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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 comprising alcohol dehydrogenase (ADH), enoate reductase (ER), and cyclohexanone monoxygenase (CHMO) that efficiently converts 2-cyclohexen-1-ol to ε-caprolactone.

We investigated the effect of oxygen transfer coefficient (k_La) and cell concentration (c_X) on the efficiency of oxidation of cyclohexanone to ε-caprolactone. The results indicated that the product formation rate was strongly dependent on the optimal setting of k_La in relation to c_X, due to the simultaneous consumption of oxygen by BVO and cell metabolism.

We further studied the effect of ethanol as an inhibitor of ADH, which catalyzes not only the first cascade step 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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