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From organic waste to biohydrogen: Approaches to enhance H_2 production by *Escherichia coli*

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Bioconversion of organic waste into biohydrogen (H_2) is a promising strategy both for inexpensive energy generation and for simultaneous waste treatment. Different waste materials, such as biodiesel production waste glycerol, lignocellulosic brewery spent grains (BSG), were used in the study. Lignocellulose, the most abundant renewable biomass with a huge amount of worldwide annual production, is an ideal candidate for biomass and H_2 production. Glucose and xylose are the two most abundant sugars derived from the breakdown of lignocellulosic biomass. Besides, Escherichia coli utilize many natural sugars to form biomass and to produce H_2. The later can be produced from formate decomposition via formate hydrogenlyase (FHL) during *E. coli* glucose, xylose or glycerol fermentation. FHL consists of formate dehydrogenase H (FDH) and membrane-associated four [Ni-Fe]-hydrogenase (Hyd) enzymes. The dilute acid pre-treatment method was used to hydrolyze the lignocellulose structure and the BSG hydrolysate (BSGH) optimal conditions for bacterial growth and H_2 production were designed. *E. coli* BW25113 parental strain and hydrogenase (Hyd) mutants with deletions of genes for key subunits of Hyd 1-4 *(ΔhyaB, ΔhybC,ΔhycE, ΔhyfG)*, respectively, as well as *ΔhyaBΔhybC* double mutant biomass formation, redox potential kinetics and H_2 production were investigated upon BSGH and glycerol utilization. Responsible Hyds for H_2 production upon both glycerol and BSGH utilization were revealed. Approaches, such as mutations in Hyd genes, heavy metals supplementation, pH, redox potential were used to enhance H_2 production upon both glycerol and BSGH utilization. The results obtained will contribute the efficient and economical biomass and H_2 production.

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