

# Optimization of carbon source for hydrogen production by *Bacillus licheniformis*

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## ABSTRACT

Interest is growing in the production of hydrogen due to increasing energy demand, depletion of fossil fuel reserves and increasing pollution loads on the environment. However, one of the limiting factors in bio hydrogen production is the low hydrogen yield. The objective of the study is to optimize the carbon source and its concentration for maximizing hydrogen production by the isolated facultative strain *Bacillus licheniformis*. Hydrogen yield of *B.licheniformis* utilizing glucose, sucrose, sugarcane bagasse hydrolysate and maize stalk hydrolysate were 0.395, 0.292, 0.404 and 0.411 mol H<sub>2</sub>/mol sugar respectively, when the initial total sugar concentrations were 10 g/L. Varying the initial sugar concentration of maize stalk hydrolysate from 5 to 20 g/L showed 15 g/L as the optimum sugar concentration with a hydrogen yield and hydrogen production rate of 0.443 mol H<sub>2</sub>/mol sugar and 10.416 mL H<sub>2</sub>/L.hr respectively.

**KEY WORDS:** Biohydrogen, *Bacillus licheniformis*.

## 1. INTRODUCTION

Hydrogen (H<sub>2</sub>) is considered as the most promising successor of fossil fuels because of its high energy content per unit volume (142 KJ/g) and zero GHG emission (Sinha, 2011). It is considered as an environmentally friendly fuel, producing only water on combustion (Dunn, 2002). Various methods adopted for the production of hydrogen are thermochemical processes, electrolytic processes, direct solar water splitting processes and biological processes. For the hydrogen-based economy to become a reality, hydrogen production must be cost effective, and this can be achieved only by using waste materials or biomass as a source of energy. Biological hydrogen production is the only option for sustainable hydrogen production because of its low energy requirements and environmental friendliness (Brentner, 2010).

Microorganisms which are capable of producing H<sub>2</sub> exist enormously in natural habitat such as soil, waste water, sludge, cow dung composts, rice straw compost and so on. Potential hydrogen producers can either be isolated or these materials can be used as source of inoculums for fermentative H<sub>2</sub> production. The easily biodegradable carbohydrates such as glucose, sucrose and starch are most widely used substrates for fermentative hydrogen production. The present study is aimed to find suitable substrate for hydrogen production using locally isolated strain of *Bacillus licheniformis*.

## 2. MATERIALS AND METHODS

**Substrates:** Glucose, sucrose, sugarcane bagasse hydrolysate and maize stalk hydrolysate were used in this study. Sugarcane bagasse and maize stalk obtained from local markets and fields, in and around Chidambaram, Tamilnadu were dried in an oven and ground well in a mixer grinder. Powdered substrates were then sieved through a sieve with a pore size of 40 mm and were stored for further use.

**Acid hydrolysis:** Five gram of the powdered substrate (maize stalk and sugarcane bagasse) was hydrolysed using 100mL of 1% (v/v) sulphuric acid for 75 minutes at 15 psi and 121°C in an autoclave (Hitech equipment, India). The hydrolysate obtained after hydrolysis was filtered using a Whattmann No.1 filter paper followed by an ordinary filter paper. The final filtrate was neutralized with 0.5M NaOH. The volume of hydrolysate equivalent to the required amount of sugar is used as the carbon source for the organism.

**Microorganism:** Hydrogen producing strain used in this study is a facultative strain of *Bacillus licheniformis* isolated from soil.

**Batch hydrogen production:** Batch hydrogen production studies were carried out in 250mL Erlenmeyer flasks with 200 mL of fermentation medium. The constituents of the fermentation medium were: (g/L) glucose (10); beef extract (1); yeast extract (2); peptone (5); and NaCl (0.5). The media was autoclaved at 121°C and 15 psi pressure for 15 minutes, after adjusting the initial pH to 6.5. The sterilized media after cooling was inoculated with 24 hr grown culture. The temperature of the system was maintained at 34°C by placing the reactor setup in a thermostat.

**Analytical Methods:** The total gas formed during the fermentation was collected in an inverted conical flask by graduated aspirator bottles. Gas collected in the conical flask was taken out using a syringe at regular time intervals for analysis using gas chromatograph (AIMIL-NUCON 5765, Mumbai, India), equipped with a thermal conductivity detector.

Total sugar was estimated using phenol sulphuric acid method. The simple sugars present in the hydrolysate and its utilization by microorganism during fermentation were analyzed using a High-Performance Liquid Chromatography (Shimadzu, Japan) equipped with C18 column and an ultraviolet detector.

### 3. RESULTS AND DISCUSSION

**Effect of different carbon sources on hydrogen production:** The effect of different carbon sources including glucose, sucrose, sugarcane bagasse hydrolysate and maize stalk hydrolysate on hydrogen production by *Bacillus licheniformis* was studied in batch at a fixed total sugar concentration of 10 g/L. Cumulative hydrogen production, hydrogen yield and hydrogen production rate are given in Table.1. Compared to the simple sugars (glucose and sucrose), hydrolysates of lignocellulosic biomass yield higher hydrogen. Previous studies have also reported a higher production of hydrogen from pre-treated lignocellulosic biomass on comparison to pure sugars (Nissila, 2014). Sucrose was found to be the least favourable substrate for hydrogen production by *B. licheniformis* with cumulative hydrogen production, hydrogen yield and hydrogen production rates of 103.20 mL H<sub>2</sub>/L, 0.292 mol H<sub>2</sub>/mol sugar and 2.457 mL H<sub>2</sub>/L.hr respectively.

Among the two lignocellulosic hydrolysates, maize stalk hydrolysate yielded maximum hydrogen of 0.428 mol H<sub>2</sub>/mol sugar compared to 0.404 mol H<sub>2</sub>/mol sugar for sugarcane bagasse. The increased hydrogen production from the hydrolysate of maize stalk may be attributed to the increased concentration of xylose in its hydrolysate compared to that in sugarcane bagasse hydrolysate, as low glucose to xylose ratio can reduce the utilization rate of glucose and thereby increase the log phase of growth (Ren, 2008). They have also demonstrated that the presence of xylose affect hydrogen yield using the strain *T. thermosaccharolyticum* as they reported limited hydrogen yield and restricted cell growth with no xylose addition. Hydrogen producing bacterial species *T. neapolitana* shows preference for glucose over xylose (Vrije, 2009).

**Effect of total sugar concentration on hydrogen production:** To investigate the influence of total sugar concentration on hydrogen production, the concentration of maize stalk hydrolysate were varied from 5 to 20 g sugar/L. Maximum cumulative hydrogen production, hydrogen yield and hydrogen production rate 437.46 mL H<sub>2</sub>/L, 0.443 mol H<sub>2</sub>/mol sugar and 10.416 mL H<sub>2</sub>/L.hr respectively were obtained for a total sugar concentration of 15 g/L. Increase or decrease in sugar concentration above or below the optimum, decreases hydrogen yield and hydrogen production rate as illustrated in Figure 1. Low hydrogen production at lower initial sugar concentration is possibly due to low rate of fermentation reactions as described by Fabiano and Perego (2001).

Among the three sugars present in the hydrolysate, the strain utilized only glucose and xylose, and the concentration of arabinose remained almost constant throughout the fermentation. It is also clear that; xylose is preferred over glucose when its concentration is higher than that of glucose. Moreover, maximum xylose consumption of 4.30 g/L corresponds to the highest hydrogen production rate and the lowest xylose consumption of 2.40 g/L corresponds to the lowest hydrogen production rate (4.758 mL H<sub>2</sub>/L.hr). These results indicate that xylose is the preferred carbon source for hydrogen production by the isolated strain *Bacillus licheniformis*.

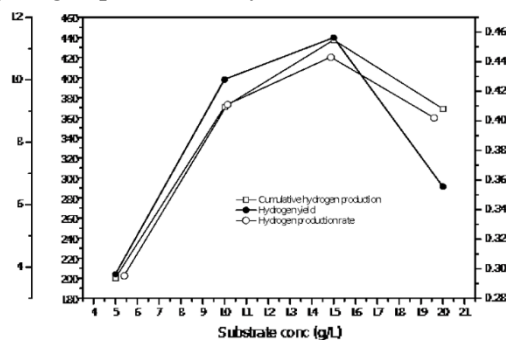


Figure.1. Effect of substrate concentration on on hydrogen production

Table.1. Effect of different carbon sources on hydrogen production

Carbon source	Cumulative hydrogen production (ml H <sub>2</sub> /L)	Hydrogen yield (mol H <sub>2</sub> /mol sugar)	Hydrogen production rate (ml H <sub>2</sub> /L.hr)
Glucose	298	0.395	7.095
Sucrose	103	0.292	2.457
Sugarcane baggasse hydrolysate	348	0.404	8.295
Maize stalk hydrolysate	370	0.411	8.831

### 4. CONCLUSIONS

Maize stalk hydrolysate yielded the maximum cumulative hydrogen production of 437 mL H<sub>2</sub>/L, when the initial sugar concentration was 15 g/L. Sugar analysis of the hydrolysate and fermentation effluent showed xylose as the preferred sugar for hydrogen production by *B. licheniformis*. Being the predominant sugar in hemicellulose, xylose is an important component in all the lignocellulosic hydrolystate, but most of the microorganisms cannot utilize it as a carbon source. Thus, the ability of *B.licheniformis* to utilize xylose is an advantage in the industrial level.

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