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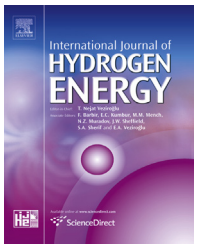

Characterization on hydrogen production performance of a newly isolated *Clostridium beijerinckii* YA001 using xylose

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ABSTRACT

A mesophilic bacterium was isolated from cow manure, and identified as *Clostridium beijerinckii* YA001 based on 16S rRNA gene sequence calculation using MEGA 5.0 and biochemical tests. This strain showed the ability to digest a wide range of carbon and nitrogen sources for hydrogen production, and it showed high hydrogen production performance using xylose as substrate. The optimum parameters for bio-hydrogen production in batch tests were pH 8.0, 1% substrate concentration, 40 °C and yeast extract as nitrogen source. The maximum hydrogen yield and the hydrogen production rate were obtained at 2.31 mol/mol xylose and 311.3 mL H_2/(Lh), respectively. These results indicate that *C. beijerinckii* YA001 is an ideal candidate for fermentative hydrogen production.

Introduction

Hydrogen production by biological methods is attractive because it is a process operated under mild conditions and many of the solid waste or waste water can be used as substrate for simultaneous energy recovery and environment treatment [1]. Fermentative hydrogen generation has shown great potential for developing into a practical bio-hydrogen production process [2]. Biomass such as wood, energy crops, agricultural wastes can be used as the substrates for fermentative hydrogen production [3,4].

Biomass is mainly composed of cellulose, hemicellulose and lignin. Both cellulose and hemicellulose is polymer of hexose or pentose. Cellulose and hemicellulose are both polymers built of either hexose or hexose and pentose sugars. Cellulose consists of D-glucose units, while hemicellulose includes pentose (such as xylose and trace amounts of arabinose) and hexose (such as glucose, mannose and galactose) sugars, which account for 55–65% to 35–45% of the saccharides in the hydrolysate of the above mentioned biomass [5,6]. Glucose, as an excellent substrate for fermentative hydrogen production, has been well studied using pure [7–9] and mixed microflora [10,11] as hydrogen producers. However, microorganisms for the conversion of pentose (xylose) to hydrogen are inefficient [12] and the information on producing hydrogen from xylose is limited [13–15]. Hence, for efficient utilization of the sugars in the hydrolysate of cellulosic

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materials for hydrogen production, it is significant to isolate bacteria that possess the ability on efficient production of hydrogen from pentose sugar, especially xylose.

In this work, we isolated, identified and characterized a bacterium with high hydrogen production performance from xylose. Parameters that are significant on hydrogen production, including initial pH [16,17], initial xylose concentration, temperature [18,19] and nitrogen source [20] were studied in batch tests.

Materials and methods

Screening for hydrogen-producing bacteria

The hydrogen-producing microorganisms were obtained from dairy manure in the suburb of Xi'an City, China. The basic medium used for enrichment, isolation and cultivation of H_2-producing strains was prepared as follows:10.0 g/L glucose, 1.0 g/L sodium glutamate, 10.0 mL/L KH_2PO_4–K_2HPO_4 buffer(pH 6.8, final concentration in culture 5.0 mmol/L), 20.0 mL/L nutrient stock solution [21]. The initial pH values of the medium were adjusted to 6.8 by 5.0 mol/L HCl or NaOH. 20.0 mL filtrate of the dairy manure soaked by deionized water was transferred into a 250 mL medium and incubated at 37 °C shaking at 150 rpm for 48 h. Then the culture was streaked onto agar plates. Single colonies were re-streaked more than three times to ensure the purity of the strains and transferred into a serum bottle containing growth media. These bottles were flushed with nitrogen gas to remove the oxygen and keep anaerobic environment, and then it was capped with rubber stopper.

Strain identification and phylogenetic analysis

The genomic DNA was extracted from cell pellets and the 16S rRNA gene of the hydrogen-producing bacterium was amplified by PCR as described in literature [22,23].The 16S rRNA gene sequence (1471 bp) of strain YA001 was obtained by PCR using a pair of universal primers, 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-TAC GGT TAC CTT GTT ACG ACT T-3′). The PCR products were purified using DNA Fragment Purification Kit (Takara, Dalian, China) and then sent to sequence. The obtained 16S rRNA gene sequences were blasted in GenBank using BLAST program [24]. A phylogenetic tree was established using the neighbor-joining method with Kimura's two-parameter method. Neighbor-joining analysis was conducted with the program MEGA 5.0 [25]. Credibility of the obtained tree was tested by bootstrap resembling analysis with 1000 replicates [26].

Hydrogen production in batch culture

The effects of parameters including carbon and nitrogen sources, initial pH, substrate concentration and temperature on hydrogen production were studied in batch tests. All batch tests were conducted in 330 mL reactor which contained 250 mL of medium and 2% inoculum (v/v). The carbohydrates (arabinose, xylose, glucose, fructose, maltose, lactose, sucrose, cellobiose, mannose, cellulose, xylan, dextran, starch) were fed as carbon source at concentration of 10.0 g/L instead of 10.0 g/L glucose in the basic medium, and the initial pH and temperature were 6.86 and 37 °C, respectively. Yeast extract, peptone and ammonium sulfate were supplemented as nitrogen source at concentration of 1.0 g/L to replace nitrogen source in the basic medium if necessary and 10.0 g/L xylose was fed as carbon source when testing the effect of nitrogen source in Table 1. The effect of the inorganic (ammonium sulfate) and organic (peptone, yeast extract and sodium glutamate) nitrogen sources were conducted at various nitrogen sources at the concentration of 1.0 g/L and using 10.0 g/L of xylose as the carbon source, initial pH of 6.86, and temperature 37 °C. The effect of pH was studied at range from 5.00 to 10.00 (with 1.00 incremental steps) using 10.0 g/L of xylose as carbon source and 1.0 g/L of yeast extract as nitrogen source at 37 °C. The effect of the xylose concentration was conducted at various levels from 5.0 to 20.0 g/L (with 5.0 incremental steps) using optimum pH at 37 °C and using 1.0 g/L of yeast extract as nitrogen source. The effect of temperature was studied from 30 to 50 °C (with 5 °C step length) using optimum pH, xylose concentration and nitrogen source. The batch reactors were run for 48–72 h until the biogas productions completed. All the reactors were placed in an orbital shaker at a rotation speed of 150 rpm. The bottles were filled with nitrogen gas before starting up and then capped with rubber stopper. All the experiments were carried out independently in triplicates, and the data shown were composed of average data with deviations.

Analytical methods

Xylose concentration was determined by a xylose assay kit (Jiancheng Biotech, Nanjing, China) using the phloroglucinol method. Dry cell weight (DCW, dcw) was determined by the method described by Niu et al. [27].

Table 1 – Kinetic parameters for hydrogen production using different substrates.

Substrate	Gompertz equation			
	HY (mL/g))	R_m (mL/(Lh))	λ (h)	R^2
Arabinose	168.1 ± 0.9	261.8 ± 22.2	18.9 ± 0.4	0.999
Fructose	125.9 ± 1.3	159.8 ± 15.2	18.3 ± 0.5	0.997
Galactose	72.3 ± 2.6	32.9 ± 4.3	26.6 ± 1.4	0.986
Glucose	80.3 ± 1.7	76.3 ± 13.1	10.1 ± 1.0	0.981
Mannose	1.8 ± 0.1	0.4 ± 0.1	15.0 ± 0.5	0.941
Rhamnose	99.7 ± 3.5	30.5 ± 2.7	17.5 ± 1.4	0.991
Xylose	91.9 ± 1.0	61.6 ± 2.5	27.5 ± 0.4	0.999
Cellobiose	108.6 ± 0.6	178.9 ± 13.9	18.8 ± 0.4	0.999
Lactose	20.7 ± 0.3	70.5 ± 8.1	17.6 ± 0.5	0.991
Maltose	82.2 ± 0.3	194.8 ± 10.8	18.3 ± 0.2	0.999
Sucrose	138.0 ± 0.4	227.0 ± 12.1	19.0 ± 0.3	1.000
Dextrin	240.8 ± 4.6	398.5 ± 12.3	12.8 ± 1.5	0.984
Starch	22.1 ± 0.1	40.0 ± 2.1	18.5 ± 0.2	0.999
Xylan	83.5 ± 2.3	47.8 ± 8.7	10.6 ± 1.6	0.976
Cellulose	20.0 ± 0.7	12.0 ± 2.5	11.0 ± 1.9	0.964
Glycerol	58.9 ± 3.1	21.0 ± 4.5	8.6 ± 2.9	0.950
YE	181.9 ± 2.9	113.8 ± 11.1	16.7 ± 0.8	1.000
Peptone	154.6 ± 1.7	67.7 ± 7.6	11.8 ± 1.5	0.987
$(NH_4)_2SO_4$	129.1 ± 2.7	150.1 ± 5.9	34.0 ± 0.6	0.996

HY: hydrogen yield, mL-H_2/g-substrate; R_m: maximum hydrogen production rate; λ lag time; YE: yeast extract.