

Design and Optimisation of 2-Oxoglutarate Dioxygenase Expression in *Escherichia coli* Strains for Production of Bioethylene from Crude Glycerol

Authors : Idan Chiyanzu, Maruping Mangena

Abstract : Crude glycerol, a major by-product from the transesterification of triacylglycerides with alcohol to biodiesel, is known to have a broad range of applications. For example, its bioconversion can afford a wide range of chemicals including alcohols, organic acids, hydrogen, solvents and intermediate compounds. In bacteria, the 2-oxoglutarate dioxygenase (2-OGD) enzymes are widely found among the *Pseudomonas syringae* species and have been recognized with an emerging importance in ethylene formation. However, the use of optimized enzyme function in recombinant systems for crude glycerol conversion to ethylene is still not been reported. The present study investigated the production of ethylene from crude glycerol using engineered *E. coli* MG1655 and JM109 strains. Ethylene production with an optimized expression system for 2-OGD in *E. coli* using a codon optimized construct of the ethylene-forming gene was studied. The codon-optimization resulted in a 20-fold increase of protein production and thus an enhanced production of the ethylene gas. For a reliable bioreactor performance, the effect of temperature, fermentation time, pH, substrate concentration, the concentration of methanol, concentration of potassium hydroxide and media supplements on ethylene yield was investigated. The results demonstrate that the recombinant enzyme can be used for future studies to exploit the conversion of low-priced crude glycerol into advanced value products like light olefins, and tools including recombineering techniques for DNA, molecular biology, and bioengineering can be used to allowing unlimited the production of ethylene directly from the fermentation of crude glycerol. It can be concluded that recombinant *E. coli* production systems represent significantly secure, renewable and environmentally safe alternative to thermochemical approach to ethylene production.

Keywords : crude glycerol, bioethylene, recombinant *E. coli*, optimization

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