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The reconstruction of metabolic pathways in selected bacterial and yeast strains for production of bioethylene from crude glycerol

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Crude glycerol, a major by-product from the transesterification of Sunflower oil with alcohol to biodiesel, can pose danger to the environment in large quantities. Studies have shown that utilization of the glycerol to afford commercial products is one of the promising options for lowering its pollution effects and biodiesel production costs. For example, its bioconversion can offer a wide range of chemicals including alcohols, organic acids, hydrogen, solvents and precursors for bioplastics. In Pseudomonas *syringae* species of bacteria, the 2-oxoglutarate dioxygenase (2-OGD) are widely known to be among the enzymes with an emerging importance in ethylene formation. However, the optimization and industrial applications of enzyme as 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 *Pichia pastoris, E. coli* MG1655 and JM109 strains. Ethylene production with a codon-optimized expression system for 2-OGD in *E.coli* using a codon optimized construct of the ethylene-forming gene was studied. The effect of 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, 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 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 ethyleneproduction.

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