

## Effect of Inoculum Ratio on Dark Fermentative Hydrogen Production

**Authors :** Zeynep Yilmazer Hitit, Patrick C. Hallenbeck

**Abstract :** Fuel reserve requirements due to depletion of fossil fuels have increased interest in biohydrogen since the 1990's. In fermentative hydrogen production, pure, mixed, and co-cultures can be used to produce hydrogen. Several previous studies have evaluated hydrogen production by pure cultures of *Clostridium butyricum* or *Enterobacter aerogenes*. Evaluating hydrogen production by co-culture of these microorganisms is an interesting approach since *E. aerogenes* is a facultative microorganism with resistance to oxygen in contrast to the strict anaerobe *C. butyricum*, and therefore has the ability to maintain anaerobic conditions. It was found that using co-cultures of facultative *E. aerogenes* (as a reducing agent and H<sub>2</sub> producer) and the obligate anaerobe *C. butyricum* for producing hydrogen increases the yield of hydrogen by about 50% compared to *C. butyricum* by itself. Also, using different types of microorganisms for hydrogen production eliminates the need to use expensive reducing agents. *C. butyricum* strain pre-cultured anaerobically at 37 °C for 15h by inoculating 100 mL of GP medium (pH 6.8) consisting of 1% glucose, 2% polypeptone, 0.2% KH<sub>2</sub>PO<sub>4</sub>, 0.05% yeast extract, 0.05% MgSO<sub>4</sub>·7H<sub>2</sub>O and *E. aerogenes* strain was pre-cultured aerobically at 30 °C, 150 rpm for 9 h by inoculating 100 mL of TGY medium (pH 6.8), consisting of 0.1% glucose, 0.5% tryptone, 0.1% K<sub>2</sub>HPO<sub>4</sub>, 0.5% yeast extract. All duplicate batch experiments were conducted in 100 mL bottles with different inoculum ratios of *Clostridium butyricum* and *Enterobacter aerogenes* (C:E) using 5x diluted rich media (GP) consisting of 2 g/L glucose, 4g/L polypeptone, 0.4 g/L KH<sub>2</sub>PO<sub>4</sub>, 0.1 g/L yeast extract, 0.1 MgSO<sub>4</sub>·7H<sub>2</sub>O. The range of inoculum ratio of *C. butyricum* to *E. aerogenes* were 2:1,4:1,8:1, 1:2,1:4, 1:8, 1:0, 0:1. Using glucose as a carbon source aided in the observation of microbial behavior as well as making the effect of inoculum ratio more evident. Nearly all the glucose in the medium was used to produce hydrogen, except at a 1:0 ratio of inoculum (i.e. containing only *C. butyricum*). Low glucose consumption leads to a higher hydrogen yield due to cumulative hydrogen production and consumption of glucose, but not as much as C:E, 8:1. The lowest hydrogen yield was achieved in 1:8 inoculum ratio of C:E, 71.9 mL, 1.007±0.01 mol H<sub>2</sub>/mol glucose and the highest cumulative hydrogen, hydrogen yield and dry cell weight were achieved in 8:1 inoculum ratio of C:E, 117.4 mL, 2.035±0.082 mol H<sub>2</sub>/mol glucose, 0.4 g/L respectively. In this study effect of inoculum ratio on dark fermentative biohydrogen production using *C. butyricum* and *E. aerogenes* was investigated. The maximum hydrogen yield of 2.035mol H<sub>2</sub>/mol glucose was obtained using 2g/L glucose, an initial pH of 6 and an inoculum ratio of *C. butyricum* to *E. aerogenes* of 8:1. Results showed that inoculum ratio is an important parameter on hydrogen production due to competition between the two microorganisms in using substrate for growth and production of by-products. The results presented here could be of great significance for further waste management studies using co-culture hydrogen production.

**Keywords :** biohydrogen, *Clostridium butyricum*, dark fermentation, *Enterobacter aerogenes*, inoculum ratio in biohydrogen production

**Conference Title :** ICEBWEM 2016 : International Conference on Energy, Biomass, Waste and Environmental Management

**Conference Location :** Montreal, Canada

**Conference Dates :** May 16-17, 2016