Fermentative biohydrogen production by *Enterobacter cloacae* from lignocellulosic biomass, a second generation feedstock!

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**Background**

Biologically H\textsubscript{2} can be produced by unique variety of microorganisms and is basically distinguished into light driven processes like photolysis or photo fermentation and light independent processes like dark fermentation, based on their light requirement. However, dark fermentative hydrogen production process has gained much attention due to the fact that this process proceeds without the presence of light and can also make use of waste organic substances as substrate. Thus, this process serves the dual purpose of waste reduction along with simultaneous production of clean hydrogen energy. In addition, other significant advantages associated with dark fermentative hydrogen process are; higher bacterial growth rates and lower energy demands (no need for light sources). In the recent past owing to the concern for food security there has been increased interest for clean energy generation from the non food competitive second generation feed stocks such as lignocellulosic biomass sugars.

This study highlights on performance of a purified anaerobe, *Enterobacter cloacae DT-1*, for fermentative hydrogen production through dark fermentation route from lignocellulosic biomass sugars.

**Experimental Details**

In present study previously isolated bacterial strain *Enterobacter cloacae* strain DT-1 was used as hydrogen producing microflora. DT-1 was grown anaerobically in BSH medium at 37°C as described previously (Subudhi et al. 2013). Alkaline treated wheat straw and acid treated wheat straw sugar (encompassing of C\textsubscript{5} and C\textsubscript{6} sugars) samples were separately supplemented into the BSH medium as carbon source. Hydrogen yield efficiency of DT-1 strain was calculated to evaluate the wheat straw biomass sugar utilization potential of DT-1 strain. Composition of biogas and production of soluble metabolites was monitored by Gas Chromatographic analysis.

**Results and Discussion**

Hydrogen production performance of DT-1 strain was first investigated from C\textsubscript{5} and C\textsubscript{6} sugars by employing synthetic glucose and xylose, DT-1 strain could effectively utilize C\textsubscript{5} as well as C\textsubscript{6} sugars to produce hydrogen through dark fermentation route. Hydrogen yield efficiency (HY) of DT-1 from glucose and xylose was; 1.4 mol H\textsubscript{2}/mol glucose and 2.2 mol H\textsubscript{2}/mol xylose, respectively. Scale up of batch fermentative hydrogen production in 20 Liter scale at regulated pH enhanced the HY efficiency of DT-1 from 2.2 to 2.8 mol H\textsubscript{2}/mol xylose (1.27 fold increase in HY from laboratory scale). 84% of maximum theoretical possible HY efficiency (3.3 mol H\textsubscript{2}/mol xylose) was achieved by DT-1. These results
demonstrated that DT-1 could significantly utilize C5 sugar. Only few microbes are known to utilize the C5 sugar.

Considering C5 sugar utilization efficiency of DT-1 strain, further research explorations were performed on optimization of hydrogen production performance of DT-1 strain by employing alkaline treated wheat straw biomass sugar (Received from DBT-ICT, Mumbai), acid treated wheat straw C5 rich prehydrolysate (DBT-IOC, Mumbai) as feedstock. Process parameters were optimized and nutrient medium was formulated for optimum hydrogen production from acid treated pretreated wheat straw enzymatic hydrolysate (second generation feedstock, obtained from DBT-ICT, Mumbai), by *Enterobacter cloacae* DT-1 strain. Hydrogen yield efficiency of DT-1 strain (obtained in laboratory scale) from acid treated wheat straw pre-hydrolysate was; 2.66 ± 0.3 mol H2/mol of reducing sugar. Hydrogen yield efficiency of DT-1 strain (obtained in laboratory scale) from pretreated wheat straw enzymatic hydrolysate (obtained from DBT-ICT, Mumbai) was; 2.2 ± 0.2 mol H2/mol of reducing sugar, which increased to 2.46 ± 0.15 mol H2/mol of reducing sugar under decreased the partial pressure.

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