Optimization Strategy for Hydrogen Production by *Escherichia coli* Using Brewery Waste

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**Abstract**
Molecular hydrogen (H₂) is efficient, ecologically friendly and renewable energy source for the future. Organic wastes utilization for H₂ production provides inexpensive energy generation with simultaneous waste treatment. Beer is one of the most consumed beverages over the world, consequently during its manufacture various by-products in huge amount are generated. Brewery spent grains (BSG), the one of by-products of brewery production, were applied for *Escherichia coli* growth and hydrogen (H₂) production. The dilute acid and alkali pretreatment methods were used to hydrolyze lignocellulose containing material; oxidation reduction potential (ORP) kinetics and H₂ production (rate and yield) were investigated with the sensitive to H₂ platinum (Pt) and titan-silicate (Ti-Si) electrodes. 1-10 % BSG were dissolved in 0.5 to 3 % acid (HCl, H₂SO₄) or alkali (NaOH, KOH) treated in steam sterilizer, and pH of BSG hydrolysate (BSGH) from 1.5 or 12 to 7.5 were adjusted. BSGH dilutions from 1 to 20 fold were applied; the optimal condition for bacterial growth and H₂ production were designed. The results were compared with the data of *E. coli* BW25113 wild-type grown on peptone medium, with 10 g L⁻¹ glycerol supplementation. Medium acidification was observed upon *E. coli* BW25113 wild-type growth on both BSGH and peptone medium with glycerol: after 6 h growth pH dropped from 7.5 to 5.5 and to 6.9, respectively. Bacterial biomass yield was ~1.8 fold less after 24 h growth on BSGH compared with that on peptone medium under glycerol fermentation. Whereas, there is no difference between bacterial specific growth rates values (μ=0.63±0.02 h⁻¹) in both cases. Subsequently, readings of ORP Pt electrode dropped from positive values down to negative ones, -400 ±10 mV value, with 0.75 ± 0.02 mmol L⁻¹ H₂ production yield at the 3rd h of growth, beginning of the log growth phase of *E. coli* BW25113 wild-type fermenting BSGH. Whereas, upon bacterial glycerol fermentation in peptone medium H₂ production was observed at the end of log growth phase with the 0.75 ± 0.03 mmol L⁻¹ H₂ production yield. In opposite, Ti-Si electrode readings drop was negligible and kept positive in contrast to glycerol fermentation in peptone medium. Taken together, our results demonstrated that usage of BSG consisting of lignocellulosic biomass needs careful process to design so as to achieve bacterial growth and sufficient H₂ production by *E. coli*.