Kinetic properties of whole-cell Baeyer-Villiger oxidation depending on the oxygen transfer rate

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The biocatalytic Baeyer-Villiger (BV) oxidation is a green and reliable way to produce carboxylic acids esters or lactones from ketones. The biocatalytic approaches of such systems can be performed either with whole-cells, purified enzyme, or their immobilized form. In the presented work, whole-cell recombinant *Escherichia coli* BL21(DE3), harboring cyclohexanone monooxygenase (CHMO) original from *Acetobacter sp.* were used as a biocatalyst. However, the use of this type of biocatalyst in such a complex system brings various challenges, which must be coped with. An efficient BV oxidation requires availability of reduced coenzyme flavin adenine dinucleotide ($FADH_2$) and reduced cofactor nicotinamide adenine dinucleotide phosphate (NADPH). Therefore, regeneration of the oxidized components of the enzyme network is necessary through glycolysis of glucose. For this purpose, oxidative regime of the biocatalyst is desirable. Although, oxygen is consumed in the catabolism of bacteria, it is also the second substrate of the BV oxidation, so that oxygen air supply should be sufficient. Bioreactor experiments showed that similar bicyclic lactones production rate 1.68 mmol L^{-1} h^{-1} were obtained for the oxygen transfer rate (OTR) 4.83 mmol L^{-1} h^{-1} at different dry cell concentrations (0.5 – 4.2 g L^{-1}). However, when OTR was increased up to 18.29 mmol L^{-1} h^{-1} the reaction rate also increased to 7.81 mmol L^{-1} h^{-1} for the highest biocatalyst concentration. This observation means that, at low OTR and higher biomass concentrations, there was not enough oxygen for the BV oxidation and a major part of dissolved oxygen was utilized in the metabolic pathways. For better understanding of BV oxidation reaction, and its further optimization, it is essential to determine the oxygen consumption by cells and in the bicyclic ketone oxidation. The specific oxygen uptake rate by bacterial cells was determined to be 1.69 mmol g^{-1} h^{-1}. If an oxygen transfer rate higher than the uptake rate was chosen, the lactones production rate could be adjusted. An optimal ratio between biocatalyst concentration (g L^{-1}) and the oxygen transfer rate (mmol L^{-1} h^{-1}) was set to 1:5. Another factor influencing enzymatic reaction rate was the bicyclic ketone initial concentration. It was shown that the affinity of CHMO to ketone substrate is high. However, the lactones production rate kept constant in substrate concentration range 0.35 – 2.25 g L^{-1} and steep reaction rate decrease was obtained at bicyclic ketone initial concentration above 4 g L^{-1}. Furthermore, optimized reaction conditions like OTR and biocatalyst concentration ratio, and initial substrate concentration will be studied to increase the productivity of the whole-cell BV oxidation.

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