Kinetics based reaction optimization of enzyme catalysed reduction of formaldehyde to methanol with synchronous cofactor regeneration - DTU Orbit (23/12/2018)

**Kinetics based reaction optimization of enzyme catalysed reduction of formaldehyde to methanol with synchronous cofactor regeneration**

Enzymatic reduction of carbon dioxide (CO₂) to methanol (CH₃OH) can be accomplished using a designed set-up of three oxidoreductases utilizing reduced pyridine nucleotide (NADH) as cofactor for the reducing equivalents electron supply. For this enzyme system to function efficiently a balanced regeneration of the reducing equivalents during reaction is required. Herein, we report the optimization of the enzymatic conversion of formaldehyde (CHOH) to CH₃OH by alcohol dehydrogenase, the final step of the enzymatic redox reaction of CO₂ to CH₃OH, with kinetically synchronous enzymatic cofactor regeneration using either glucose dehydrogenase (System I) or xylose dehydrogenase (System II). A mathematical model of the enzyme kinetics was employed to identify the best reaction set-up for attaining optimal cofactor recycling rate and enzyme utilization efficiency. Targeted process optimization experiments were conducted to verify the kinetically modelled results. Repetitive reaction cycles were shown to enhance the yield of CH₃OH, increase the total turnover number (TTN) and the biocatalytic productivity rate (BPR) value for both system I and II whilst minimizing the exposure of the enzymes to high concentrations of CHOH. System II was found to be superior to System I with a yield of 8 mM CH₃OH, a TTN of 160 and BPR of 24 μmol CH₃OH/U·h during 6 hours of reaction. The study demonstrates that an optimal reaction set-up could be designed from rational kinetics modelling to maximize the yield of CH₃OH, whilst simultaneously optimizing cofactor recycling and enzyme utilization efficiency. This article is protected by copyright. All rights reserved.

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