

## Hydrogen production and metabolic flux analysis of metabolically engineered *Escherichia coli* strains

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*Escherichia coli* can produce H<sub>2</sub> from glucose via formate hydrogen lyase (FHL). In order to improve the H<sub>2</sub> production rate and yield, metabolically engineered *E. coli* strains, which included pathway alterations in their H<sub>2</sub> production and central carbon metabolism, were developed and characterized by batch experiments and metabolic flux analysis. Deletion of *hycA*, a negative regulator for FHL, resulted in 2-fold increase of FHL activity. Deletion of two up-take hydrogenases (1 (*hya*) and 2 (*hyb*)) increase H<sub>2</sub> production yield from 1.20 to 1.48 mol/mol glucose. Deletion of lactate dehydrogenase (*ldhA*) and fumarate reductase (*frdAB*) further improved the H<sub>2</sub> yield; 1.80 mol/mol glucose under high H<sub>2</sub> pressure or 2.11 mol/mol glucose under reduced H<sub>2</sub> pressure. Batch experiments at varying concentration of glucose (2.5–10 g/L) and yeast extract (0.3 or 3.0 g/L) were conducted for the strain containing all these genetic alternations, and carbon and energy balances were analyzed. The metabolic flux analysis revealed that deletion of *ldhA* and *frdAB* directed most of the carbons from glucose to the glycolytic pathway leading to H<sub>2</sub> production by FHL, not to the pentose phosphate pathway.