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Corn Straw as Substrate for Producing Ethanol by Solid-State-Fermentation

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Abstract

The technology of Solid-State-Fermentation (SSF) with corn straw by *Pichia ohmeri* T021 was studied in this article. After being crushed, the corn straw powder was added into vitriol solutions to hydrolysis, which the quality proportion of corn straw: water: vitriol (98%) is 20:80:1. The mixtures was incubated at 120℃ for 1 hour, and the hydrolysis rate reached 19%. Following, the mixture was adjusted to pH 4.5 by sodium carbonate and added cellulase (25IFPU/g). The hydrolysis rate reached 15% after the mixture was incubated at 50℃ for 25 h.

The mixture which hydrolysed by vitriol was inoculated by *Pichia ohmeri* T021 (5×10^7cell/g) and added cellulase (25 IFPU/g) at the same time. The ethanol yield reached 2.99g per 100 gram substrate after the fermenting grains was incubated at 33℃, pH 4.5 for 5 days.

Key words: Solid-State-Fermentation, Corn Straw, Ethanol

1. Introduction

Ethanol is one of the largest output fermentation products in the world. With the sharp increase in petroleum prices, all the countries have developed their ethanol industries energetically based on the energy security. Thus, the yield of fuel ethanol has continuously risen. Also, its proportion to world ethanol output continues to increase accordingly. Sugarcane, beet molasses or starchiness are currently the major feed stocks for ethanol fermentation, but these materials can be used as food or feedstuffs. This shows that it is necessary to seek new feed stocks such as lignocelluloses for fermentation [1, 2]. There are approximately 100 billion tons of renewable lignocellulose's resources available in the world per year. To take straw as an example, there are over 0.7 billion tons of unutilized straw annually in China, including about 0.22 billion tons of corn straw. Most of these resources are burned in the fields, creating air pollution, except that a small amount of them are used as ruminant feedstuffs [3].

The constitution of carbohydrate fraction within corn straw (%): glucose 39.0, mannose 0.3, galactose 0.8, xylose 14.8 and arabinose 3.2. Besides, it contains some lignin, mineral and protein with 15.1%, 4.3%

and 4.0% respectively. If carbohydrate can be transformed into ethanol effectively, utilization of corn stover will have a promising future [4, 5].

Therefore, a new technology of the SSF to make ethanol from corn straw was studied in this paper. In this SSF method, celluloses and hemicelluloses within corn straw partly converted into ethanol, and the remnants were used as stuffs in order to make solid substrate become loose. It is beneficial to transmit material and heat, and solid continuous distillation so as to improve the conversion rate of ethanol. Moreover, fermentation distiller' grain can be used as feedstuffs to reach zero-discharge standards.

2. Materials and Methods

2.1 Microorganism

The microorganism used in this study was *Pichia ohmeri* T021. The strain was conserved in Fermentation Engineering Lab, Shandong Institute of Light Industry.

2.2 Corn straw's hydrolysis and SSF

After the corn straw was crushed, the powder was added into vitriol solutions. The mixtures were incubated at different temperature for different time and the hemicellulose was hydrolysed. The major ingredients being investigated included the granularity, hydrolysis temperature and time, the vitriol solutions' thickness, and the content of solid matter. After filtration, the filtrate was collected for measurement of carbohydrates. The effects of granularity, hydrolysis temperature and time, vitriol solutions' thickness, and the content of solid matter on the hydrolysis' rate were investigated.

After the mixture hydrolysis by acid, it was neutralized by sodium carbonate, and the solid-state substrate and cellulase solution were added to acetate buffer solution together. The mixture was incubated at different conditions for some time and the cellulose would be hydrolysed. The major ingredients being investigated included the hydrolysis temperature and time, the acetate buffer solution's pH, and the initial cellulase concentration. After being hydrolysed by cellulase, the filtrate was collected for measurement of carbohydrates. The effects mentioned above were investigated.

The above strain of yeast was maintained on malt extract agar at 5℃ and growth was carried out in a resultant medium containing 2% peptone, 1% yeast extract, and 2% glucose (pH 6.0). After the corn straw powder was hydrolysis by vitriol and neutralized by sodium carbonate, *Pichia ohmeri* T021 was inoculated in the solid-state substrate (5×10^7cell/g) and cellulase was added at the same time. The fermenting grains cultured anaerobically. The major ingredients being investigated again included the fermentation temperature and time, the initial pH, and the initial cellulase content. When the fermentation completed, the ethanol was distilled and collected for measurement of ethanol yield. The effects mentioned above were investigated.

2.3 Analysis methods

Measurement of enzyme activity Cellulase activity was measured with hydrolysis filter paper as a substrate. Enzyme solution (0.5mL) was added to 1.5mL of substrate solutions, which contained 0.05g cellulase in an acetate buffer solution (pH 5). After mixture was incubated at 50℃ for 60 min, it was then centrifuged and the amount of reducing sugar produced in the supernatant was determined by the dinitrosalicylic acid (DNS) method. One unit of cellulase activity was defined as the amount of enzyme that produced 1 μ mol glucose per 60 min at 50℃ and a pH of 5.

The components of hydrolyte liquid by acid Total carbohydrates were determined after hydrolysed by vitriol. Carbohydrate was assayed on an HPLC by injection of 20 μ l of sample solution after filtration through 0.45 μ m. The components were separated on the Shodex Packed column (SHODO. CO. LTD) under isocratic conditions with 16mM NaOH as the eluent at a flow rate of 0.5 mL min^{-1} and a column temperature of 80℃. Peaks were detected with a pulsed amperometric detector. After each run, the column was regenerated by washing it for 10 min with 200mM NaOH. Standard solutions of glucose, xylose, and mannose were also injected [6, 7].

Total sugar determination The total sugar determined by dinitrosalicylic acid (DNS) method [8]. The hydrolysis rate was defined as:

$$\eta = \frac{a \times 0.9 \times n}{m \times (1-b)} \times 100\%$$

η, Hydrolysis rate; *a*, Reducing sugar, mg/mL; *n*, the dilution multiple; *m*, the weight of corn straw, g/L; b, the moisture content.

Ethanol yield determination 100 g draff after fermentation was diluted by 100 mL distilled water. After distilled the mixture, 100mL distilled liquid was collected to determine the ethanol yield through GC, colorimetric, and density bottle methods.

Observation by electron microscope. The corn straw powder's structure was observed by SEM (20kV).

3. Results and Discussions

3.1 Hydrolysis by acid

Fig.1 showed the effect of granularity of the corn straw on hydrolysis during different period with the hydrolysis temperature controlled at 120℃ and the solid material content at 20%. According to Fig.1, after

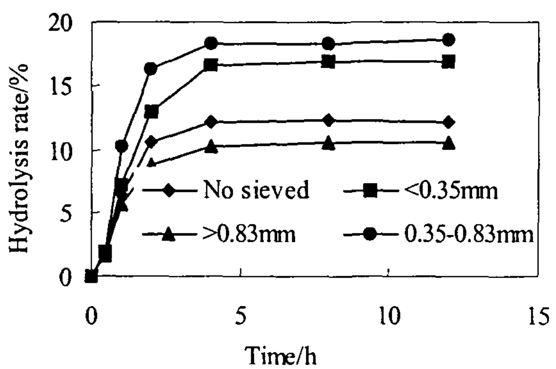

Fig. 1 Effect of the granularity on hydrolysis

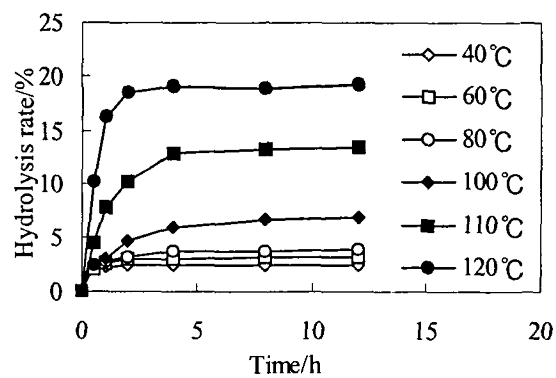

Fig. 2 Effect of the temperature on hydrolysis

hydrolysis for 4 h, the hydrolysis rate of corn straw that passed 20-eye sieve (granularity < 0.83mm) reached the max while below 20 eyes (0.83~2mm) reached the min, which indicated the smaller that granule was, the more sufficient that the hydrolysis was. But at the same time the more energy was badly needed. Considering overall, the corn straw powder should be sieved through a 20-eye sieve before hydrolysis as substrate.

Fig. 2 showed the effect of hydrolysis temperature. We can conclude that under the condition of certain vitriol solutions (1%), with the temperature hoisting, the hydrolysis rate aggrandized rapidly. When the corn straw hydrolyzed at 40℃ for 8 h, the hydrolysis rate can arrive at 2.2%, or at 100℃ for 4 h, the hydrolysis rate can arrive at 6.2%, while at 120℃ for only 2 h, the hydrolysis rate can arrive at about 19.5%. The results indicate that the hydrolysis temperature evidently affects the hydrolysis rate and Hoisting the temperature can observably increase the hydrolysis rate and save the hydrolysis time.

Fig. 3 showed the effect of vitriol solution' concentration on hydrolysis. When the vitriol solutions' concentratiton reached 1.0%, the hydrolysis rate ultimately arrived at the maximum. The rate did not go up with the vitriol concentration increasing.

Fig. 4 showed the effect of the solid-state material's content on the hydrolysis under the condition of the temperature controlled at 120℃ for 4 hours and vitriol solutions' concentration controlled at 0.5% and 1.0% respectively. According to Fig.4, the more that the solid material was contained, the lower the hydrolysis rate was. When the solid material content was below 20%, the hydrolysis rate was high and ultimately stabilized, while the solid material content was more than 20% to 30%, the hydrolysis rate reduced rapidly.

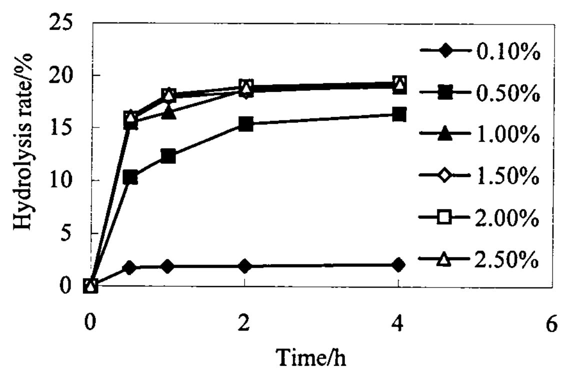

Fig. 3 Effect of vitriol solutions' concentration on hydrolysis

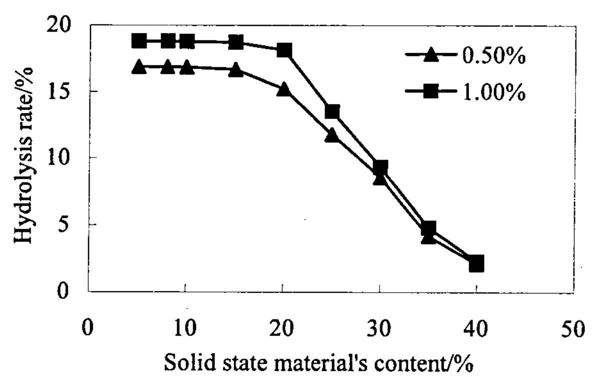

Fig.4 The effect of solid-state material's content to hydrolysis

Table. 1 The components of hydrolyte (g/L)

Conditions \ Sugar	Xylose	Glucose	Arabinose	Galactose	Total
120℃,1h	13.87	1.53	2.33	0.82	18.55
120℃,2h	14.12	1.92	2.65	0.93	19.62
120℃,4h	14.15	1.99	2.64	0.93	19.71
110℃,2h	13.03	1.12	2.04	0.55	16.74
110℃,4h	13.37	0.97	2.17	0.63	17.14

At the end of hydrolysis by acid, the filtrate was collected for measurement of carbohydrates by HPLC method. Table 1 turned out that the primary reducing sugar in the filtrate was glucose, including a little xylose, arabinose, and galactose else.

Fig. 5 is the micrograph of corn straw taken by the electronic microscope before and after hydrolysis. From A we can see clearly that the surface structure of corn straw is very tight and the fasciculus array parallel, which maybe prevent the cellulose enzyme from affecting the cellulose in corn straw. From B we can see that the little cell made up by cellulose is empty after the hemicellulose hydorlysed by acid and the loose structure will be prone to more hydrolysis by cellulase.

In conclusion, the hydrolysis with acid was controlled by the temperature most, vitriol solution and time less, and solid-state matterial's content least. The optimal technology conditions were that the vitriol solution concentration, hydrolysis temperature, hydrolysis time and material's content were controlled at 1.0%, 121℃, 60min and 20% respectively.

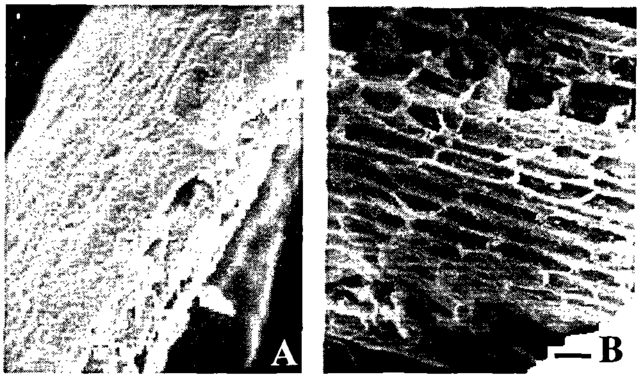

Fig. 5 Scanning electron micrograph of corn straw surface. Bar=10μm

A: without hydorlysis.

B: hydrolysed by vitriol solution.

3.2 Hydrolysis by cellulase

Fig. 6 showed the effect of cellulose content at 50℃, pH 4.85. With the cellulase increasing the hydrolysis rate increased rapidly until the cellulase concentration reached 25 IFPU/g, the mount of cellulase had less effect on hydrolysis. According to Fig. 7, the hydrolysis rate was higher when pH was controlled

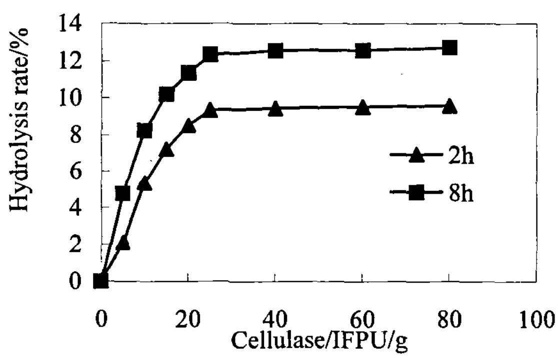

Fig. 6 Effect of cellulase concentration on hydrolysis

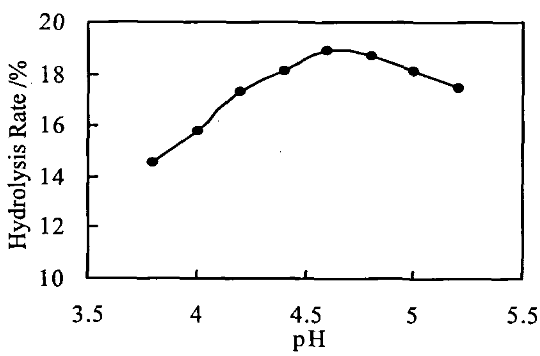

Fig. 7 Effect of pH on hydrolysis

during 4.4-4.8 and the maximum hydrolysis rate was about 19% with the pH 4.6. Fig. 8 showed the affect of hydrolysis temperature. From 0 to 24 hours, with the hydrolysis time increasing the hydrolysis rate increased gradually. However, after 24 hours, the hydrolysis rate didn't increase any longer.

Fig.9 is the scanning electron micrograph of corn straw surface. Comparing Fig. 5 and Fig.9, we can observe that hydrolysis by vitriol solution or by cellulase directly can both damage the complicate surface structure of corn straw. The surface structure of corn straw which hydrolysis by acid and cellulose one by one was badly damaged and looser than that after hydrolysed by cellulase directly.

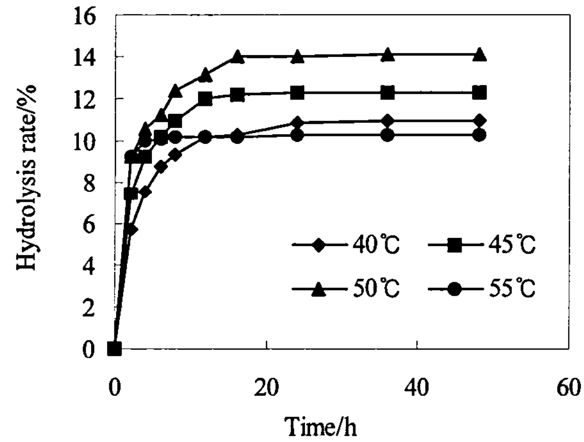

Fig. 8 Effect of hydrolysis temperature on hydrolysis

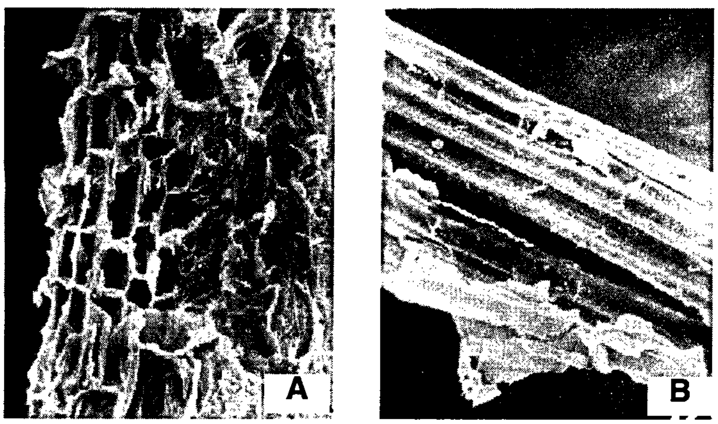

Fig. 9 Scanning electron micrograph of corn straw surface. Bar=10μm

A: hydorlysis directly by cellulase.

B: hydrolysed by vitriol solution and cellulase in turn.

3.3 The SSF with corn straw

As been shown above, the cellulase concentration and fermentation time determine the fermentation cost and the ethanol yield. In order to improve ethanol yield and save money, it is necessary to determine the optimum cellulase concentration and fermentation time. As shown in Table 2, 3 days fermentation and 25U/g cellulase showed the optimum condition. Prolonging the fermentation time and increasing the cellulase concentration did not increase the ethanol yield, instead, it would increase the cost.

The effect of temperature on the fermentation was tested. As shown in Table 3, at 25℃ the ethanol yield was more than other temperature. More time will benefit to the fermentation, instead, will improve the cost.

To determine the optimum fermentation condition such as initial pH and so on, a relative experiment was made, and the result as shown in Table 4. After analysed the data by Mathematica Soft, it shows that the best initial pH is 4.3, temperature is 33℃, cellulase concentration is 25.3IFPU/g, fermentation for 5.5days, and the yield is about 2.99g/d. After fermentation, there are still nutrient materials such as protein, starch, sugar, and fat in the distiller' grain, and it can be very useful to livestock [9].

Table. 2 Effect of cellulase concentration on ethanol yield (g/100g)

Time/d \ Cellulase content (U/g)	0	10	20	25	30	35	40	50
1	0.56	0.60	0.55	0.57	0.58	0.61	0.64	0.68
2	0.93	0.99	1.11	1.13	1.16	1.17	1.15	1.32
3	1.01	1.21	1.94	2.11	2.21	2.20	2.34	2.44
4	1.13	1.56	2.49	2.75	2.74	2.84	2.76	2.80
5	1.10	1.89	2.56	2.75	2.76	2.83	2.78	2.82

Table. 3 Effect of temperature on ethanol yield (g/100g)

Time/d \ Temperature/℃	25	27	29	31	33	35	37	39
1	0.35	0.40	0.53	0.68	0.65	0.59	0.48	0.21
2	0.90	1.00	1.22	1.36	1.33	1.13	0.87	0.33
3	1.11	1.34	1.68	1.98	2.11	2.01	1.66	1.41
4	1.22	1.56	1.87	2.22	2.45	2.66	2.00	1.45
5	1.45	1.57	2.14	2.23	2.67	2.88	2.22	1.64
6	1.46	1.67	2.17	2.20	2.55	2.90	2.34	1.80

Table. 4 Analysis of fermentation condition

Group	Temperature/℃	Days/d	Initial pH	Cellulase/ IFPU/g	Enthol/g/100g
1	32	5	4.5	20	2.10
2	32	6	5.0	25	2.30
3	32	7	5.5	30	2.48
4	35	5	5.0	30	2.84
5	35	6	5.5	20	2.55
6	35	7	4.5	25	2.42
7	37	5	5.5	25	1.70
8	37	6	4.5	30	2.10
9	37	7	5.0	20	2.21
$\overline{K}_1$	2.29	2.21	2.21	2.29	
$\overline{K}_2$	2.60	2.32	2.42	2.14	
$\overline{K}_3$	2.00	2.37	2.24	2.47	
R	0.60	0.16	0.21	0.33	

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