Growth and Hydrogen Production Properties of *Escherichia Coli* During Fermentation of the Mixture of Glucose, Glycerol and Formate at Different pHs

June 3, 2017, 12:15 - 2:15 PM

**Exhibit Hall D, Exhibit and Poster Hall**

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**Disclosures**

**Abstract**
*Escherichia coli* perform dark mixed acid fermentation, during which dihydrogen (H₂) is generated by hydrogenases (Hyd) [1]. *E. coli* encodes four reversible membrane bound [Ni-Fe]-Hyd enzymes having different role in H₂ metabolism during dark fermentation depending on pH [1]. Growth of *E. coli* was investigated during fermentation of the mixture of glucose, glycerol and formate at different pHs. Especially, at pH 7.5 and 6.5 specific growth rate (µ) in wild type and mutants with defects in different Hyd enzymes was in the same range. But at pH 5.5 µ was 0.716 h⁻¹ which was ~1.5 fold lower than at high pH. Surprisingly, in *hycE* mutant (lacks large subunit of Hyd-3) µ was decreased by ~8 fold. It has been shown that in batch tests at pH 7.5 *E. coli* wild type strain evolved H₂ during long time (~220 h) but at pH 5.5 H₂ production time was less (~150 h). This is prolonged period if compared to single carbon sources fermentation. Hyd-3 was responsible for H₂ production at all tested pHs. Interestingly, during ~ 24 h at pH 7.5 the oxidation reduction potential measured by Ti-Si-redox electrode dropped down to -150 mV in Hyd mutants compared to wild type. In addition, when cells were grown at pH 7.5 medium pH dropped with 1 unit only in *hyfG* (lacks large subunit of Hyd-4) mutant compared to wild type. Taken together, it can be concluded that when the mixture of glucose, glycerol and formate is present H₂ evolution by *E. coli* was prolonged till 10 days. This is important if applying in large scale H₂ gas production. Moreover, at pH 5.5 the role of Hyd-3 in *E. coli* growth was revealed. At high pH reducing reactions are stronger than at low pH. These data might suggest that when the mixture of glycerol, glucose and formate is present the role of Hyd enzymes in H₂ metabolism and cell growth, in general, is changed. This might be important to understand the role of Hyd enzymes in cell physiology mainly the role of Hyd-3 in maintaining proton motive force at low pH.