Hydrogen Production from Corncob by Thermoanaerobacterium Thermosaccharolyticum M18

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Abstract. This work experimentally used the corncob to product hydrogen with *Thermoanaerobacterium thermosaccharolyticum* M18. The initial pH of culture medium, particle size of the corncob and NaOH pretreatment condition were researched and optimized to get high yield and efficiency of hydrogen production. Moreover, the structure and composition of the corncob before and after the pretreatment were analyzed and compared by SEM and a fiber quality analyzer. The optimal operation conditions for high-efficient hydrogen production were: medium initial pH 7, particle size of the corncob 0.25mm (60 mesh), 2% sodium hydroxide solution hydrolysis at 90°C for 120 minutes.

Introduction

Hygrogen is being further production and developed as a kind of clean and renewable energy, also has potential to replace fossil fuels. While, most studies of hydrogen production have been used to treat easily degradable carbohydrates such as glucose, lactose, xylose, sucrose and molasses [1-4]. However, these raw materials are too expensive. Using cheaper raw materials will surely accelerate the pace of scaled-up production of biological hydrogen [5]. Corn has wide cultivating areas in China and annual yield up to billions of kilograms. Corncob is the by-product of corn. A small part of the corncobs are used to sugar production, pulp manufacture, cattle food. While most of corncobs are abandoned, which cause serious waste of resources [6]. Therefore, hydrogen production from corncob can lower the cost of production and recycling of waste resources, which advanced the development of clean energy and have large social and economic benefits.

Corncob is mainly composed of cellulose, hemicellulose and lignin, which are closely intertwined [7]. Because of the structure is complex and stability, contains a high content of lignin, corncob cannot be well utilized by most microorganism. While, *Thermoanaerobacterium thermosaccharolyticum* M18 was confirmed having a great degradation capacity to cellulose. This strain could efficiently uptake microcrystalline cellulose, xylan, filter paper, and sodium carboxymethylcellulose for hydrogen production, as well as unpretreated corn stover, rice straw, and corncob [8]. But cellulose is surrounded by lignin, we should pretreat the corncob to remove or destroy the lignin. To date, pretreatment approaches include steam-explosion [9], acid pretreatment [10-11], ozone [12], smash, caustic pretreatment. Among these approaches, acid pretreatment has no obvious effect on removal of lignin and some acid hydrolysate such as furfural inhibit hydrogen production [13-14]. Steam-explosion can not remove lignin. Ozone due to its high cost, can not large-scale application. While caustic pretreatment can remove lignin well and won't produce inhibitors. So this study utilized smash and caustic pretreatment.

In this study, firstly, smashed the corncob and researched the effects of different particle sizes on hydrogen production. Then used NaOH pretreatment in different conditions to pretreat the corncob and researched the hydrogen production influencing factors, including concentration of NaOH, hydrolyzing temperature, hydrolyzing time. The last, observed and analyzed morphological structure of the corncob with SEM and determine proportion of cellulose, hemicellulose, and lignin. This study optimize the hydrogen production, which can provide a reference for the scaled-up production.

Materials and methods

The strain M18 was isolated and identified as *Thermoanaerobacterium thermosaccharolyticum*. It was grown at 60°C in a medium containing(/L): 1.0 g $(NH_4)_2SO_4$, 3.0 g K_2HPO_4, 1.5 g KH_2PO_4, 0.5 g $MgCL_2·6H_2O$， 1.0 g Nacl, 0.2 g KCL, 0.5 g L-Cysteine, 2.0 g Yeast extract powder, 5 g corncob, 1 ml trace element solution, 1 ml vitamini solution, 0.5 ml 0.1% (w/v) resazurin [8]. In this nutrient solution, the concentration of the corncob is 5g/L. Corncob used in this study was harvested from a farm in Harbin. The air-dried corncob was grounded into particles with sizes of bigger than 10 mesh (1.7-4 mm), 10-40 mesh (0.3-1.7 mm), 40-60 mesh (0.25-0.3 mm), 60-80 mesh (0.18-0.25 mm), 80-100 mesh (0.15-0.18 mm), smaller than 100 mesh (0-0.15 mm) then oven-dried at 60°C to constant weight.

Initial pH of the medium. All fermentations were carried out in 100 ml glass anaerobic bottles with working volume of 50 ml. Put 50 ml nutrient solution and 0.25 g corncob powder in the bottle. The initial pH were adjusted to 5.0, 6.0, 7.0, 8.0, 9.0 in five comparing fermentation solutions with 2% NaOH and 2% HCL. All fermentation experiments were carried out in duplicate to check data quality. Nitrogen-blow for anaerobic environment. Then sterilized at 121°C for 15 min. M18 was used as the inoculum and inoculated at 5% (v/v). The fermentation temperature was kept at a constant value of 60°C. Withdraw the samples after 72 h, determining total gas production and hydrogen production.The volume of biogas was measured by plunger displacement method.

Different particle sizes of the corncob powder. Dried and smashed the corncob in six different sizes:1.7-4 mm, 0.3-1.7 mm, 0.25-0.3 mm, 0.18-0.25 mm, 0.15-0.18 mm. The initial pH of all fermentations were adjusted to 7.0. React for 96 h, every 12 h withdraw the samples detemining total gas production, hydrogen production, pH, volatile fatty acid concentration.

NaOH pretreatment of the corncob. NaOH solution hydrolyzed the corncob at a solid-liquid ratio of 1:10 (g dry weight to ml). Then washed the corncob powder to neutral and dried. Put 50ml nutrient solution and 0.25 g corncob powder in the bottle. Study three factors that influence the hydrogen production: concentration of NaOH (0.5%, 1%, 1.5%, 2%, 2.5%, 3%), hydrolyzing temperature (45°C, 60°C, 75°C, 90°C, 105°C, 120°C), hydrolyzing time (30 min, 60 min, 90 min, 120 min, 150 min). The structure and composition were measured by SEM and cellulose analyzer. The volatile fatty acids were detected by GC (4890D, Agilent Cooperation, USA) equipped with a hydrogen flame-ionization detector and a 2.0 m stainless steel column packed with GDX103. The gas samples were measured by GC (GC-SC2, Shanghai Analytical Apparatus) equipped with a thermal conductivity detector and a 2.0 m stainless steel column packed with TDS-01 and N_2 at a flow rate of 70 ml min^{-1} as carrier gas[15].

Results and discussion

Initial pH of the medium. From Fig.1., there was no gas production when pH=5, the corncob wasn't degraded. The total gas yield, H_2 yield and the proportion of H_2 achieved the maximum respectively at pH=7, were 39.29 mmol/L and 8.04 mmol/L, the proportion was 20.46%. While when the pH up to 9, total gas and H_2 yield and proportion of H_2 were all decreased, were 33.04 mmol/L, 6.30 mmol/L and 19.07%. The results showed that M18 is unable to withstand the acid environment and also don't adapt to the alkaline environment. Due to the growth of bacterial cells and the enzymes activity in metabolic reactions were all inhibited, which affected M18 to absorb nutrients, lead to low biomass and hydrogen production potential dropped. Therefore, the optimal initial pH was 7.

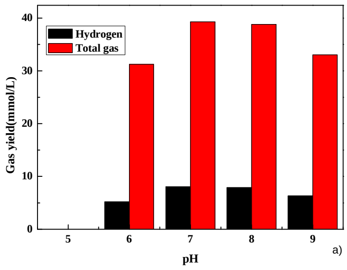

Fig.1. Total gas and H_2 yield at different initial pH

Particle size and reaction time. Fig.2. and fig.3. showed that total gas and H_2 yield increased rapidly in 24-48 h, after 48 h, gas yield slowly increased with the passing of time, but after 72 h had no noticeable change. And increased as the particle size decreased, but when the size smaller than 0.25 mm, the gas yield had no noticeable increase. Continue to grind the corncob had little impact to the hydrogen production. The highest gas yield and H_2 yield reached to 40.18 mmol/L and 8.22 mmol/L. The maximum proportion of hydrogen reached to 20.5%.

Fig.4. showed that fermentation terminal liquid products mainly were acetate and butyrate, belongs to butyrate-type fermentation. Valerate and propionate were a little. 24-60h, acetate and butyrate increased rapidly, after 72 h gradually stabilized. Because the substrate reduced and metabolite increased. When fermentation time after 72 h, the decrease of pH was not evidence, represented fermentation was completed. When the partical size was 1.7-4 mm acetate and butyrate were 6.24 mmol/L and 3.62 mmol/L, while fermentation completed at pH=5.92. However, as the particle size decreased, the acetate and butyrate increased and pH decreased, fermentation was more sufficient. What's more, the ratio of butyrate and acetate increased from 1.15 to 1.72. The ratio of acetate and butyrate can measure the effect of hydrogen production, the bigger the better.

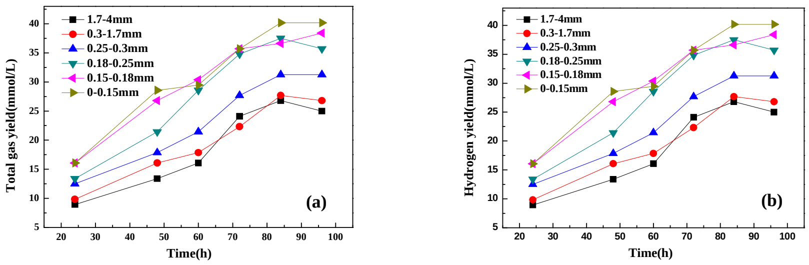

Fig.2. Total gas and H_2 yield at different fermentation time: (a) Total gas yield (b) hydrogen yield.

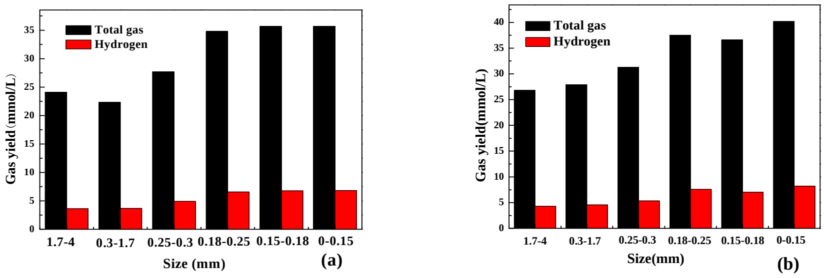

Fig.3. Gas yield at different particle size: (a) fermentation time was 72 h (b) fermentation time was 84 h

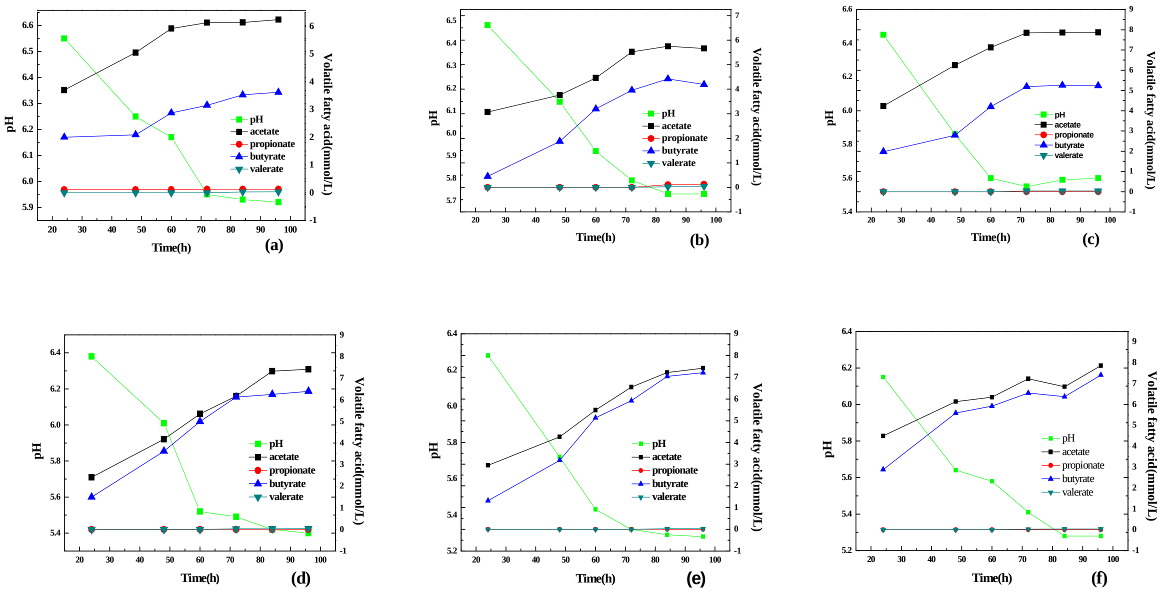

Fig.4. Volatile fatty acid concentration at different fermentation time: (a) Partical size was 1.7-4 mm (b) 0.3-1.7 mm (c) 0.25-0.3 mm (d) 0.18-0.25 mm (e) 0.15-0.18 mm (f) 0-0.15 mm

NaOH pretreatment. From fig.5. H_2 yield and proportion increased with the increase of NaOH concentration, H_2 yield were 11.05 mmol/L and 11.33 mmol/L respectively at 2% and 3% NaOH concentration, while the proportion of H_2 were 22.50% and 22.26%. What's more, volatile fatty acid had no obvious increasing after the concentration of 2%. It showed that after the concentration of 2%, Continuing increasing the NaOH concentration had no obvious benefit to the hydrogen production. What's more, volatile fatty acid had no obvious increasing after the concentration of 2%. From.fig.6., H_2 yield and proportion increased with the increase of hydrolyzing time. After 120min, continue to hydrolyze the corncob, H_2 yield and proportion increased slowly. Considering the cost and efficiency, hydrolyzing the corncob for 120 min. From.fig.7., H_2 reached the highest yield 12.81 mmol/L and total gas yield was 58.04 mmol/L at 120^{o}C, while were 12.44mmol/L and 55.36mmol/L at 90^{o}C, only a little increment. Heated to above 100^{o}C need using too much energy and cost more, which hardly applied in practice. Therefore, choose 90^{o}C as the hydrolyzing temperature.

Above all, the optimal condition of NaOH pretreatment is 2% NaOH, 90^{o}C, 120min. The H_2 yield and total gas yield were 12.44 mmol/L and 55.36 mmol/L. While the proportion of H_2 was 22.47%.

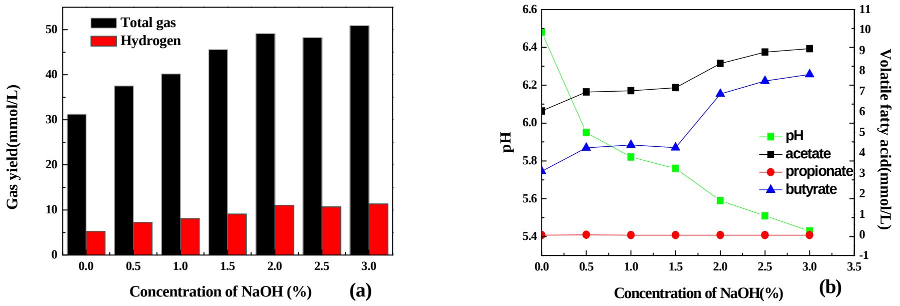

Fig.5. Different concentration of NaOH: (a) gas yield (b) pH and volatile fatty acid concentration

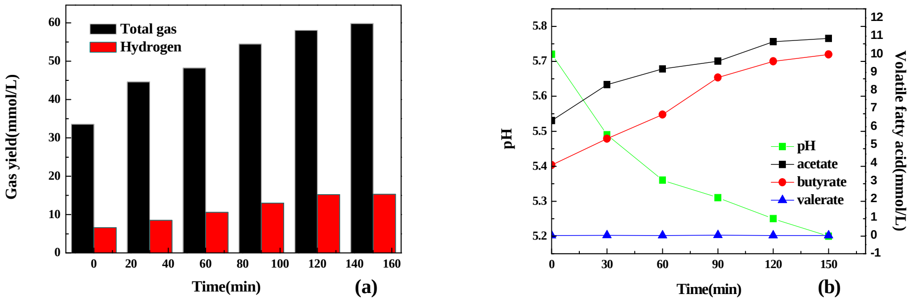

Fig. 6. Different hydrolyzing time:(a) gas yield (b) pH and volatile fatty acid concentration

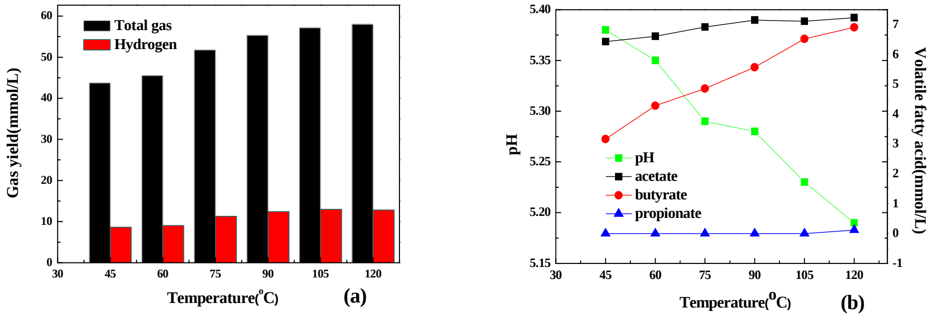

Fig. 7. Different hydrolyzing temperature:(a) gas yield (b) pH and volatile fatty acid concentration.

Ligin decreased with the concentration and hydrolysis time increasing. NaOH has strong ability in removing lignin and decreasing crystallinity, chemical bond between lignin and carbohydrate ruptured. Part of hemicellulose also be removed, while most of cellulose are reserved. From table 1, after the pretreatment, hemicellulose and lignin propotion was decreased 16.56% and 35.53% while cellulose propotion increased 56.22%.

Table 1. Composition of the corncob

	Cellulose （%）	Hemicellulose （%）	Lignin （%）	Others （%）
Untreated corncob	40.2	32.6	15.2	12
NaOH pretreatment (2% NaOH,90°C,120 min）	62.8	27.2	9.8	0.2

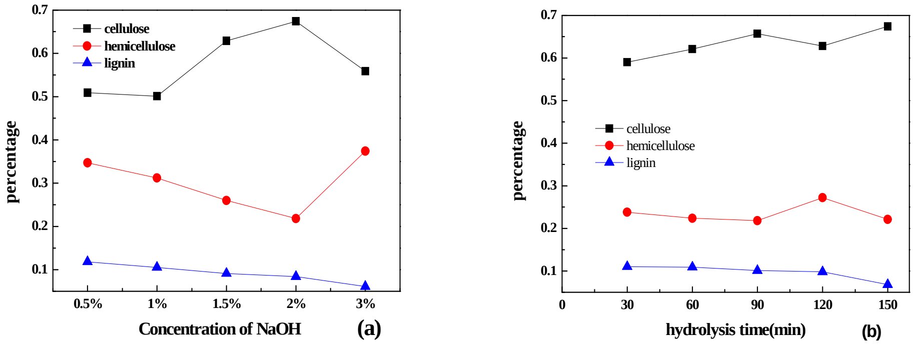

Fig. 8. Composition of the corncob at different pretreatment conditions: (a) concentration of NaOH (b) hydrolysis time

Scan electro microscope was applied to analyze the impact of NaOH pretreatment on the corncob structure. The structure was looser after pretreatment, a lot of holes and cracks appeared in the corncob. What's more, wax layer on the surface of corncob fell off and the fibre exposed, the surface was rough while it was smooth before pretreatment. The lignin was removed or destroyed.

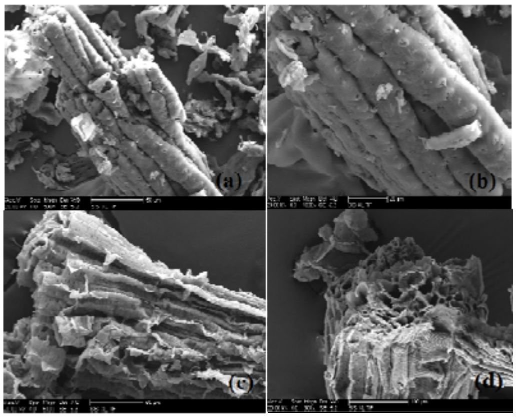

Fig. 9. The structure of corncob (a), (b) before NaOH pretreatment (c), (d) after NaOH pretreatment

Conclusion

The optimized operation conditions for hydrogen production from corncob by Thermoanaerobacterium thermosaccharolyticum M18 were obtained in this study, which were particle size 0.25 mm (60 mesh), NaOH concentration 2%, hydrolyzing temperature 90 ^{o}C and hydrolyzing time 120 min. Under this optimal conditions, H_2 yield, total gas yield and H_2 proportion were 12.44 mmol/L, 55.36 mmol/L and 22.47%, respectively. The initial composition of the corncob were 40.2% cellulose, 32.6% hemicelluloseand 15.2% lignin. However, after optimized NaOH pretreatment, the composition of lignin, hemicelluloses and cellulose decreased to 9.8% 27.2% and 62.8%, respectively. These optimized conditions were of great guiding significance for practical scaled-up hydrogen production from corncob.

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