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Research Article

Biohydrogen production from xylose by the newly isolated *Clostridium beijerinckii* PS-3

Pongsak Noparat [1,2]*

[1]Program in Environmental Science, Faculty of Science and Technology, Suratthani Rajabhat University, Suratthani 84100, Thailand
[2]Renewable Energy and Environment Research for Local Community Unit (REERCU), Suratthani Rajabhat University, Suratthani 84100, Thailand
***E-mail**: pongsak@sru.ac.th

Abstract

The optimal condition for hydrogen production using *Clostridium beijerinckii* PS-3 was at an initial pH of 6.3–6.7 and cultivation at room temperature (30±2ºC). Among various types of substrate tested, the hydrogen yield of 1.73 mol/mol-xylose was about 1.2, 1.6 and 5.6 fold higher than those of glucose, starch and cellulose, respectively. From the xylose concentrations (5-30 g/L) tested, the optimum value was 10 g/L, giving the cumulative hydrogen production and hydrogen yield of 3254 ml/L and 2.18 mol/mol-xylose, respectively. Soluble metabolites from xylose were composed mostly of butyric acid (39.0-48.7mM), acetic acid (35.0-39.9 mM), ethanol (30.2-35.3 mM) and propionic acid (4.9-6.5 mM).

Keywords: hydrogen production, xylose, *Clostridium beijerinckii*

Introduction

Hydrogen is an alternative energy source that is cost-effective, environment-friendly and renewable. It has high specific energy content per unit mass (a high energy yield of 122 kJ/g), which is 2.75 times greater than hydrocarbon fuels (Chong et al., 2009a). Among various hydrogen production processes, the biological method is known to be less energy intensive as it can be carried out at ambient temperature and pressure (Lavarack et al., 2002) and at high temperature (Prasertsan et al., 2009).

Renewable biomass is the best non-petroleum based resource that is generated from various agro-industries as waste materials (Chong et al., 2009b). Felled oil palm trunk (OPT) (25 years old) provides an abundant biomass in oil palm plantations, especially in Southern Thailand. The composition of the OPT used was 31.28-42.85% cellulose, 19.73-25.56% hemicellulose, 10.74-18.47% lignin, 1.63-2.25% protein, 1.60-1.83% fat, 1.12-1.35% ash and with trace amount of minerals (0.01-0.40%) (Noparat et al., 2011). Glucose is easily biodegraded during hydrogen production by fermentation, giving the values of 1.2-2.52 mol/mol-glucose (Ishikawa et al., 2006; Xing et al., 2008; Chin et al., 2003). Conversely, fermentative hydrogen production from xylose is generally inefficient due to the lack of efficient hydrogen-producing bacteria for this substrate. Therefore, it is necessary to find a certain bacterial strain that prefers xylose to glucose in hydrogen production. Besides the bacterial strain, fermentative hydrogen production is influenced by many factors such as pH, temperature and substrate (Zhu and Yang, 2004; Lin et al., 2008; Lo et al., 2008).

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A number of studies have examined the potential of using hydrogen-producing bacteria, mainly in the genus *Clostridium*, from various sources such as anaerobic sludge digesters (Chen et al, 2005) and compost piles (van Ginkel et al, 2001). *Clostridium* sp. are able to degrade both simple sugars such as glucose and xylose and more complex substrates such as rice winery wastewater (Yu et al., 2002), sugarcane bagasse hydrolysate (Fangkum and Reungsang, 2011), palm oil mill effluent (O-Thong et al., 2007) and oil palm sap (Noparat et al., 2011). In the present study, the potential of newly isolated *Clostridium beijerinckii* PS-3 for hydrogen-producing from xylose was investigated.

Materials and methods

Microorganism and growth medium

C. beijerinckii PS-3, newly isolated from oil palm sap (Noparat et al., 2011), was grown in the synthetic medium (Chimtong et al., 2009) at 30±2°C under anaerobic condition for 18 h and kept at 4°C as stock culture for 1 month. Prior to cultivation, *C. beijerinckii* PS-3 was activated by transferring 1 mL of the stock culture at a cell concentration of OD_{660} = 0.5 into 5 mL of fresh synthetic medium. Then, a serum bottle was flushed with nitrogen to create anaerobic condition and incubated at 30±2°C for 18 h. The synthetic medium contains 10 g/L xylose, 1.5 g/L KH_2PO_4, 2.9 g/L K_2HPO_4, 2.1 g/L urea, 4.5 g/L yeast extract, 0.05 g/L $MgCl_2.6H_2O$, 0.0075 g/L $CaCl_2{\cdot}2H_2O$ and 0.015 g/L $FeSO_4{\cdot}7H_2O$ (Chimtong et al., 2009).

Optimization for Production of Hydrogen

Using batch fermentation, 5 mL of bacterial suspension at an exponential phase (OD_{660}=0.5) was transferred into 45 mL synthetic medium (Chimtong et al., 2009) supplemented with xylose as a carbon source to a final concentration of 10 g/L. To determine the effects of the operating parameters on hydrogen production, the xylose concentration, pH value and temperature were varied. The initial pH was set in the range of 5.9-7.3 in serum bottles using 2 N HCl and 2 N NaOH. The incubation temperature varied from 25 to 45°C. The xylose varied from 5 g/L to 30 g/L. All the experiments were done in triplicate. Culture broth from the serum bottles was taken for determining the pH, substrate utilization and fermentation end products. The volumetric H_2 production (ml/L) was calculated from the total gas production and the concentration of H_2 in the headspace. The hydrogen yield was calculated as mol/mol-xylose.

Analytical methods

The evolved biogas was collected in the headspace of a serum bottle. The total volume at each time interval was measured at room temperature by syringe. Hydrogen in the biogas was measured by gas meter (MX2100 OLDHAM, MULTIGAS Type MX2100) (O-Thong et al., 2008). The culture broth was centrifuged at 10,000×g for 10 min. Fermentation end products (volatile fatty acids (VFA) and ethanol) in the supernatant were determined by gas chromatography (GC HP 6890 series) equipped with a flame ionization detector (FID) and Innowax column (dimensions 30 m × 320 um × 0.25 um). The GC-FID conditions were set according to Miyazaki et al. (2008). The concentrations of xylose were determined using high performance liquid chromatography (HPLC) (Agilent 1200 series), equipped with Aminex HPX-87H ion exclusion column. The HPLC was operated with a refractive index detector under conditions of 0.005 M H_2SO_4 as effluent for 0.6 mL/min at 65°C (Delgenes et al., 1996). Data showed the average of three replicated assessments.

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Results and discussion

Hydrogen production with various xylose concentrations

When xylose was used as the carbon source, the initial xylose concentration (5, 10, 15, 20, 25 and 30 g/L) had an influence on hydrogen production in the synthetic medium. The optimum xylose concentration for hydrogen production by *C. beijerinckii* PS-3 at 30ºC and pH 7 was found to be 10 g/L (Figure 1.), where the cumulative hydrogen production and hydrogen yield were 2675 ml/L and 1.79 mol/mol-xylose , respectively (Table 1). A significant decrease in the hydrogen yield was obtained when the xylose concentration was raised from 10 g/L to 15 g/L, beyond which the yields were not significantly different (1.33-1.37 mol/mol-xylose) at 15 to 25 g/L xylose. These results suggested that higher substrate concentrations may induce a shock load (van Ginkel et al., 2001). In addition, the metabolism for hydrogen production was related to that of volatile fatty acid production. The lower hydrogen yield at a higher substrate concentration suggested that the carbon flux was more directed to the production of reduced by-products such as organic acids and alcohol (van Ginkel et al., 2001). In view of the cumulative hydrogen production and hydrogen yield, 10 g/L xylose was selected for the following experiments.

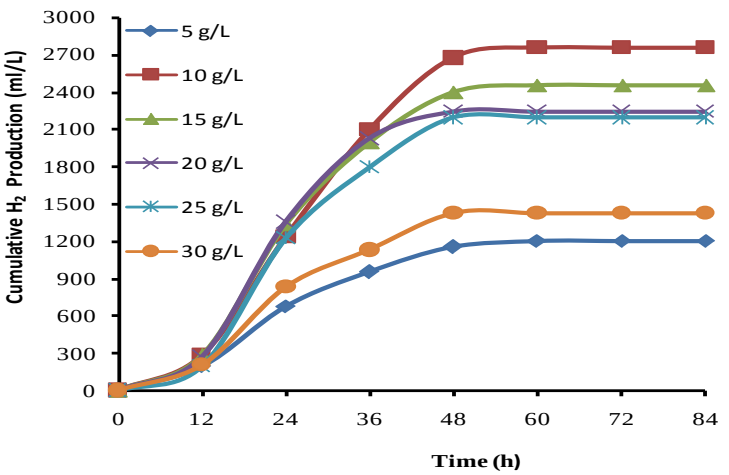

Figure 1. Effect of xylose concentrations on cumulative hydrogen production by *Clostridium berjerinckii* PS-3 in synthetic medium under anaerobic condition at 30ºC.

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Table 1. Comparison of hydrogen production and soluble metabolites occurred during cultivation of *Clostridium berjerinckii* PS-3 in synthetic medium under different conditions

Conditions	H_2 (ml/L)	H_2 yield (mol/mol -xylose)	H_2 rate (ml/L/h)	Ethanol (mM)	Acetic acid (mM)	Butyric acid (mM)	Propionic acid (mM)
A: Xylose concentration (g/L)							
5	1150	1.54	24.0	22.8	35.2	28.6	6.5
10	2675	1.79	55.7	30.5	39.8	45.6	9.8
15	2399	1.34	50.0	26.5	34.7	39.2	8.1
20	2245	1.37	46.8	24.3	35.4	45.5	7.9
25	2191	1.33	45.6	24.3	35.4	45.5	7.9
30	1427	0.96	29.7	18.8	21.2	25.7	5.4
B: pH value							
5.9	2548	1.71	53.1	29.8	30.2	32.4	6.1
6.3	3120	2.09	65.0	33.6	34.9	44.5	9.4
6.7	2978	2.00	62.0	32.5	34.8	39.0	7.9
7.1	2365	1.59	49.3	22.5	33.7	42.8	8.2
7.3	2267	1.52	47.2	23.3	33.5	41.3	8.7
C: Temperature (^oC)							
25	2468	1.65	51.4	31.8	36.2	42.4	8.4
30	3254	2.18	67.8	35.3	39.9	48.7	9.5
37	2650	1.78	55.2	32.2	34.8	44.5	9.2
45	2345	1.57	48.9	24.5	33.7	32.6	7.9

Effect of initial pH

In order to determine the optimum pH of the medium, the cultivation of *C. beijerinckii* PS-3 at the initial pH of 4.7, 5.3, 5.9, 6.3, 6.7, 7.1, 7.3 and 8.1 with fixed xylose concentration of 10 g/L was investigated. The pH values were adjusted with 0.10 M phosphate buffer. As shown in Figure 2. The optimum pH range was 6.3-6.7 where the cumulative hydrogen production and hydrogen yield were not significantly different (2978-3120 ml/L and 2.00-2.09 mol/mol-xylose, respectively) (Table 1).

It should be emphasized that no hydrogen production was observed at pH 4.7 as the high acidity (low pH) had destroyed the cell's ability to maintain internal pH. This finding was consistent with most previous studies, in which the optimum pH value for hydrogen-producing system occurred in the pH range of 5.0-7.0 (Li and Fang, 2007). The results clearly demonstrated that the initial pH affects the hydrogen production considerably. The effect of pH may be on the inhibition of the hydrogenase activity that resulted from a higher pool of reduced ferredoxin in the cells which is reoxidized via NAD(P)H-ferredoxin oxidoreductase (Dabrock et al., 1992). In addition, this might be attributed to the carbon repression encountered during complex anaerobic metabolism (Venkata et al., 2007).

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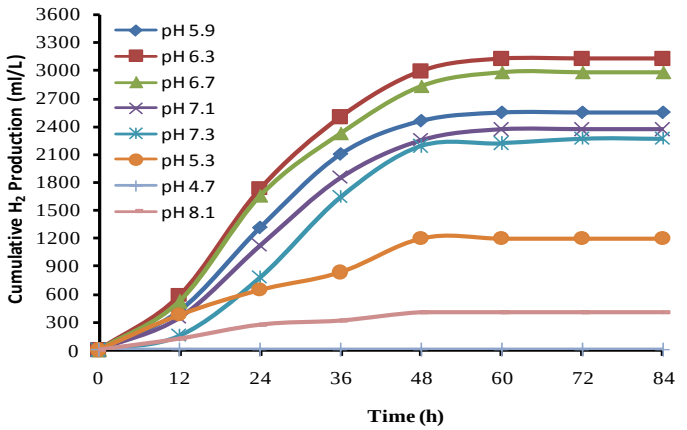

Figure 2. Effect of initial pH on cumulative hydrogen production by *Clostridium berjerinckii* PS-3 in synthetic medium under anaerobic condition at 30°C.

Effect of temperature

Temperature is often a critical factor in the performance of a fermentative process. The hydrogen production by *C. beijerinckii* PS-3 at temperatures ranging from 25°C to 45°C are given in Figure 3. and Table 1. The maximum cumulative hydrogen production (3254 ml/L) and the hydrogen yield (2.18 mol/mol-xylose) were obtained at 30°C. The hydrogen yields dropped by 18% and 28% compared to those at 37°C (1.78 mol/mol-xylose) and 45°C (1.57 mol/mol-xylose), respectively. These results demonstrated that *C. beijerinckii* PS-3 can grow and produce hydrogen both at 30°C and 45°C, hence it could be thermoterolant. At high temperatures, the denaturation rate of the enzymes in the fermentative hydrogen production process increased (Chittibabu et al., 2006), which could lead to the inactivation of hydrogenase and a decrease in the hydrogen yield.

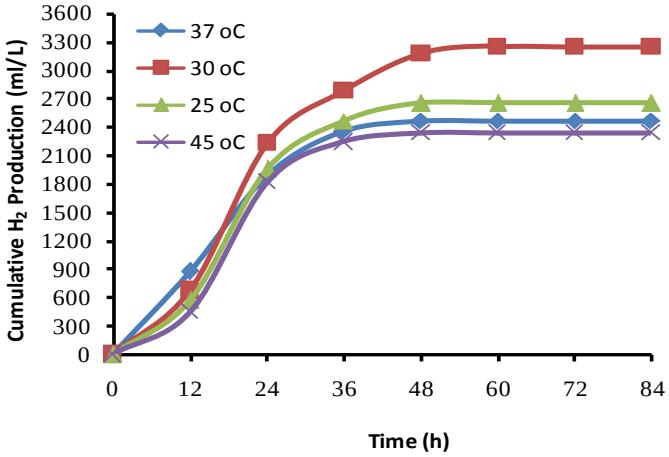

Figure 3. Effect of temperature on cumulative hydrogen production by *Clostridium berjerinckii* PS-3 in synthetic medium under anaerobic condition at 30°C.

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The hydrogen production capability of *C. beijerinckii* PS-3 was compared with those of other fermentative hydrogen-producing microorganisms (Table 2). Due to the drastic differences in conditions, substrate and other process conditions, it was difficult to compare the hydrogen yields. Nevertheless, the characteristic values of hydrogen production by *C. beijerinckii* PS-3 were 1.1-4.1 fold higher than most other hydrogen-producing strains including mixed cultures. The maximum hydrogen yield of this strain using xylose was 15% lower than that obtained from *C. beijerinckii* Fanp3 using glucose (Pan et al., 2008).

Table 2. Comparison of *Clostridium berjerinckii* PS-3 with various fermentation hydrogen-producing microorganisms.

Microorganisms	Xylose concentration (g/L)	Maximum H_2 yield (mol/mol-sugar)	References
Enterobacter sp. CN1	16.15	2.00	Datar et al. (2007)
Clostridium sp. HR-1	12	1.63	Long et al. (2010)
Enterobacter cloacae IIT-BT 08	10	0.95	Xu et al. (2010)
Clostridium tyrobutyridum ATCC 25755	10	0.77	Lo et al. (2008)
Clostridium beijerinckii PS-3	10	2.18	This study

Conclusion

Optimization for hydrogen production from *Clostridium beijerinckii* PS-3 revealed that the optimal initial pH was 6.3–6.7 at 30±2^{o}C and 10 g/L xylose. Under this condition, the maximum cumulative hydrogen production of 3254 ml/L and the maximum hydrogen yield of 2.18 mol/mol-xylose were achieved. Therefore, *C. beijerinckii* PS-3 is a potential candidate for fermentative hydrogen production from xylose and ability application from xylose in the lignocellulosic hydrolysate.

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