

Greater Importance of Structural Changes Over Lignin Removal in Impacting the Enzymatic Hydrolysis of Crop Straws

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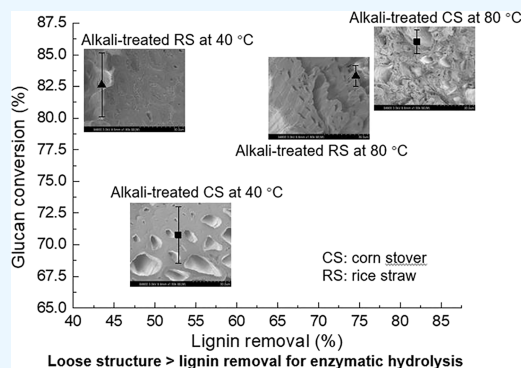
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ABSTRACT: The importance of structural changes and lignin removal in influencing the enzymatic hydrolysis of crop straws was investigated. Alkaline pretreatment of corn stover (CS) and rice straw (RS) was conducted to remove lignin. It was found that alkaline pretreatment caused more lignin removal from CS than RS under the same condition, and CS and RS treated at 40 °C (CS40 and RS40) attained less lignin removal than those treated at 80 °C (CS80 and RS80). However, CS40 achieved glucan conversion efficiency of 70.77% which was 11.85% less than that of RS40, and similar glucan conversion efficiencies were obtained for RS 40 and RS80. Structural observation showed that the connection among single fiber bundle of CS40 was tighter than that of RS40, while those of RS40 and RS80 were loose. It meant that structural changes might be more important than lignin removal to influence enzymatic hydrolysis of crop straws.



1. INTRODUCTION

Bioconversion of crop straws into biofuels and bioproducts is one of the appealing and promising ways due to the potential alternative of fossil fuels and the reduction of greenhouse gas emission.¹ Like other lignocelluloses, crop straws are also mainly composed of cellulose, hemicellulose, and lignin which endow their recalcitrance for being degraded. It has been prevalently thought that lignin is the chief factor to negatively influence the enzymatic hydrolysis of lignocellulose due to its unproductive adsorption of cellulase and accessing blockage of cellulase to cellulose.² However, some researchers pointed out that cellulose accessibility determined the enzymatic hydrolysis efficiency of lignocellulose.³

Generally, the cellulose accessibility is characterized by the BET method, conventional SEM observation, Simons' staining method, water retention value (WRV), and so on. Compared with the first two methods which require dry samples, the latter two methods can measure the original state of treated lignocellulose without drying.⁴ It was reported that the drying process could induce fiber hornification and lead to the shrinkage of all pores in lignocellulose to reduce cellulose accessibility.^{4,5} In order to characterize the prototypical feature of treated crop straws, the WRV detection method which could predict the recalcitrance of the wet sample was employed,⁶ and new cryo-SEM was applied to observe the longisecton and transection of untreated and treated samples. Corn stover (CS) and rice straw (RS) which are not only the top two crop straws in China,⁷ but also the second and fourth crop straws in the world,⁸ are selected as samples. This study pretreated CS and RS under the same condition and compared their composi-

tional changes, surface structural changes, and enzymatic saccharification efficiencies. It showed that lignin removal might not be as important as structural feature to influence enzymatic hydrolysis of crop straws, and limited washing times combined with pH adjustment was more suitable than several washing times for achieving good enzymatic digestion of alkali-treated CS and RS.

2. RESULTS AND DISCUSSION

2.1. Physicochemical Changes of Crop Straws after Alkaline Pretreatment. Except 5.81% higher relative glucan content of the untreated RS than that of untreated CS, the differences in the relative xylan, araban, and acid-insoluble lignin contents are in the range from 0.34 to 1.10% (Table 1). After alkaline pretreatment under the same condition, the alkali-treated CS has the similarly relative glucan, xylan, and araban contents as the alkali-treated RS but lower relative acid-insoluble lignin content than the alkali-treated RS. According to the retention of glucan, xylan, and araban and the removal of lignin, it can be found that alkaline pretreatment at 40 °C exhibits milder influence on the composition of CS and RS than that at 80 °C. After alkaline pretreatment at 40 and 80 °C,

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Table 1. Composition of Untreated and Treated CS and RS

samples ^a	glucan (%)		xylan (%)		araban (%)		acid-insoluble lignin (%)	
	content	retention	content	retention	content	retention	content	removal
CS	33.49 ± 0.40		23.54 ± 0.37		4.25 ± 0.02		25.82 ± 0.27	
CS40	44.43 ± 0.13	89.95 ± 0.26	25.78 ± 0.79	74.24 ± 2.26	4.68 ± 0.02	74.70 ± 0.28	17.95 ± 1.10	52.87 ± 2.90
CS80	51.96 ± 0.46	85.03 ± 0.75	26.93 ± 0.27	62.69 ± 0.62	4.35 ± 0.04	56.13 ± 0.48	8.46 ± 0.00	82.05 ± 0.00
RS	39.30 ± 0.01		22.44 ± 0.01		4.72 ± 0.00		25.48 ± 1.50	
RS40	45.97 ± 0.08	83.06 ± 0.15	24.13 ± 0.05	76.37 ± 0.15	4.91 ± 0.04	73.82 ± 0.67	20.28 ± 0.15	43.50 ± 0.41
RS80	52.38 ± 0.20	75.18 ± 0.29	26.02 ± 0.00	65.41 ± 0.00	4.47 ± 0.09	53.30 ± 1.10	11.49 ± 0.92	74.56 ± 2.04

^aThe numbers 40 and 80 are temperatures (°C) for treating CS and RS.

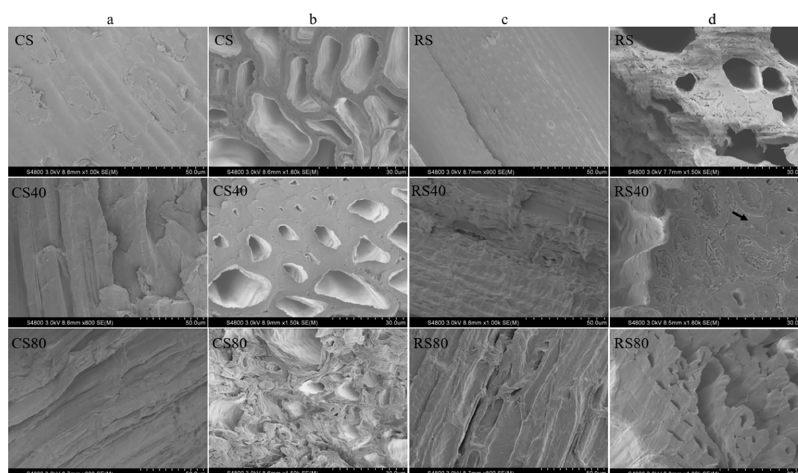


Figure 1. Longisection and transection micromorphologies of untreated and alkali-treated CS and RS observed by cryo-SEM. (a) Longisection micromorphologies of untreated CS and alkali-treated CS at 40 °C (CS40) and 80 °C (CS80), (b) transection micromorphologies of untreated CS, CS40, and CS80, (c) longisection micromorphologies of untreated RS and alkali-treated RS at 40 °C (RS40) and 80 °C (RS80), (d) transection micromorphologies of untreated RS, RS40, and RS80. The note indicated by the arrow in RS40 should be the unevaporated water.

CS has higher glucan and araban retention and lignin removal but lower xylan retention than RS. It means that glucan and araban in CS have stronger recalcitrance for alkaline pretreatment than those in RS, while xylan and lignin in CS are more vulnerable during alkaline pretreatment than those in RS.

The longisection and transection of untreated and treated samples were captured by cryo-SEM to show the changes in the surface structure. From Figure 1a,c, the longisection's graphs show that the untreated CS and RS have a smooth and compact surface, and alkaline pretreatment causes rough surfaces and makes their filamentary structures (CS40, CS80, RS40, and RS80) exposed. From Figure 1b,d, the transection's pictures show that the fibrous bundles in the untreated CS and RS are close, next to each other, and the single fibrous bundle becomes thicker after alkaline pretreatment (CS40, CS80, RS40, and RS80), which corresponds to the cellulosic swelling by alkaline pretreatment.⁹ These structural changes should be largely related to the removal of lignin which should play a key role in maintaining the microstructure of lignocellulose.¹⁰ It can be also found that the single fibrous bundle in the alkali-treated CS is still tightly adjacent to each other, while that in the alkali-treated RS is detached. Additionally, the fibrous bundles of CS treated at 40 °C have a well-preserved shape (CS40), while those at 80 °C exhibit a broken shape (CS80). However, those of RS treated at 40 °C (RS40) and 80 °C (RS80) both keep good morphologies.

WRV has been pointed out as an index for indirectly indicating the porosity and cellulosic accessibility of plant biomass.¹¹ From Figure 2, the WRVs of alkali-treated CS and

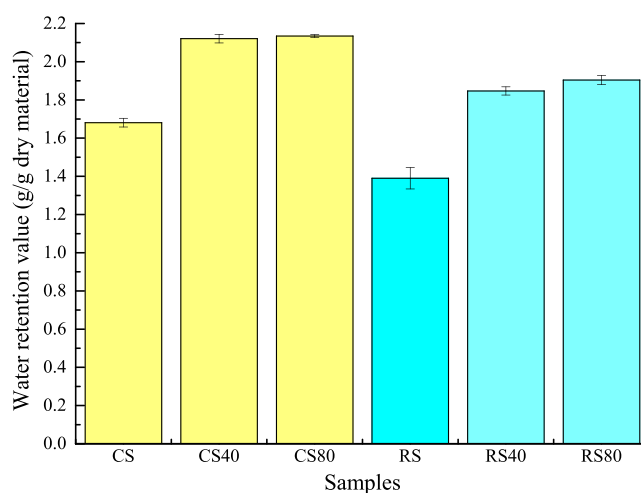


Figure 2. WRVs of untreated and alkali-treated CS and RS. CS40 and CS80 are short for alkali-treated CS at 40 and 80 °C, and RS40 and RS80 are short for alkali-treated RS at 40 and 80 °C.

RS are higher than those of untreated CS and RS, which corresponds to the previous study.⁶ The increased WRV usually indicates the incremental porosity and cellulosic accessibility to the enzyme.¹¹ It is also found that the alkali-treated CS samples have higher WRVs than the alkali-treated RS samples, while the alkali-treated CS at 40 °C possesses a similar WRV as that at 80 °C and so do alkali-treated RS at 40 and 80 °C. It should be pointed out that WRV might not be

used for differentiating different samples due to the combination effect of specific biomasses and pretreatment methods.⁶ The water affinity of lignocellulosic components should be cellulose > hemicellulose > lignin, and cellulose does not provide much contribution to WRV as hemicellulose and lignin.¹¹ Hemicellulose has great relation to WRV.⁶ From Table 1, CS and RS treated at 40 °C have almost the same xylan and araban contents as CS and RS treated at 80 °C, respectively, which might be the reason for the similar WRVs (Figure 2).

2.2. Effect of Alkaline Pretreatment on Enzymatic Hydrolysis. The enzymatic hydrolysis of alkali-treated CS and RS which were washed for seven times to make pH paper display neutral color is shown in Figure 3a. The enzymatic

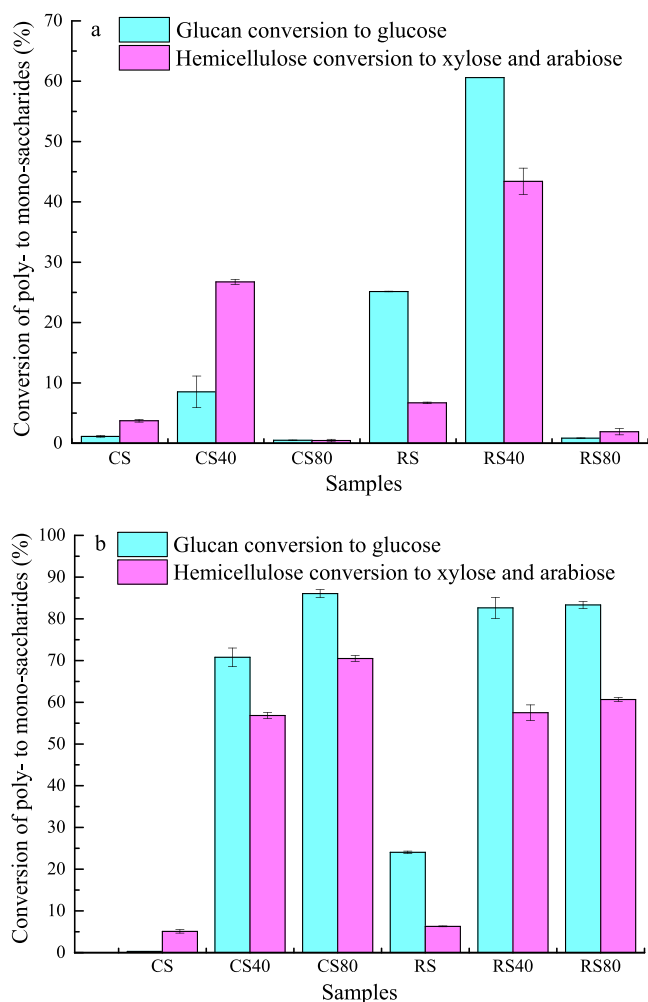


Figure 3. Enzymatic hydrolysis of untreated and alkali-treated CS and RS. (a) Seven times washed CS and RS treated by alkaline solution at 40 and 80 °C (CS40, CS80, RS40, and RS80) without pH adjustment. (b) Three times washed CS40, CS80, RS40, and RS80 with pH adjustment to 4.8.

hydrolysis efficiencies of CS and RS treated at 40 °C are higher than those of untreated CS and RS, respectively, while those of CS and RS treated at 80 °C are lower. Moreover, RS treated at 40 °C obtains glucan conversion of 60.6% and hemicellulose conversion of 43.4%, which are 52.1 and 16.7% more than CS treated at 40 °C. From Figure 2, the alkali-treated CS has higher WRV than the alkali-treated RS, which hints that the alkali-treated CS would retain more alkaline solution than the

alkali-treated RS, even if the same washing procedure is adopted to remove residual alkaline solution in alkali-treated CS and RS. Higher temperature for treating CS and RS would make deeper infiltration of alkaline solution into CS and RS, which might result in difficult alkalinity removal through washing for seven times. Although the alkali-treated CS and RS after seven times washing could make pH paper show neutral color, actually they were still alkaline after being checked by a pH meter. The addition of acetate buffer (pH 4.8) could easily lower down the pH of alkali-treated RS at 40 °C to be close to 4.8, followed by alkali-treated CS at 40 °C, but had little impact on obviously reducing the pH of alkali-treated CS and RS at 80 °C.

The enzymatic hydrolysis of alkali-treated CS and RS which were washed for three times with pH adjustment to 4.8 is present in Figure 3b. The alkali-treated CS and RS attain glucan conversion from 70.8 to 86.0% and hemicellulose conversion from 56.8 to 70.5%, which are significantly higher than the seven times washed alkali-treated CS and RS (Figure 3a). It means that several times washing alkali-treated CS and RS to eliminate their alkalinity is not necessary, and limited washing combined with pH adjustment should be more suitable for post treatment of alkali-treated CS and RS for subsequent enzymatic saccharification. The glucan and hemicellulose conversion of alkali-treated CS at 40 °C are 15.3 and 13.7% less than those of alkali-treated CS at 80 °C, while those of alkali-treated RS at 40 °C are similar to those of alkali-treated RS at 80 °C. It has been reported that lignin has negative influence on the enzymatic hydrolysis of lignocellulose due to its unproductive adsorption of cellulase and blocking the access of cellulase to cellulose.² More lignin is removed from CS than RS after alkaline pretreatment at 40 °C, and the alkali-treated CS has lower relative lignin content than the alkali-treated RS (Table 1). The alkali-treated CS at 40 °C achieves inferior enzymatic hydrolysis efficiency than the alkali-treated RS at 40 °C. The alkali-treated RS at 40 °C has less lignin removal and contains 8.79% more relative lignin content than the alkali-treated RS at 80 °C (Table 1), but these two samples attain equal enzymatic hydrolysis efficiency. It hints that lignin removal and presence should not be labeled as the chief factors to inhibit enzymatic hydrolysis of lignocellulose. From Figure 1b,d, it can be seen the single fiber bundle of alkali-treated CS at 40 °C connected with each other tightly, which might cause inconvenient access of cellulase to cellulose. Nevertheless, the single fiber bundle of alkali-treated CS at 80 °C was seriously broken and that of alkali-treated RS linked loosely. The loose structural feature might play a more important role in influencing the enzymatic hydrolysis of alkali-treated lignocellulose than lignin removal.

3. CONCLUSIONS

The loose microstructure plays a more important role than lignin removal in enhancing the enzymatic saccharification of crop straws. Limited number of washings combined with pH adjustment is more suitable than several times washing for realizing maximum enzymatic hydrolysis of crop straws.

4. MATERIALS AND METHODS

4.1. Materials. CS and RS in particle size ≥ 0.42 mm were washed for three times to remove dust and then oven-dried at 60 °C to a constant weight. Cellulase powder of 161.25 FPU/g containing xylanase activity of 110 U/g was purchased from

Imperial Jade Bio-technology Co. Ltd. (China). Sodium hydroxide (NaOH) and glacial acetic acid are analytically pure.

4.2. Alkaline Pretreatment. The raw materials of CS and RS were added separately into 250 mL of conical flasks containing 50 mL of 2% NaOH solution. The solid-to-liquid ratio was 1:10. The flasks were placed into a water bath shaker setting at 40 or 80 °C and 150 rpm. After being shaken for 2 h, the solid residues were obtained through filtration. A part of the treated solid residues was washed with water at a solid-to-liquid ratio of 1:10 for seven times to make pH paper exhibit neutral color. Another part of the treated solid residues was washed for three times and then adjusted to pH 4.8 by glacial acetic acid. After solid–liquid separation, the wet solid residues were sealed to store at –20 °C for the subsequent enzymatic hydrolysis. The moisture contents of the wet solid residues were confirmed by the weight method of dry material subtraction from the wet material.

4.3. Enzymatic Hydrolysis. The wet alkali-treated solid residue containing 0.3 g of dry material was loaded into 25 mL conical flasks. According to the moisture content of the wet alkali-treated solid residue, 0.05 M acetate buffer (pH 4.8) was supplemented to form a solid–liquid ratio of 1:10. The enzymatic hydrolysis was performed at 50 °C, 150 rpm for 72 h with cellulase loading of 20 FPU/g cellulose.

4.4. Analytical Methods. The compositions of untreated and treated materials were analyzed according to the NREL protocol.¹² The WRVs of untreated and treated materials were measured by the TAPPI UM256 procedure. The cryo-SEM photographs of the longisection and transection of wet untreated and treated materials were captured with a cryo-scanning electron microscope (S4800, Hitachi) at an accelerating voltage of 2.0 kV. The sugar concentration was determined by the HPLC method.¹³ The conversion of glucan and hemicellulose to monosaccharides was calculated by the following equations.

$$\begin{aligned} \text{Glucan conversion} \\ = \frac{0.9 \times C_{\text{glucose}} V + 0.95 \times C_{\text{cellobiose}} V}{M_{\text{glucan}}} \times 100 \end{aligned} \quad (1)$$

$$\begin{aligned} \text{Hemicellulose conversion} \\ = \frac{0.88 \times C_{\text{xylose}} V + 0.88 \times C_{\text{arabinose}} V}{M_{\text{xylan}} + M_{\text{araban}}} \times 100 \end{aligned} \quad (2)$$

Where C_{glucose} , $C_{\text{cellobiose}}$, C_{xylose} , and $C_{\text{arabinose}}$ are the concentrations of glucose, cellobiose, xylose, and arabinose in the enzymatic hydrolysate (g/L), respectively, V is the volume of the enzymatic hydrolysate (L), M_{glucan} , M_{xylan} , and M_{araban} are the masses of glucan, xylan, and araban in the substrates (g), respectively, and the constants are the dehydration coefficients of glucose and cellobiose polymerized to glucan, and xylose and arabinose synthesized to xylan and araban, respectively.

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Notes

The authors declare no competing financial interest.

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