion of cyclic ketones to lactones. In this study, an *Acidovorax*-derived CHMO gene was expressed in *Pseudomonas taiwanensis* VLB120. Upon purification, the enzyme was characterized *in vitro* and shown to feature a broad substrate spectrum and up to 100% conversion in 6 h. Further, we determined and compared the cyclohexanone conversion kinetics for different CHMO-biocatalyst formats, i.e., isolated enzyme, suspended whole cells, and biofilms, the latter two based on recombinant CHMO-containing *P. taiwanensis* VLB120. Biofilms showed less favorable values for K_S (9.3-fold higher) and k_{cat} (4.8-fold lower) compared to corresponding K_M and k_{cat} values of isolated CHMO, but a favorable K_I for cyclohexanone (5.3-fold higher). The unfavorable K_S and k_{cat} values are related to mass transfer- and possibly heterogeneity issues and deserve further investigation and engineering, in order to exploit the high potential of biofilms regarding process stability. Suspended cells showed an only 1.8-fold higher K_S, but 1.3- and 4.2-fold higher k_{cat} and K_I values than isolated CHMO. This together with the efficient NADPH regeneration via glucose metabolism makes this format highly promising from a kinetics perspective.

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