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The Effect of Sugarcane Bagasse`s Size on the Properties of Pretreatment and Enzymatic Hydrolysis

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Abstract. The influence of milled bagasse particle size on their reducing sugar and lignin content during dilute acid hydrolysis followed by enzymolysis was investigated. The biomass crystal structures of hydrolyzed residues and enzymolyzed substrates were studied with X-ray diffractometry (XRD). The results showed that the conversion ratio of reducing sugar declined with decreasing milled bagasse particle size. The conversion ratio of reducing sugar after acid hydrolysis decreased from 31.3% to 28.9%. The smaller of the milled bagasse particle size was, the higher of the klason lignin content of hydrolyzed residuals was, which resulted in a decline in conversion ratio of reducing sugar during enzymolysis. In this study, the optimal size of milled bagasse particles was 10 to 20 meshes. The total reducing sugar conversion ratio was 61.5%, consisting of 31.3% in hydrolysis and 30.2% in enzymolysis. After hydrolysis, the specific surface area and pore size increased, and the fiber length was shortened. The inner microfiber bundles were exposed, which improved the accessibility of cellulase and the efficiency of enzymolysis.

1.Introduction

Plant fiber is an important renewable resource that is mainly composed of cellulose, hemicellulose, and lignin. Lignin and hemicellulose are connected by covalent bonds, and cellulose is encrusted with lignin and hemicellulose.[1] Due to its complex structure and the crystalline structure of cellulose, fibers are resistant to enzymatic hydrolysis.[2] However, pretreatment of lignocellulosic materials improves the sugar yield, which can also increase the specific surface area of cellulose.[3] Acid pretreatment is a common chemical pretreatment method.[4] Acid dissolves hemicellulose in wood fiber and improves the accessibility of cellulose.[5] Due to the highly concentrated acid, the equipment used for pretreatment must have high corrosion resistance. So, diluted acid with mild reaction conditions and less inhibitors are widely used.[6] Many factors influence the efficiency of cellulase, such as the size of milled bagasse particles, lignin and hemicellulose content, crystallinity, and the accessibility of cellulose.[7] The shape and size of milled bagasse particles determine the external specific surface area, and the porosity and capillary structure in fiber determine the internal specific surface area.[8] Efficient pretreatment methods effectively improve the internal and external surfaces of milled bagasse particles, especially the internal specific surface area. The external surface can also be improved by reducing the size of milled bagasse particles.[9]

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In order to improve the ethanol conversion ratio during enzymatic hydrolysis and fermentation[10], the bagasse was milled and pretreated with diluted acid. The substrates were subjected to enzymolysis. The influence of particle size on reducing sugar conversion *via* pretreatment and enzymolysis was analyzed.

2.Experimental

2.1. Materials and Pretreatment

The bagasse was provided by a factory. The raw materials were washed, dried, milled, and screened. The chemical composition of the different bagasse particles was determined and listed in Table 1.

Table 1. Composition of differently sized bagasse particles

Particle Size (Mesh)	Glucan (%)	Xylan (%)	Klason Lignin (%)	Acid-Soluble Lignin (%)
10 - 20	49.44	25.39	18.23	0.30
20 - 40	50.99	25.04	20.03	0.29
40 - 60	50.15	24.69	22.85	0.28
60 - 80	50.05	23.65	23.14	0.30

2.2. Dilute Acid Hydrolysis

The dilute acid hydrolysis process was conducted in the electrical heated digester. The samples was treated at 160℃ for 15min with the solid liquid ratio of 1:15 and the H_2SO_4 concentration of 0.8%. After hydrolysis, the hydrolyzed samples were filtered through a 400-mesh cloth. The filtrate was tested for pH and stored at 4 ℃. The residuals were washed and dehydrated to 30% consistency and stored in a self-sealed plastic bag.

2.3.Enzymolysis

Enzymolysis reactions were conducted in a 50-ml Erlenmeyer flask with 1 g of the pretreated residuals, 100 ml of buffer (citric acid and sodium citrate buffer solution, 0.05mol/L, pH 4.8), and 20 FPU of cellulase enzyme. The flasks were stirred for 48 h at 50 ℃ and 200 rpm. After, the sample flasks were placed in a boiling water bath for 10 min to stop enzymolysis. Then, the samples were cooled in ice water and centrifuged for 5 min at 12000 rpm. The reducing sugar content of the supernatant was measured. The enzymolyzed substrates were washed, air-dried, and stored in plastic bags.

2.4.Reducing Sugar Analysis

The total reducing sugar content in hydrolyzed filtrates and enzymolyzed supernatants was calculated by ion chromatography. The conversion ratio of reducing sugar was calculated as follows:

$$C_{RSA}(\%)=W_1/(W_0 \times C_0) \times 100 \quad (1)$$

$$C_{RSA}(\%)=W_2 \times Y_1/(W_3 \times C_0) \times 100 \quad (2)$$

$$C_{TRS} (\%) = C_{RSA} (\%) + C_{RSE} (\%) \quad (3)$$

where C_{RSA} refers to the conversion ratio of reducing sugar in acid hydrolysis, C_{RSE} refers to the conversion ratio of reducing sugar in enzymolysis, C_{TRS} refers to the conversion ratio of total reducing sugar, C_0 refers to the total reducing sugar content in raw materials, W_0 refers to the weight of raw materials, W_1 refers to the reducing sugar weight in hydrolyzed filtrate, W_2 refers to the reducing sugar weight in enzymolyzed supernatant and W_3 refers to the weight of enzymolysis samples.

2.5.X-ray Diffraction (XRD) Analysis

Crystal structures in the different bagasse particles were determined by an X-ray diffractometer. The relative crystallinity (C_r) was calculated using the Segal formula (Eq. 1):

$$C_r (\%) = (I_{002} - I_{am})/I_{002} \times 100 \quad (4)$$

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where I_{002} is the diffraction peak intensity of the 002 area and I_{am} refers to the scattering intensity of non-crystalline background with a diffraction angle of 18.

3.Results and Discussion

3.1.Effect of Bagasse Particle Size on Reducing Sugar Conversion

The sugar yield and the related conversion of RSA and RSE were listed in table 2 and table 3, separately.

Table 2. Sugar yield in hydrolyzed filtrates and enzymolysis supernatants

Particle Size	Hydrolyzed Filtrate					Enzymolysis Supernatant		
	Xylose	Glucose	Arabinose	Galactose	RSA	Xylose	Glucose	RSE
10-20	20.12	1.02	1.14	1.03	23.31	2.49	30.30	32.79
20-40	20.19	1.15	1.22	1.04	23.60	2.46	30.08	32.54
40-60	18.64	0.96	1.24	0.94	21.78	1.61	28.22	29.83
60-80	18.42	0.80	1.36	0.72	21.30	1.40	26.01	27.41

Table 3. Conversion ratio of RSA, RSE, and TRS

Particle Size	RSA	RSE	TRS
10-20	31.3	30.2	61.5
20-40	30.9	29.4	60.3
40-60	29.1	27.9	57.0
60-80	28.9	26.4	55.3

As showed in table 3, RSA and RSE conversion declined with decreased bagasse particle size. Since the smaller biomass particles allow better degradation, which releases more reducing sugars was dissolved.[11] However, the reducing degree was not very high. For example, the RSA conversion rate decreased from 31.3% to 28.9% when the bagasse particle size decreased from 10-20 mesh to 60-80 mesh. The smaller bagasse particles may have lower xylan content and more resistance from lignin (Table 1). Chen *et al.*[12] found that when the pretreatment time was less than 3 min, the reaction mainly happened on the milled bagasse surface. Thus, for small bagasse particles with high specific surface areas, the conversion ratio of reducing sugar was high.

In addition, when the bagasse particles were small, the corresponding conversion ratio of RSA and RSE was also small. The RSA and RSE conversion ratios of 10-20 mesh and 20-40 mesh particles were approximately 30 ±0.4%. The RSE conversion ratio for 40-60 mesh and 60-80 mesh particles were 27.9% and 26.4% respectively, which decreased noticeable from the previous two samples. The maximum conversion ratio of was 61.5% in the 10-20 mesh samples, which mainly depended on RSE conversion.

3.2.Effect of Particle Size on the Lignin Content of Hydrolyzed Residuals

Figures 1 and 2 present the lignin content and its effects on reducing sugar conversion. As a filling and binding agent, lignin combines with and reinforces cellulose fibers *via* physical and chemical bonds in the cell wall.[13] As for the three components of lignocellulose, hemicellulose is the most easily degraded, while lignin is the most difficult to degrade.[14] But, nucleophilic molecules in lignin (such as the C_1 and C_6 of benzene ring) reacted with nucleophilic reagents and dissolved[15] during sulfuric acid treatment. The maximum RSE conversion ratio dropped 3.8% with increasing lignin content.

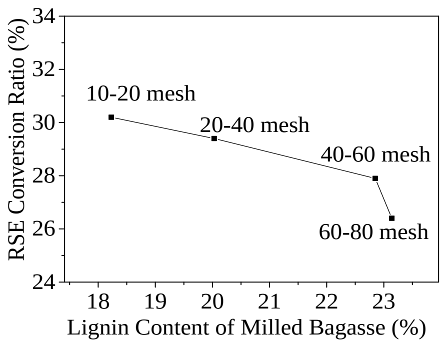

Figure 1. Effect of klason lignin content in milled bagasse on the RSE conversion ratio

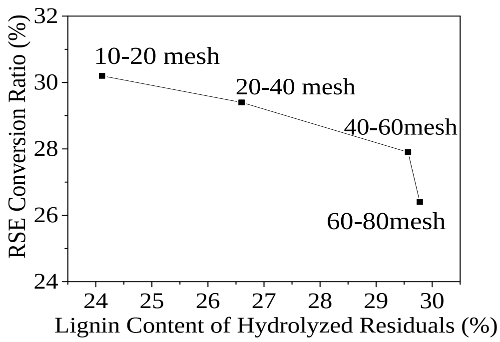

Figure 2. Effect of klason lignin content in hydrolyzed residuals on the RSE conversion ratio

Yu *et al.*[16] proposed that lignin prevented cellulase attachment to cellulose resulting in the low efficiency of enzymolysis. Masarin *et al.*[17] found that RSE conversion is inversely proportional to lignin content in hydrolyzed residuals. Lindedam *et al.*[18] also found that RSE conversion is proportional to carbohydrate content. In agreement with these results, when the lignin content of hydrolyzed residuals was high in this study, the RSE conversion ratio was low, probably because the lignin inhibited cellulase adsorption.[19]

3.3.Effect of Particle Size on Hydrolyzed Residuals Crystallinity

From the figure 3, it can be seen that the crystal structure of the four kinds of hydrolyzed residuals was similar. They were all composed by the crystalline region and amorphous area the same as milled bagasse particle. The crystallinity of milled bagasse particles was 37.0%, while the crystallinity of 10-20 mesh, 20-40 mesh, 40-60 mesh, and 60-80 mesh hydrolyzed residuals was 47.1%, 46.5%, 43.9%, and 43.6%, respectively. The increased relative crystallinity was due to the partial hydrolysis of amorphous cellulose and complete of hemicellulose hydrolysis.[20] The crystallinity of the 10-20 mesh and 20-40 mesh particles was similar, as was the 40-60 mesh 60-80 mesh particles, with the former set being about 3% higher than the latter. This result suggests that in a certain size ranges, milled bagasse particles react similarly to pretreatment.

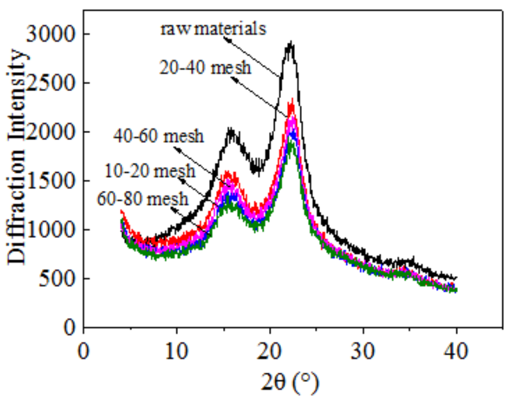

Figure 3. XRD spectra of milled bagasse particles after acid hydrolysis

The crystallinity of the 10-20 mesh hydrolyzed residuals was the highest, and the RSE conversion ratio was also the highest (30.2%). Similarly, the crystallinity and RSE conversion ratio (26.4%) of 60-80 mesh hydrolyzed residuals were the lowest. Higher crystallinity indicated that hemicellulose hydrolysis during pretreatment was better, which increased the accessibility of cellulose and improved the relative crystallinity of cellulose. It meant that when the crystallinity of the hydrolyzed residuals were higher, the enzymolysis was not more difficult[21]. Therefore, the efficiency of enzymatic hydrolysis was influenced by many factors including the crystallinity of hydrolyzed residuals.

Conclusions

1. With decreasing bagasse particle size, the conversion ratio of RSA and RSE declined. The conversion ratio of RSA fell from 31.3% to 28.9%. The conversion ratio of RSA and RSE was not increased by reducing the size of milled bagasse particles.
2. Cellulose and the lignin showed little degradation during hydrolysis. The size of milled bagasse particles was smaller, and the klason lignin in hydrolyzed residuals was higher, which decreased the reducing sugar conversion ratio. The optimal size of milled bagasse particles was 10-20 mesh, which produced a TRS conversion ratio of 61.5%, including 31.3% RSA conversion and 30.2% RSE conversion ratio.
3. Hydrolyzed residuals produced from 10-20 mesh particles showed the highest crystallinity and RSE conversion ratio. After acid hydrolysis, most hemicellulose was degraded and dissolved. The lignin was also degraded. These changes were very beneficial to enzymolysis.

Acknowledgements

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