

Bio-hydrogen production from acetic acid steam-exploded corn straws

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ABSTRACT: Hydrogen was produced by simultaneous saccharification and fermentation from acetic acid steam-exploded corn straw (ASECS) using *Ethanoligenens* sp. B49. The effects of acetic acid concentration and enzyme loading were examined with respect to maximum specific hydrogen production rate and hydrogen productivity. In simultaneous saccharification and fermentation, enzyme loading was more effective than acid concentration. Under 16% acetic acid concentration and 120 U/g and 180U/g enzyme loading, maximum hydrogen yield and maximum specific hydrogen production rate were 72 mL/g ASECS and 103 mL/g VSSd respectively.

Keywords: B49, hydrogen, corn straw, simultaneous saccharification and fermentation

1. INTRODUCTION

Hydrogen is considered to be an ideal energy alternative for the future. Compared with conventional hydrogen generation process (thermochemical and electrochemical), bio-hydrogen production processes are more environment-friendly and less energy intensive. Hydrogen could be produced from renewable materials, such as waste water, organic wastes, corn straws and waste water sludge, etc.^[1-4]. Bio-conversion of dissoluble Carbohydrate, for example, glucose, waste molasses, into hydrogen has been well studied^[5-7]. However, little information on hydrogen yield from cellulosic biomass is available up to now. Cellulosic biomass is a promising source due to its abundance and low cost and it has been used for fermentation of many products, such as ethanol, etc.^[8,9]. Yao-Ting et al.^[10] reported to converse wheat straw wastes pre-treated with dilute HCl by microwave heating into bio-hydrogen and Vrije et al.

^[11] used Miscanthus for hydrogen production by 12% NaOH (w/w) pre-treatment at 70°C. But these pre-treatment were expensive.

A successful biological conversion of biomass to hydrogen depends strongly on the processing of raw materials to produce feedstock which can be fermented by the microorganisms. Straw has a complex crystal structure in which cellulose is integrated with hemi-cellulose and embedded in a lignin matrix. Various pre-treatment methods have been described to promote the accessibility of the polysaccharides in a lignocellulose complex for enzymatic hydrolysis and acid hydrolysis and steam explosion are considered to be efficient^[3,12-14]. Acetic acid steam-exploded corn straw (ASECS) was used for biological hydrogen production using *Ethanoligenens* sp. B49 by simultaneous saccharification and fermentation in this experiment. Since the chemical reagents used in the pretreatment

are expensive, and acetic acid is one of the end liquid products of the bio-hydrogen fermentation. If acetic acid could be efficient, then the end liquid products of the bio-hydrogen fermentation could be considered for the pretreatment instead of other pure chemical reagents, for example, NaOH, HCl, H₂SO₄, and so on. It would be economic and environmentally friendly. A series of batch experiments were made to investigate the effects of two factors, such as acetic acid concentration and enzyme loading on hydrogen production and to determine the optimum operational condition based on specific hydrogen production rate.

2. MATERIALS AND METHODS

2.1 Seed microorganism and cellulase

The anaerobic *Ethanoligenens* sp. B49 was used for hydrogen production.

The microorganism was enriched in the medium ATCC 1191 of which contained the following: glucose 3g, yeast extract 2g, KH₂PO₄ 1.5g, Na₂HPO₄·12H₂O 4.2g, NH₄Cl 0.5g, MgCl₂·6 H₂O 0.18 g, Vitamin Solution 0.5 ml (see below), Resazurin (0.1%) 1.0 ml (see below), Wolfe's Modified Mineral Elixir 5.0 ml (see below), Reducing Solution 40.0 ml (see below), Distilled deionized water to 1.0 L, pH 6.8-7.5.

Reducing Solution:

0.2 N NaOH 200.0 ml, Na₂S·9H₂O 2.5 g, L-Cysteine·HCl 2.5 g

Vitamin Solution:

Biotin 20.0 mg, p-Aminobenzoic acid 50.0mg, Folic acid 20.0mg, Pantothenic acid calcium salt 50.0mg, Nicotinic acid 50.0mg, Vitamin B12 1.0mg, Thiamine·HCl 5.0mg, Pyridoxine hydrochloride 100.0mg, Thiocetic acid 50.0mg, Riboflavin 5.0mg.

Wolfe's Modified Mineral Elixir:

Nitrilotriacetic acid 1.5g, MgSO₄·7H₂O 3.0g, MnSO₄·H₂O 500.0mg, NaCl 1.0g, FeSO₄·7H₂O 100.0mg, Co(NO₃)₂·6H₂O

100.0mg, CaCl₂ 100.0mg, ZnSO₄·7H₂O 100.0mg, CuSO₄·5H₂O 10mg, AlK(SO₄)₂ 10mg, Boric acid 10.0mg, Na₂MoO₄·2H₂O 10mg, NaSeO₃ 1mg, Distilled water 1.0L.

The enzyme activity of the cellulase (Richeng Co. Ltd., Heilongjiang, China) is 10000 U/g.

2.2 Substrate preparation

The corn straw used in all the experiments was obtained from Harbin field (Heilongjiang, China). It was dried at 60°C for 1 day. The original length was between 2 and 5cm. It was milled and screened to between size of 20 to 80 mesh prior to the pretreatment. The stream-exploded vessel was the Sterilizer of our laboratory.

2.3 Batch experiments

A series of batch experiments were made in serum vials with a volume of 80ml at 39°C and 2.0g of ASECS was used for fermentation in each batch. The microorganism enrichment and other processes were all performed by Hungate-Bryant technique.

2.4 Analytical methods

Gas samples of from serum bottles were measured by gas chromatography (102 G, Shanghai Analysis Instrument Company, China) equipped with a thermal conductivity detector. A stainless steel column packed with Molecular Sieve 5A was used for detecting H₂. Nitrogen was used as carrier gas at a rate of 25 ml/min. The contents of reduced sugars were determined using the dinitrosalicylic(DNS) technique. The complex components of cellulose, hemi-cellulose and lignin contents of substrate were measured following the methods described by Goering and Van Soest^[15]. The pH was determined by pH meter (pHS-3C).

2.5 Kinetic parameters

The cumulative hydrogen production

data were fitted to a modified Gompertz equation ^[16,17] which was a suitable model for describing the progress of cumulative hydrogen production in a batch experiment:

$$H = P \cdot \exp \left\{ - \exp \left[\frac{R_m \cdot e}{P} (\lambda - t) + 1 \right] \right\}$$

where H is the cumulative hydrogen production (ml), P is the maximum hydrogen production potential (ml), R_m is the maximum hydrogen production rate (m/h), λ is lag-phase time (h) and e is 2.718281828. The analysis software Origin 7.5 was used to fit the equation and determine P, R_m and λ . The hydrogen yield (ml/g ASECS) was obtained by dividing P by the dry weight of ASECS used for fermentation. The maximum specific hydrogen production rate (ml/g VSSd) was obtained by dividing R_m by the volatile suspended solids (VSS) that is obtained by subtracting ash from dry SECS.

3. RESULT AND DISCUSSION

3.1 Acetic acid steam explosion

The chemical composition of corn straw was shown in Table 1. Cellulose was the major component followed by hemicellulose and lignin. Prior to the steam explosion experiments, 25g of corn straw was soaked in 0%, 0.4%, 0.8%, 1.2%, 1.6% (v/v) acetic acid solution for 72h. The mixture was then introduced into the steam-exploded vessel, at 1.5MPa for 10min and then a valve on the top of the vessel was opened suddenly to bring the reactor rapidly to atmospheric pressure. Then the ASECS was washed to neutral and was dried for future use. After pretreatment at different acetic acid explosion, the scrubbing filtrate was collected and the total reducing sugars were analyzed, and the results were summarized in Figure 1. While the acetic acid concentration increased from 0% to

16%, the total reducing sugar in the filtrate increased 4.27 times, from 15.8 mg/g to 69.2mg/g, respectively. Acetic acid concentration did significantly influence the release of total reducing sugar from the biomass. So, acetic acid, one of the bio-hydrogen fermentation end liquid products, was available to be used in the pretreatment of the corn straw.

Table 1: Average composition of used corn straw

Constituents	% of dry weight
Hemicellulose	16.5
Cellulose	41.7
Lignin	9.6
Ash	1.0
Water	4.0

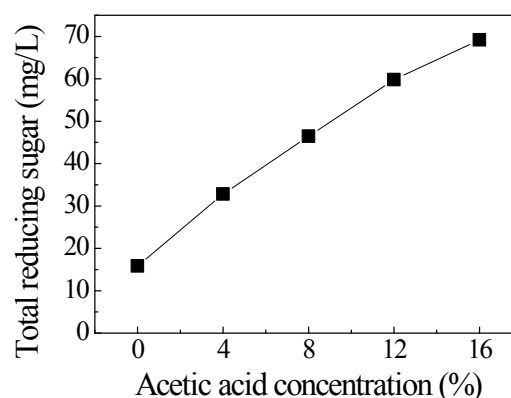


Figure 1: The total reducing sugar during the pretreatment

3.2 Simultaneous saccharification and fermentation and kinetic parameters for hydrogen production from ASECS

Strain B49, used in this experiment, identified as a novel *Ethanoigenens* strain, is gram-positive, no-spore-forming, no encapsulate, strict anaerobe. The optimal temperature and pH for B49 to grow and produce hydrogen from glucose was 37°C and 4.8, respectively. Doubling time was 7.2h. Strain B49 can utilize glucose, wheat, soybean, corn, potato starch, and molasses in wastewater, but can not utilize cellulose. This strain produced hydrogen from glucose with maximum hydrogen

production rate of 25 mmol H₂ h⁻¹ g⁻¹ dry cells and a hydrogen yield of 1810ml l⁻¹ medium^[18].

Pre-enzymolysis fermentation is always used for the utilization of cellulosic materials. But there is one research showing that simultaneous saccharification and fermentation was a promising method for hydrogen production by steam-exploded straw for its shorter lag-phase time and larger maximum specific hydrogen production rate^[3]. So, in the following experiment, bio-hydrogen was produced from ASECS by simultaneous saccharification and fermentation. Table 2 represents the orthogonal design of the experiment and the kinetic parameters for hydrogen production by simultaneous

saccharification and fermentation.

When acetic acid concentration was 0%, the maximum hydrogen production was 56ml/g when enzyme loading was 180U/g. When acetic acid concentration was 16%, the maximum hydrogen production was 72ml/g when enzyme loading was 120U/g. While the acetic acid concentration increased from 0% to 16%, the hydrogen yield increased 1.3 times. The lag-phase time was between 1.4h~5.2h. While the acetic acid concentration increased from 0% to 8%, the average lag-phase time decreased from 4.36h to 2.52h. When acetic acid concentration was 8%, 12%, 16%, the average lag-phase time was 2.52h, 2.54h, 2.58h, respectively. So, acetic acid steam explosion did significantly influence the bio-hydrogen fermentation.

Table 2: The orthogonal design L₂₅(5³) and kinetic parameters for hydrogen production from ASECS by simultaneous saccharification and fermentation

Acetic acid % (v/v)	Enzyme loading (U/g)	Hydrogen production (mL/g)	Maximum hydrogen production rate (mL/g VSSd)	Lag-phase time (h)	R ²
0	60	23	39	5	0.99873
	90	31	56	5.2	0.99952
	120	52	66	4.3	0.99795
	150	55.5	72	3.4	0.99693
	180	56	91	3.9	0.99709
4	60	39	63	4.2	0.99559
	90	51.5	59	2.4	0.99648
	120	56.5	70	3.1	0.9966
	150	59	78	3.3	0.99721
	180	62	82	3.5	0.99782
8	60	43	57	2.6	0.9954
	90	54	62	2.4	0.99596
	120	58	88	3.0	0.99613
	150	65	88	2.5	0.99896
	180	61	91	2.1	0.998
12	60	48	59	2.9	0.99758
	90	56	68	2.9	0.9949
	120	66	75	1.4	0.9948
	150	68	90	2.2	0.99415
	180	66	93	3.3	0.99109

16	60	48	61	2.8	0.99417
	90	60	65	2.5	0.99312
	120	72	91	2.7	0.98982
	150	71	81	3.1	0.98888
	180	64	103	1.8	0.99423

3.3 The influence of the acetic acid concentration and enzyme loading

Figure 2 describes the hydrogen production with different acetic acid concentration and different enzyme loading. The optimal ratio between enzyme and substrate is very important for the efficient use of cellulase enzyme. The result shows that when the acetic acid concentrations were 0% and 4%, the hydrogen production increased while the enzyme loading increased from 60U/g to 180U/g. When acetic acid concentration was 8%, 12%, 16%, the maximum hydrogen production were the largest when the enzyme loading was 150U/g, 150U/g, 120U/g, respectively. Hydrogen yield decreased with increasing enzyme loading. When enzyme loading was between 60U/g and 150U/g, the hydrogen production increased when acetic acid concentration increased.

As mentioned above, both acetic acid concentration and enzyme loading could affect the hydrogen yield. The intuitive analytic results of the orthogonal design about the hydrogen yield of simultaneous saccharification and fermentation was presented in table 3. R of the acetic acid concentration was 19.5 while R of enzyme loading was 24.4. The result shows that enzyme loading was more effective than the acetic acid concentration in simultaneous saccharification and fermentation by B49.

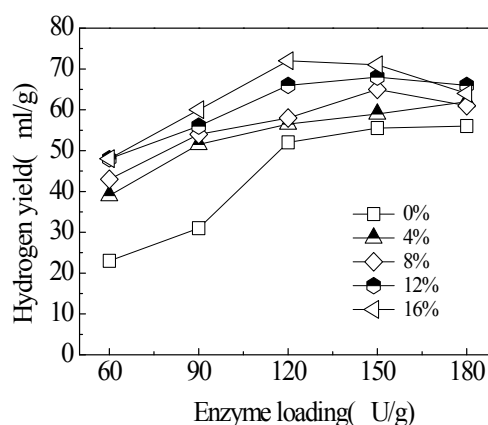


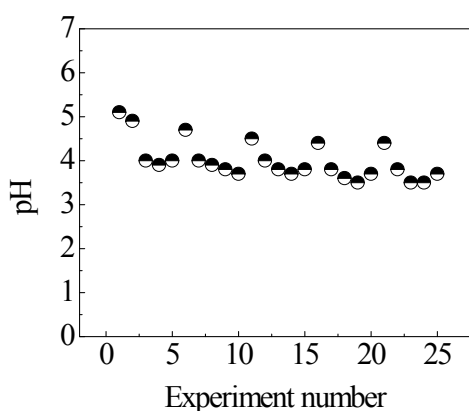
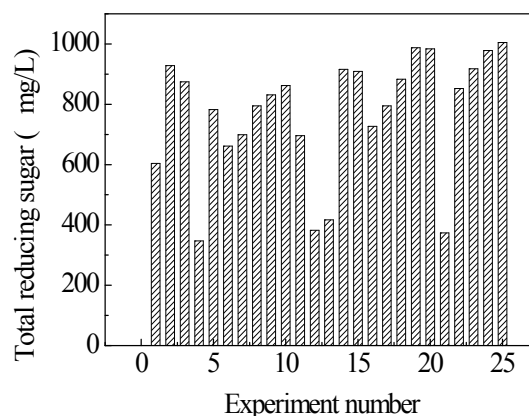
Figure 2: The hydrogen yield at different acetate acid concentration and enzyme loading

3.4 End pH of bio-hydrogen fermentation

Before bio-hydrogen fermentation, NaOH and dilute hydrochloric acid were used to adjust pH to 7.5. End pH of the bio-hydrogen fermentation was summarized in Figure 3. The result shows that the range of end pH is 3.5 to 5.1. The optimal pH for B49 to grow and produce hydrogen from glucose was 4.8, and it could even grow at pH 3.3. Total reducing sugar of the fermentation liquid was then analyzed and the result was described in Figure 4. It shows that there was still a certain amount of total reducing sugar in each experiment. It is probably that the enzyme added inhibited the growth and the hydrogen production of B49.

Table 3: Intuitive analytic results of the orthogonal design $L_{25}(5^2)$ about hydrogen yield of simultaneous saccharification and fermentation

Level	Influencing factor			
	Acetic acid concentration		Enzyme loading	
	K	$\bar{K}$	K	$\bar{K}$
1	217.5	43.5	201	40.2
2	268	53.6	252.5	50.5
3	281	56.2	306	61.2
4	304	60.8	323	64.6
5	315	63	303	60.6
R	19.5		24.4	

**Figure 3:** The end pH of bio-hydrogen fermentation**Fig 4:** The total reducing sugar of the end liquid

4. CONCLUSION

It is feasible for hydrogen production by simultaneous saccharification and fermentation from ASECS by *Ethanoligenens* sp. B49. The results in this experiment indicate that acetic acid can be used in the pretreatment of the corn straw and it could also affect the hydrogen yield. Hydrogen yield of acetic acid steam-exploded corn straw was 1.3 times higher than steam-exploded corn straw. And the lag-phase time decreased with the increase of the acetic acid concentration. Both two factors were effective to the hydrogen yield. Compared with acetic acid concentration, enzyme loading was more effective in simultaneous saccharification

and fermentation. But enzyme added might inhibited the growth and the hydrogen production of B49.

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