

The Influence of Pretreatment on the Preparation of Fuel Ethanol from Corn Stalk

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Abstract. In this research, the effects of three different pretreatment methods, physical pulverizing, steam blasting and hydrogen peroxide oxidizing, on ethanol preparation from corn straw were compared. The results showed that the content of reducing sugar in corn straw briquette with grinding and steam blasting pretreatment were 2 and 1.5 times higher than that without pretreatment. In the final product, the concentration of ethanol and rate of alcohol increased about 3.8 and 2 times, respectively. Besides, the reducing sugar content, ethanol content and alcohol yield in corn stalks soaked in hydrogen peroxide were 7 times higher than the untreated. The cellulose can be effectively isolated after being soaked in hydrogen peroxide with a concentration of 2.5% for 72 hours, as well as better degradation of lignin and hemicellulose. The amount of ethanol and the yield of alcohol were 1.9 and 3.3 times higher than physical pulverization and steam blasting. In brief, it is declared that hydrogen peroxide pretreatment can easily destroy the lignocellulosic cellulose of maize straw and improve the conversion rate of cellulose, which might be beneficial for the production of fuel ethanol.

1 Introduction

China is a large agricultural country with abundant corn straw yield. But the structure of corn straw is made of cellulose, hemicellulose and lignin, which is dense and complex and either saccharification or biological transformation is very difficult. In order to improve the solution rate of sugar in cellulose enzymolysis liquid [1], pretreatment is necessary to break the structure of lignocellulose, reduce its crystallinity, and improve the cellulose accessibility, as well as cellulose hydrolysis rate.

Reported pretreatment methods involves in mechanical crushing pretreatment, steam explosion pretreatment, and acid or alkali treatment, etc. Among which, mechanical crushing pretreatment can reduce the crystalline region of cellulose to expand, raw material surface area, make the cellulase quickly penetrate into the internal cellulose. Steam explosion method is to make the lignin soften under high temperature and pressure, and through rapid decompression, the cellulose crystal and fiber bundles will burst, and lignin and cellulose will be separated, to improve the straw cellulose hydrolysis rate [2]. The cellulose crystallinity damage is very limited and the lignin structure change is very weak. Acid or alkali treatment mainly uses acids, alkali or organic solvents as pretreatment reagent to remove lignin, or break the connection of wood carbon water complexes, or destroy

the crystal structure of cellulose [3]. Studies on the production of ethanol from switchgrass and hemp were shown that using switch grass and marijuana as raw materials to produce ethanol can obtain the alcohol with 16g.L⁻¹ and 11g.L⁻¹ ethanol concentration respectively after physical crushing pretreatment, and the concentration of ethanol in the control group without pretreatment was 14.6g.L⁻¹ and 8.66g.L⁻¹[4]. Study on the enzymatic hydrolysis of wheat straw pulverized pretreatment that the surface area of straw pulverization increases with the increase of comminution degree, and the surface binding point increases with the increase of enzyme hydrolysis rate [5]. The relationship between the pretreatment of steam blast and the hydrolysis rate of corn stalk cellulase was found, cellulose hydrolysis rate increased with the increase of the blasting pressure and increase pressure time, when the steam pressure is 1.6MPa and 9min dimensional pressure time, obtained cellulase hydrolysis yield was 75.76%[6]. When 5% NaOH containing 2.5% hydrogen peroxide, the soaking time was 72h, solid-liquid ratio of 1 to 20, the lignin removal rate is 61.52%, and the reducing sugar yield is 39.30% [7].

Because of the difference of analytical methods from different researchers, as well as the different treatments, the effect of ethanol preparation from even the same cellulosic materials are indeed different. In this study, corn stalk was used as the raw material. Based on previous studies, the best pretreatment conditions were

*Fund project: national natural science foundation project (41561108).

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selected through preliminary experiments. The corn stalk that was crushed to 0.2-0.4 cm, the bursting pressure is controlled at 80KPa and the blasting time is 30min and pretreatment with the concentration of 2.5% hydrogen peroxide. Then, the effect of several pretreatments on the ethanol production of corn straw was compared with the standard and analytical method.

2 Materials and methods

2.1 Materials and sampling

The corn stalk used in the experiment was collected from the farmland nearby Kunming.

Sodium dihydrogen phosphate-citric acid buffer solution was purchased from Fujian, Xiamen Haibiao Technology Co. Ltd. Cellulase (Novozymes (Chinese) Biotechnology Co., Ltd.), enzyme activity was 50000U.g⁻¹. Hydrogen peroxide (Shanghai Taopu Chemical Factory), by adding a proper amount of distilled water to adjust the concentration of 2.5%.

The method of (Van Soest) fiber determination was used to determine the content of cellulose, lignin and ash content in corn straw [8].

The method of reducing sugar content determination was adopted by DNS (3, 5-Dinitrasalicylic acid) colorimetry [9].

2.2 Experimental design

In this study, four experimental groups were designed, and each group was repeated three times. 50 grams corn straw cutting to 1~2cm was hydrolyzed and fermented in jar as the control group (group A).

Physical crushing corn straw to 0.2~0.4cm as the experimental group (group B1), in each, 50g raw material was put into a jar.

The Steam explosion pretreatment: adding distilled water into 50g corn stalk which was cut to 1~2cm, under blasting pressure 80KPa, steam explosion 30min (group B2).

Hydrogen peroxide pretreatment: corn stalk was cut to 1~2cm, according to 1:20 of the solid-liquid ratio, and putting into 2.5% hydrogen peroxide for 72h (group B3).

Hydrolysis: 1mol.L⁻¹ sodium citrate buffer was added to adjust the pH value to 4.8, and 35IU.g⁻¹ cellulase was added 50000U.g⁻¹. After mixing, the mixture was heated in a magnetic heating mixer, and the temperature is 50°C. Keeping for 48h, and measured the reducing sugar content.

Fermentation: The corn stalk was hydrolyzed and then removed, the temperature cooled to 30, and then the inoculation rate was 5% yeast. Its seal, after mixing in the temperature of 35°C, stirring speed 160r.Min⁻¹ fermentation under the condition of 72h. The formula [10] of ethanol yield can be calculated as:

$$Y = \frac{m_1}{m_0} \times 100\%$$

In the formula: m₀ and m₁ are the qualities of glucose (g) and ethanol (g) after hydrolysis; Y is ethanol yield.

Distillation: connected to a distillation unit for distillation.

3 Results and analysis

3.1 The effects of different pretreatment methods on the components of corn straw

It can be found in table 1, the content of corn straw cellulose, hemicellulose and lignin after steam explosion pretreatment were all decreased. Besides, the structure of the cell wall was destroyed, and the crystallinity polymerization of cellulose declined, and the hemicellulose was decompose into monosaccharides and oligosaccharides, as part of the lignin dissolved and the accessibility of cellulose enzyme is improved [11]. After pretreated with 2.5% hydrogen peroxide, the content of cellulose from corn stalks increased and the content of hemicellulose and lignin decreased, which might show that the soaking of H₂O₂ solution can break the chemical and hydrogen bond, which still caused the corn straw swelling and the lignin and hemicelluloses are dissolved. The specific surface area increased with the reducing of cellulose DP, while the crystalline region became indefinite area [12].

Table 1. Effects of Different Pretreatment Methods on Corn Stalk Components (%).

Pretreatment	Cellulose	Hemicellulose	Lignin	Ash	Moisture
—	38.17	23.01	18.25	4.37	7.98
Physical crushing	38.17	23.01	18.25	—	—
Steam explosion	35.91	19.63	10.74	—	—
Hydrogen peroxide	41.98	9.02	7.90	—	—

3.2 The study of effect on enzymatic hydrolysis with different method

Table 2. Reduced sugar content of different methods of pretreated corn stalks (%).

Component	A	B1	B2	B3
Reducing sugar content	1.1	2.6	1.7	7.7

It can be seen in table 2 that reducing sugar content of corn stalks without pretreatment (group A) is lowest, while others increased. Among that, reducing sugar content in group B1 is 2.6%, which was twice higher than group A. It might reflect that cellulose lignin of pretreated corn straw with ultra-fine grinding was destroyed, as the reactivity of the enzymolysis process was improved. The raw sugar content of corn stalk pretreated by steam blast was 1.7 percent, which was 1.5 times as much as before.

In group B3, the content of reducing sugar was 7.7%, which was 7 times higher than control group. It might reflect that when the corn stalks was soaked in hydrogen

peroxide solution, the lignin would be effectively dissolved and separated, which caused the cellulose be hydrolyzed more easily, so that the enzymolysis rate of raw materials was improved.

3.3 The impact on alcohol yield

The amount of alcohol yield with different pretreatments is shown in Table 3.

Table 3. The alcohol concentration (g/L) and the amount of alcohol yield (g) with different pretreatments.

	A	B1	B2	B3
Alcohol concentration	0.75	2.80	1.60	5.25
Amount of alcohol yield	0.28	1.07	0.58	1.95

Can be seen from table 3, after grinding pretreatment of the experimental group B1 the alcohol yield and ethanol producing content compared with the control group A, increased 3.8 times, shows that through mechanical crushing broken to A certain extent the lignocellulose, thus improve the rate of wine. The yield and the amount of ethanol of group B2 with steam explosion pretreatment were 1.60g/L and 0.58g, increasing by 2 times than the control group A. It shows that the sugar content increased significantly by the steam explosion pretreatment lasting for 30min, the yield of sugar is obviously increased and the yield is better.

In the experimental group B3 with hydrogen peroxide pretreatment to the corn straw, the yield and ethanol production is nearly 7 times higher than the control group A. After hydrogen peroxide pretreatment, the lignin of corn straw was degradation by peroxidase catalytic, increasing sensitivity of hydrolysis and improving lignocellulose conversion efficiency for glucose.

After soaking in the concentration of 2.5% hydrogen peroxide for 72h, the reducing sugar content, the amount of ethanol and the yield was significantly higher than other pretreatment methods. It shows that the lignin was easier to degradation and the cellulose hydrolysis rate was significantly improved after hydrogen peroxide pretreatment. The degradation effect to cellulose is better, which is beneficial to ethanol fermentation and the ethanol content was higher than that of other pretreatment methods.

3.4 Compared with other research results

Kim et al. [13] added carbon dioxide in steam explosion and used it to treat wood fiber raw material with 73% humidity, residing at 160°C and 21.4MPa for 30min, the rate of hydrolysis reached 84.7%. Pang et al. [14] proposed a combined pretreatment process (SE-MI) which steam explosion and microwave radiation. Compared with the single pretreatment by steam explosion, the enzymatic hydrolysis rate of corn stalks pretreated by SE-MI increased significantly. Yu Shengshuang [15] studied the effects of corn stalks by microwave-steam explosion combined with pretreatment, the results showed that the best pretreatment conditions for the straw length of 2 ~ 3cm, burst pressure of 2.0MPa, pressure time of 15min,

microwave radiation power was 470-500W, microwave radiation time was 3min, water content was 20%, cellulose hydrolysis rate reached 89%. Yang et al. [16] studied the alkaline oxide pretreated corn stalks and obtained in the solid-liquid ratio, fermentation time, alkaline oxide, pretreatment time were 1:20, 5d, 1%, and 3d respectively. The content of reducing sugar reached 15.92%. Varga E et al. [17] used corn stalks as a substrate treated by wet oxidation, fermentation of ethanol 120h with the method of simultaneous saccharification in 30FPU.g⁻¹ dry substrate plus enzyme, the production of ethanol reached 52.3 g.L⁻¹. MJ Zhang et al. [18] used corn cob as the substrate pretreated by acid-base, and started with 19% (w/v) substrate concentration under 30 FPU.g⁻¹ dextran addition, in Synchronous saccharification and fermentation process via fed in batches, at last supplied the total substrate concentration to 25% (w / v), ethanol concentration reached 84.7 g.L⁻¹ after 96 h.

Compared with the previous studies, the rate of wine production was higher than that obtained in this experiment. The reason may be that in the steam explosion pretreatment, the burst pressure set in this paper is 80KPa, which is far less than the burst pressure used in previous studies. The effect of steam explosion is affected by factors such as pressure and dwell time. Another reason is that only a single steam explosion method and adding H₂O₂ to corn stalk pretreatment, and previous studies using a combination of pretreatment [14-17] method. In the steam explosion process, adding some chemicals, such as H₂SO as a catalyst, or pre-steaming steam pretreatment SO₂ pre-treatment of the material can increase the hydrolysis rate of hemicellulose, hemicellulose hydrolysis degree significantly increased, while also reducing enzyme inhibitors, and improve the proportion of mono saccharides in the sugar solution [19]. Therefore, in the pretreatment of corn stalks, the choice of combination of physical and chemical methods or joint treatment is more conducive to improve the rate of enzymatic hydrolysis of corn stover.

4 Discussion

It is easy to hydrolyze starch, root mould, aspergillus, etc., which can hydrolyze starch into monosaccharide, and many yeast can also produce amylase and glycosylase. But as a result of semicrystalline starch particles consisting of alternating crystalline region and amorphous area, structure compact crystalline region limits the chemical reagent to the penetration of starch molecules inside, reaction occurs only in the particle surface, leading to low reactivity and reaction efficiency of starch [20]. Is the preparation of starch derivatives are generally use the chemical methods such as acid, alkali, oxidation, pasting, heat treatment, extrusion, radiation, high temperature physical method and enzyme solution methods such as preprocessing to improve the conversion of starch to starch or improve the performance of the product [21-27]. The physical process of pressure is under the condition of pressure or vacuum heat treatment to wet and heat treatment of starch, the starch in the wet and heat treatment process, due to the interaction of heat and

moisture of damaged starch α -1,6 glycosidic bond, at the same time the α -1,4 glycosidic bond is cracking [20]. Li Guanglei [28] steamed blasting treatment of indica rice starch, molecular weight of indica rice starch decreased and the degree of polymerization of starch molecular chains decreased with the increase of burst pressure, burst time and moisture content of indica rice. Wang Chunyan et al. [29] Studies have shown that corn starch oxidized by hydrogen peroxide carboxyl and carbonyl content increased, the higher the carboxyl content, the more the degree of oxidation, the higher the degree of molecular degradation, the lower the viscosity. Under the action of oxidant, the hydroxyl groups on C2, C3 and C6 are easily oxidized. Under different conditions, the hydroxyl groups are oxidized to aldehyde, keto and carboxyl groups and reduced the number of hydroxyl groups in the starch molecule, Associated blocked, weaken the sub-hydrogen bonds between the binding capacity; at the same time some of the oxidants can make the end of the starch molecule glucose ring structure broken in the C1 position of the oxygen atoms in the ring and at the C1 position formed aldehyde, To reduce the degree of polymerization [20], increasing the enzymatic hydrolysis activity, thereby increasing the starch production rate of raw materials.

Cellulose is a chain of polymers linked by glucose via β -1, 4-glucosidic linkages. There are three active hydroxyl groups, one primary hydroxyl group and two secondary hydroxyl groups on each glucosyl ring in the cellulose chain. Hydroxyl can be integrated into molecules and intermolecular hydrogen bonds, which have a profound influence on the morphology and reactivity of the cellulose chains. In particular, the intermolecular hydrogen bonds formed between the hydroxyl group at the C-3 position and the oxygen on the adjacent molecular ring. Not only enhance the linearity and rigidity of the molecular chains of cellulose, but also its molecular chains arranged closely to form a highly ordered crystalline zone [30]. Drawing on the mechanism of pretreatment of starch raw materials to increase liquor yield, corn stalk cellulose was pretreated with acid and alkali, the β -1, 4-glycosidic bond between two adjacent glucose monomers was opened, the sugar bonds ruptured and the degree of polymerization decreased. Oxidative degradation takes place at the C-2 position mainly, the C-3 position and the free hydroxyl position at the C-6 position of the glucosyl ring. After oxidation, C-2, C-3 and C-6 form carbonyl groups, Base, carboxyl, then the molecular chain caused by degradation through the β -alkoxy elimination reaction, glycosidic bond breaking, thereby increasing the contact area of cellulase and improve alcohol production rate.

5 Conclusion

Steam blasting and hydrogen peroxide pretreatment can improve the reducing sugar content, mainly because of steam explosion to separate the lignin and cellulose, increase the contact area of cellulase to cellulose and hydrogen peroxide can effectively removing the lignin in the corn straw, to improve the purity of cellulose, thus improve the conversion efficiency of glucose. After

pretreatment of corn stalk has markedly improved wine rate, rate of steam explosion pretreatment makes wine increased 2 times, hydrogen peroxide pretreatment makes wine rate increased 7 times, mainly because the pretreatment of lignocellulose into simple sugars, by adding yeast fermenting sugar into alcohol. Steam blasting and hydrogen peroxide pretreatment can increase the rate of maize straw to produce wine, but weak acid pretreatment process, furan, such as inhibitor, influence wine effect, so in the future we need to develop a variety of ways the pretreatment technology of combining, through the complementary advantages between different pretreatment methods to solve the shortage of the single processing technology, in addition, we also can increase the rate of wine by optimizing the fermentation conditions.

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