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Enhancing ε-Caprolactone Production via a Multienzyme Cascade in E. coli: Impact of Oxygen Transfer and Ethanol as a Competitive Inhibitor

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ε-Caprolactone is an important monomer in the industry, which can be polymerized into polycaprolactone. Traditional chemical synthesis of lactones uses Baeyer-Villiger oxidation (BVO) of ketones using peracids, which has disadvantages such as low selectivity and formation of harmful by-products. In this study, we present a sustainable alternative via whole-cell biocatalysis using a three-enzyme cascade (ADH, ER, CHMO) that efficiently converts 2-cyclohexanone-1-ol to ε-caprolactone.

We investigated the effect of oxygen transfer coefficient (kLa) and substrate concentration (cs) on the efficiency of oxidation of cyclohexanone to ε-caprolactone. The results indicate a strong dependence of the cascade rate on the correct ratio between kLa and cs, due to the simultaneous metabolic consumption of oxygen by the cells themselves.

We further studied the effect of ethanol as an inhibitor of ADH, which catalyzes not only the first step of the cascade but also the reaction of cyclohexanone to the undesired by-product cyclohexanol. Our study showed that there is an optimal concentration of ethanol that inhibits the side reaction without significantly suppressing the desired ADH activity. These results contribute to a better understanding of kinetic interactions within multi-enzymatic reaction networks and provide a basis for the design of more efficient biocatalytic processes.

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