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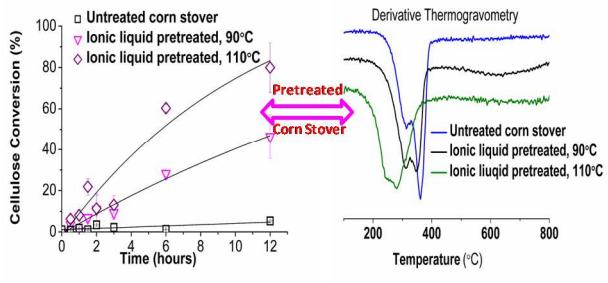

Effects of biomass pretreatment on the enzymatic hydrolysis and thermal degradation of corn stover were compared

A Comparative Study of Enzymatic Hydrolysis and Thermal Degradation of Corn Stover: Understanding Biomass Pretreatment

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Abstract

Impacts of ionic liquid and alkaline pretreatments on the enzymatic hydrolysis and thermal degradation of corn stover were investigated to better understand the mechanism of biomass pretreatment. Corn stover samples were pretreated at a 5 wt.% biomass loading in 1-butyl-3-methylimidazolium acetate ([C4mim][OAc]) at different temperatures varying from 50 to 110°C for 6 h. NaOH solutions of three different concentrations were used to pretreat the corn stover samples: 0.5 wt%, 1.0wt% and 4.0wt% at temperatures of 35 and 50°C for 6h. Different pretreatment conditions enabled various degree of alteration to their structures. Structural factors which led to enhanced enzymatic digestibility and to variations in thermal degradation behavior were compared and discussed. It was found that biomass pretreatment enhanced enzymatic digestibility of corn stover that was correlated with a drop in thermal degradation temperatures and increased amount of materials degrading at lower temperatures. Pretreatment did not always lead to a decrease in activation energy of the thermal degradation of biomass samples. The activation energy was mainly influenced by cellulose crystallinity. XRD analysis also indicated that [C4mim][OAc] interacted with (101) and $(10\bar{1})$ lattice plans of cellulose during the initial dissolution process.

Keywords: enzymatic hydrolysis; thermal stability; ionic liquid pretreatment; NaOH pretreatment; lignocellulosic biomass

1. Introduction

Lignocellulosic biomass is widely accepted as a renewable source of precursors and sugars which can be converted to chemicals and fuels via biochemical or thermo-chemical approaches.[1] Production of liquid fuels from lignocellulosic biomass via the biochemical conversion process often begins with a pretreatment step that is necessary to mitigate biomass recalcitrance to enzymatic hydrolysis.[2] Progress has been made on understanding the biomass pretreatment by analyzing changes in chemical composition and physical structures during pretreatment processes and their contributions to the enzymatic hydrolysis.[3] However, suitable pretreatment techniques have not been developed due to complex interactions between plant cell walls and pretreatment that occurs over many length scales.[1, 4] A better understanding of the mechanism of biomass pretreatment requires continuous improvement on biomass structural characterizations.[3, 5]

A number of studies have also shown promise in upgrading bio-oil produced via pyrolysis after physio-chemical pretreatments of biomass.[6-10] The thermal degradation and the pyrolysis kinetics of biomass are analyzed by thermogravimetric (TG) analysis and derivative thermogravimetry (DTG). More recently, there has been an increase in the number of studies utilizing thermogravimetry data to understand alterations in the biomass structures as a result of pretreatment.[11-18] Biomass components [19-21] show different thermal behaviors in TG and DTG data. It is reported that hemicelluose decomposes in the temperature range of 100-380°C, while cellulose decomposes in the range of 250-380°C. The degradation of lignin takes place over a

wide temperature range: 180-900°C.[22] Lignocellulosic biomass displays two distinct peaks in DTG curves;[12] the first peak is generated by degradation of hemicelluose and some of the lignin; the second peak corresponds to degradation of cellulose and some of the lignin. DTG data of xylan from beachwood demonstrated the side chains were less stable than that of main chains during ionic liquid (IL) pretreatment.[12] In an investigation of the mechanism of aqueous IL pretreatment of biomass samples, cellulose pretreated by neat 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_3$]) showed a significant reduction in thermal stability while the cellulose pretreated by aqueous [BMIM][$MeSO_3$] exhibited little change in thermal stability.[15] However, the cellulose pretreated by aqueous [BMIM][$MeSO_3$] showed better enzymatic hydrolysis efficiency than that pretreated by the neat [BMIM][$MeSO_3$].[15] This prompted further investigation which discovered that the aqueous [BMIM][$MeSO_3$] pretreatment led to a higher accessible surface area and more binding sites for cellulases.[15]

These studies suggest that TG–DTG data of pretreated biomass offer important complementary information of the structural changes that occurred as a result of the pretreatment. In this work, the impact of the IL pretreatment on the enzymatic hydrolysis and thermal degradation of corn stover was studied with an attempt to reveal common factors affecting both processes. NaOH pretreatment was also included to compare with that of the IL process. The characteristics of IL and NaOH pretreatments were summarized in a recent review.[2] TG–DTG data of pretreated corn stover were analyzed in conjunction with x-ray diffraction (XRD) and compositional

analysis. It was expected that this study will advance our understanding of biomass pretreatment/recalcitrance via evaluation of the impacts of biomass pretreatment by both enzymatic hydrolysis and thermal degradation. Additionally, an investigation on the impact of biomass pretreatment on the thermal behavior of biomass samples is also beneficial to the thermo-chemical conversion process.

2. Materials and Methods

2.1 Materials

Corn stover was harvested from a local farm of Yan Qing City of Beijing, China. Biomass samples were ground and sieved (-40/+60 cut). The size range of the obtained biomass particles is between 0.3-0.45mm. Non-structural materials were extracted with a Soxhlet extractor using water and ethanol for 12h, respectively. The ionic liquid 1-butyl-3-methylimidazolium acetate ([C4mim][OAc]) was purchased from Lanzhou Institute of Chemical Physics, China. Cellulase from *Trichoderma reesei* ATCC 26921 (activity≥1unit/mg solid provided by the manufacture, lot# C8546), Avicel PH101 (lot# BCBJ4592V), xylan from beechwood (lot#BCBH7762V), alkaline lignin (lot#MKBJ0452V) were purchased from Sigma–Aldrich. All other chemicals used in this study were also purchased from Sigma-Aldrich.

2.2. Compositional analysis of corn stover

Cellulose and xylan contents were determined by a two-step acid hydrolysis and subsequent HPLC analysis, based on the standard NREL procedure (NREL/TP-510-42618). The sugar composition of the hydolysates was determined by high performance liquid chromatography (HPLC) using a refractive index detector

(Hitachi, Tokyo, Japan). A Sugar-pak1 column (Waters, Milford, MA, USA) was used at 80°C with ultrapure water as the eluent at a flow rate of 0.5mL/min. The lignin content was determined with the acetyl bromide method using an averaged extinction coefficient of 17.747 L g^{-1} cm^{-1} for corn stover. [23] The percentage reported in Table 2 is based on dry matter.

2.3 IL pretreatment.

Corn stover samples were pretreated in [C4mim][OAc] at 5wt.%. A biomass solution was prepared by combining 0.5g of biomass with 9.5 g [C4mim][OAc] in a 50 mL glass centrifuge tube. The mixture was heated in an oil bath without stirring at different temperatures, 50, 70, 90 and 110ºC, for 6 h. After pretreatment, the pretreated biomass samples were washed at least four times with water. The recovered solids were lyophilized for 48h and then stored in a sealed plastic bag at 5ºC for analysis. The weight loss percentage of the corn stover samples after IL pretreatment was in the range from 20 to 30.

2.4 NaOH Pretreatment

Corn stover samples (0.5g) were mixed with 9.5 ml NaOH solution with different concentrations for 6 h. At 35ºC, NaOH solutions of three different concentrations were used to pretreat the corn stover samples: 0.5 wt%, 1.0wt% and 4.0wt%. At 50ºC, a 4.0wt% NaOH solution was used. After the pretreatment, the biomass samples were washed at least four times with water. The recovered solids were lyophilized for 48h and then stored in a sealed plastic bag at 5ºC for analysis. The weight loss percentage after 0.5 wt% and 1.0wt% NaOH pretreatment was in the range from 20 to 30. After

pretreatment by the 4.0wt% NaOH, it was from 40 to 50.

2.5 XRD measurement

Pretreated and untreated samples were analyzed by XRD. The samples were scanned on a D8 ADVANCE diffractometer equipped with a sealed tube Cu Kα source. The operating voltage and current were 40 kV and 40mA and the x-ray wavelength was 0.15406nm. Scans were collected from 2θ = 5 to 60º with step size of 0.03 at 4 s per step. A peak deconvolution method was used to extract the crystallinity. Built-in Gaussian functions in Origin 8 were used to fit the peaks. The obtained biomass crystallinity was normalized by cellulose content in each corn stover sample to get the cellulose crystallinity. To minimize the uncertainty introduced by biomass pretreatment, samples obtained from 3 parallel pretreatments under the same condition were mixed for the XRD measurement. To ensure the reproducibility of the XRD data, microcrystalline cellulose (Avicel PH101) was measured each time along with other biomass samples.

2.6 TG measurement

Thermal analysis was performed by using a thermogravimetric analyzer (DTG-60A, SHIMADZU). The temperature was calibrated with indium, zinc, aluminum and gold. Samples of 5 to 10mg placed in alumina crucibles (70μL) were heated from 50 to 800ºC at a rate of 10ºC/min in the presence of nitrogen (30ml/min). The Coats and Redfern method was used to extract the apparent activation energy (E_a) from the TG data in this study. Biomass pyrolysis was approximated as a one-step global reaction with a reaction order of one [24] : $\ln\left[\frac{-\log(1-\alpha)}{T^2}\right] = \ln\frac{AR}{\beta E_a}\left[1-\frac{2RT}{E_a}\right]-\frac{E_a}{RT}$

(1)

In equation 1, α is the degree of conversion and is given by $\alpha = \frac{w_t - w_0}{w_t - w_\infty}$, w_t, w_0, and w_∞ are the mass at the initiation of the degradation process, mass at temperature T and the final mass at a temperature at which the mass loss remains almost unchanged, respectively. The term A is the pre-exponential factor (1/s), E_a is the apparent activation energy (kJ/mol), R is the gas constant (8.314 J/mol K) and T is the absolute temperature (K). The plot of $\ln\left[\frac{-\log(1-\alpha)}{T^2}\right]$ vs. 1/T gives a straight line and the slope represents the value of $\frac{E_a}{R}$.

2.7 FTIR measurement

FTIR was recorded from a FTIR spectrophotometer (NICOLET 6700, Thermo, USA). The samples (KBr pellets) for analyses were prepared by mixing 20 mg material powder with 200 mg KBr. Thirty two scans were taken from 4000 to 400 cm^{-1}.

2.8. Enzymatic saccharification

Enzymatic hydrolysis reactions were performed in 15mL centrifuge tubes on a rotary shaker (Scientific Industries, INC., SI-1402) at 30 rpm and 50ºC in volumes of 10mL (citrate buffer, pH=4.8) with a cellulase concentration of 50 mg protein/g glucan. 50mg of corn stover was added to each tube. Solutions (50 μL) were periodically removed and immediately placed in a freezer (-20ºC) to quench the enzymatic hydrolysis. The total reducing sugar concentration of the supernatants was measured by the DNS assay against a glucose standard.

3 Results

Table 1 Influence of pretreatment on the main components of corn stover

	Xylan	Cellulose	Lignin	(xylan+cellulose)/lignin	(xylan+cellulose)/lignin from FTIR
untreated	24.8±1.6%	35.4±0.8%	19.7±1.5%	3.05	0.224
IL,50°C	21.4±0.9%	35.0±0.2%	18.6±1.7%	3.03	0.276
IL,70°C	21.0±0.6%	34.5±0.2%	17.0±1.1%	3.26	0.278
IL,90°C	20.9±0.6%	37.4±0.1%	15.8±0.1%	3.69	0.305
IL,110°C	13.9±0.2%	39.8±0.1%	13.5±0.6%	3.98	0.403
0.5% NaOH, 35°C	20.9±0.5%	38.3±0.1%	18.4±0.6%	3.21	0.296
1.0% NaOH, 35°C	20.3±1.0%	39.2±0.6%	17.4±1.1%	3.41	0.310
4.0% NaOH, 35°C	14.0±0.6%	50.3±1.2%	10.5±0.8%	6.09	0.379
4.0% NaOH, 50°C	12.8±0.7%	53.5±0.3%	9.7±0.7%	6.83	0.482

Table 1 presents the influence of IL and NaOH pretreatments on the composition of corn stover samples. More xylan and/or lignin were lost to the liquid phase than cellulose during the pretreatment, as reported before.[25, 26] An efficient pretreatment process preserves the hemicelluloses and cellulose for subsequent hydrolysis while removing the lignin from the biomass.[27] It is interesting to compare the ratio of the amount of xylan (a major biopolymer in hemicelluloses of corn stover[25]) and cellulose to that of lignin as a function of pretreatment condition.[25] As shown in Table 1, this ratio increases with increasing pretreatment severity for both processes. This ratio can also be derived from FTIR data by comparing the area of the peaks at 897 to that of 1510cm^{-1} (supporting information).[28, 29] Shown in Table 1, the ratio obtained from FTIR data is consistent with that of the compositional analysis. This also demonstrates that FTIR offers a convenient way of evaluating biomass pretreatment efficiency.[30]

Figure 1a and 1b present DTG curves of IL and NaOH pretreated samples, respectively. To better describe the data, the peak position on the DTG curves at lower temperature side is denoted as T_{maxL} and the peak position at higher temperature side is denoted as T_{maxH}. The ratio of the area of the peak at lower temperature side to the

sum of the areas of the peaks is named as ARL; and the ratio of the area of the peak at higher temperature side to sum of the areas of the peaks is named as ARH. For untreated biomass samples, the area of the lower temperature peak represents mainly the weight loss of hemicelluloses and the one at higher temperature corresponds to mainly the weight loss of cellulose. After the pretreatment, the components corresponding to the two degradation peaks become less well defined.[12, 13] As shown in Table 2, with the increase of pretreatment temperature from 50 to 90°C, the T_{maxH} decreases slightly. After pretreatment at 110°C, the T_{maxH} exhibits a significant decrease. Meanwhile, the T_{maxL} hardly varies with pretreatment temperature. A decrease in T_{maxH} indicates that thermally recalcitrant biomass is becoming easier to decompose [18]. Importantly, the ARL increases continually from 0.61 to 0.84 while the ARH decreases from 0.39 to 0.16. This suggests that the fraction of materials decomposing at lower temperatures increases as a result of the pretreatment. In comparison to the ARL and ARH, the variation of the thermal degradation temperature is not very sensitive to structural changes that occurred at temperatures lower than 90°C.

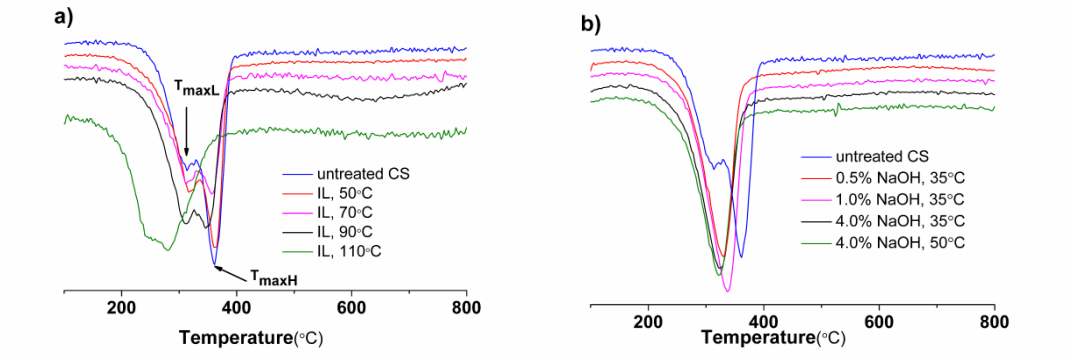

Figure 1. DTG curves of IL pretreated corn stover (a) and NaOH pretreated corn stover (b). The data are shifted vertically with respect to each other for the sake of clarity.

Table 2 Influence of pretreatment on the thermal stability of corn stover

	T_{maxL}(°C)	ARL	T_{maxH}(°C)	ARH
untreated	316±7.1	0.61±0.08	363±1.7	0.39±0.09
IL, 50°C	322±5.4	0.77±0.05	362±1.5	0.23±0.07
IL, 70°C	317±7.5	0.83±0.07	358±3.0	0.17±0.1
IL, 90°C	313±9.5	0.84±0.01	353±4.2	0.16±0.02
IL, 110°C	237±8.0	0.04±0.005	274±3.8	0.96±0.01
0.5% NaOH, 35°C	308±6.4	0.65±0.09	332±0.7	0.35±0.1
1.0% NaOH, 35°C	314±3.1	0.64±0.05	340±0.4	0.36±0.05
4.0% NaOH, 35°C	301±6.4	0.57±0.07	328±0.5	0.43±0.08
4.0% NaOH, 50°C	303±2.0	0.64±0.03	325±0.5	0.36±0.04

After IL pretreatment at 110°C, one peak dominates the DTG curve with a T_{maxH} of 274°C and a RAH of 0.96. These changes in the thermal degradation behavior of corn stover samples strongly suggest that IL pretreatment leads to degradation of biomass components and cellulose decrystallization (see Figure 2a). The impact of pretreatments on cellulose crystallinity will be discussed later.

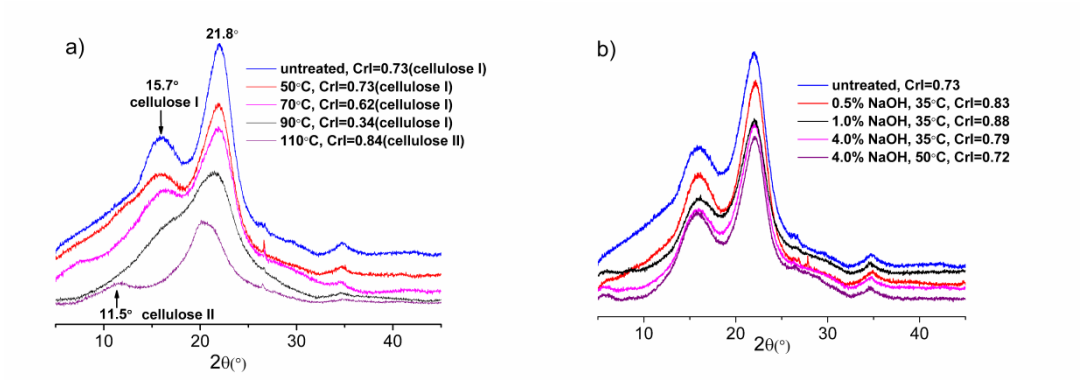

Figure 2. XRD curves of IL pretreated corn stover (a) and NaOH pretreated corn stover (b). The crystallinity index (CrI) refers to cellulose crystallinity.

After NaOH pretreatment, the DTG curves of all the samples show one peak. It

has been reported that removal of hemicelluloses transforms the two peaks on DTG curves into one peak.[13, 14, 17] However two peaks are still resolved using a peak fitting program (Origin 8) in current study. It is remarkable that there are significant drops in T_{maxH} after the NaOH pretreatment despite an increase in cellulose crystallinity (except for the sample pretreated in 4.0% NaOH at 50°C) (Figure 2b). The value of T_{maxL} also decreased modestly after the pretreatment. The ARL and ARH do not vary much from those of untreated sample. Possible reasons of the decrease in thermal degradation temperatures are discussed below. The NaOH pretreatment caused more breaking of the chemical linkages in biomass than that of the IL pretreatment, and the changes in interactions of cellulose with hemicelluloses and lignin affected their thermal behaviors.[21] A recent computer simulation work showed that hemicellulose branches of arabinose, glucuronic acid and glucuronate strengthen the primary cell wall by strongly coordinating to hydrogen bond donor sites on the cellulose surface.[31] Therefore the breaking of chemical linkages in plant cell walls most likely caused the changes in thermal degradation temperatures. It is reported that alkali pretreatment cleaves α and β-aryl ether bonds in lignin and removes acetyl group and various uronic acid substitutions in hemicelluloses.[26] The removal of acetyl group is demonstrated by FTIR data (supporting information) of all the NaOH pretreated samples.

Another important parameter derived from the TG data is the activation energy of the thermal degradation process. In this work, the average activation energy is obtained using the Coats and Redfern method assuming a reaction order of one.[24]

Figure 3 shows the Coats and Redfern plot used for the derivation of the average activation energy. The temperature range over which the activation energy was derived was from 250 to 370°C for untreated corn stover and IL pretreated ones at 50, 70 and 90°C. For IL pretreated one at 110°C, it was from 177 to 277°C. The temperature range was from 265 to 350°C for NaOH pretreated samples. The R^2 of fitting to a straight line is larger than 0.99 for all the samples. It is noted that these temperature ranges cover most of the weight loss peaks on the DTG curves. Therefore the activation energy obtained represents the weighted average of most of the cellulose and hemicelluloses and a fraction of lignin.[32] The activation energy of untreated corn stover is 60.8kJ/mol, which is close to a literature value, 66.5kJ/mol.[33]

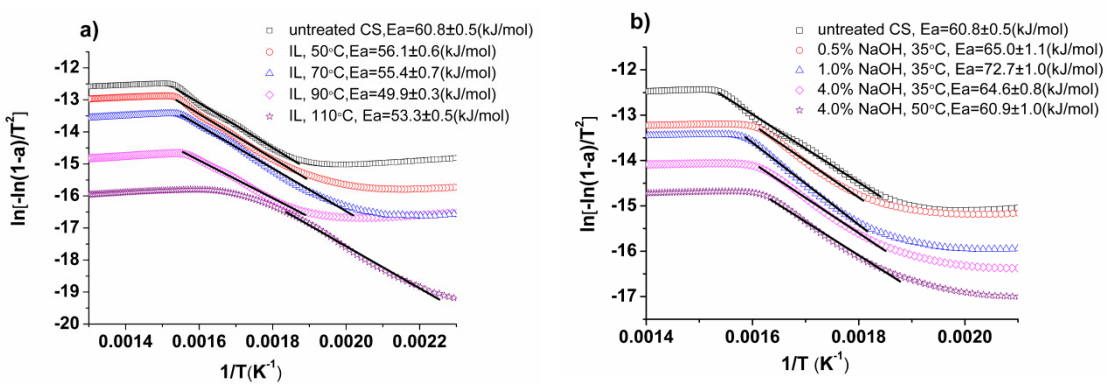

Figure 3. The Coats and Refern plot for derivation of apparent activation energy of IL pretreated corn stover (a) and NaOH pretreated corn stover (b). The continuous lines on the curves are a fit to a straight line. The data are shifted vertically with respect to each other for the sake of clarity.

With increasing the IL pretreatment temperature, the activation energy decreases, as shown in Figure 3a. However, it increases after IL pretreatment at 110°C. The values of the activation energy, lignin content, (cellulose+xylan)/lignin ratio as a functions of pretreatment conditions were listed in Table 3 to better understand which

factor affects the activation energy most. While it is reasonable to claim that the decrease in cellulose crystallinity and lignin content and the increase in (cellulose+xylan)/lignin ratio correlate with the decrease in activation energy; the increase in the activation energy after pretreatment at 110°C can only be explained by an increase in cellulose crystallinity. Figure 3b shows the activation energy of corn stover samples pretreated by NaOH. All the NaOH pretreated samples have higher or similar activation energy in comparison to the untreated sample. The higher activation energy is also correlated with higher crystallinity (Figure 2b). However its relations with lignin content and (cellulose+xylan)/lignin are opposite to those of IL pretreated samples. After NaOH pretreatment at 50°C, the activation energy decreases, this is correlated with a decrease in crystallinity (Figure 2b). The results suggest that the cellulose crystallinity outweighs the lignin content and (cellulose+xylan)/lignin ratio in determining the activation energy, i.e., it plays a major role in determining the activation energy of the corn stover samples.

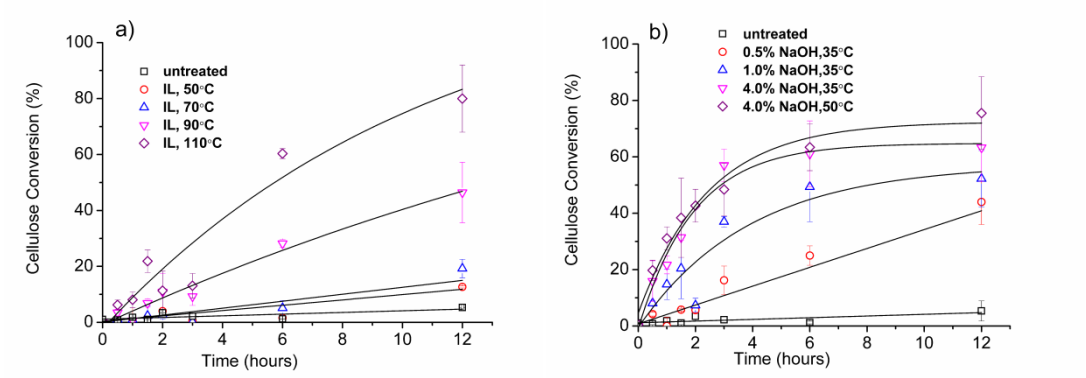

Figure 4. Sugar release obtained from corn stover samples pretreated by IL (a) and NaOH (b) over 12h saccharification. The continuous lines are a guide to the eye. The reducing sugar conversion was obtained by normalizing the measured glucose concentration with theoretical glucose concentration from cellulose.

Enzymatic saccharification was carried out on untreated and pretreated corm stover samples. Figure 4a presents the hydrolysis data of corn stover samples pretreated by IL and Figure 4b shows the data of NaOH pretreated samples. The sugar conversion increases only slightly for samples pretreated with IL at 50 and 70°C. This is consistent with small changes in biomass composition (Table 1) and in cellulose crystallinity (Figure 2a). After pretreatment at 90 and 110°C, the sugar conversion increases significantly as a result of delignification and variation in cellulose crystalline structure. As shown in Figure 2a, the cellulose crystallinity decreased from 0.73 to 0.34 after IL pretreatment at 90°C; it transformed into cellulose II structure after pretreatment at 110°C as indicated by the peak at around 12.0°.[34] A transformation into cellulose II structure suggests that native crystalline cellulose in corn stover was solubilized in IL at 110°C. At lower pretreatment temperatures, the decrease in crystallinity of cellulose I is due to swelling of cellulose with IL molecules.[35] It is interesting to note that with increasing pretreatment temperature, the broad peak at around 15.7°, which is a composite peak corresponding to (101) and $(10\bar{1})$ lattice plans,[36] on the XRD curves decreases in intensity while the main peak at around 21.8° corresponding to (002) plane remains less affected (Figure 2a). This indicates that IL preferentially attacks (101) and $(10\bar{1})$ lattice plans. This suggests a cellulose microfibril model which has a square cross section with (101) and $(10\bar{1})$ terminating surfaces.[37] It further indicates that the hydrogen-bonded sheets in cellulose I[36] persist while the originally ordered cellulose chains within these sheets are disturbed by IL molecules. It is expected that only the lamella structure remain

with further increase of pretreatment severity and finally amorphous cellulose should be obtained before a transformation into cellulose II. This requires proper manipulation of IL pretreatment conditions and they were not observed in this work. It is worth to mention that lamella structures were observed during formation of cellulose II crystals in water by computer simulation studies. [38]

The cellulose conversion reaches to about 80% after 12h enzymatic hydrolysis of the corn stover sample pretreated at 110°C for 6h. Similar conversion rate has been reported before for IL pretreated biomass samples.[28, 29] For NaOH pretreated samples, the cellulose conversion after 12h hydrolyses ranges from 40 to 80%, which is also consistent with literature data.[39] There are two major differences between IL and NaOH pretreated samples based on the data presented in this work. One is the content of acetyl groups. As mentioned above, the removal of acetyl group is demonstrated by FTIR data (supporting information) of all the NaOH pretreated samples. Studies have shown that removal of acetyl groups enhances enzymatic digestion.[40] In addition, the change in thermal degradation temperatures implied more breaking of the chemical linkages during NaOH pretreatment, which is consistent with the characteristics of alkaline pretreatment.[26] The second one is cellulose crystallinity. All of the samples keep native cellulose I structure with higher or similar cellulose crystallinity than that of untreated corn stover (Figure 2b). In contrast, IL pretreatment led to a decrease in cellulose crystallinity or a change in cellulose crystalline structure. The observed saccharification data are the combined effects of changes in chemical structures such as delignification, breakage of chemical bonds and in physical structures such as

decrystallization.

Table 3 Relations between activation energy, biomass components and enzymatic hydrolysis

	CrI	Lignin (%)	(xylan+cellulose)/ lignin	Activation Energy (kJ/mol)	Cellulose Conversion at 12h
untreated	0.73	19.7±1.5	3.05	60.8±0.5	0.053±0.01
IL, 50°C	0.73	18.6±1.7	3.03	56.1±0.6	0.13±0.003
IL, 70°C	0.62	17.0±1.1	3.26	55.4±0.7	0.19±0.03
IL, 90°C	0.34	15.8±0.1	3.69	49.9±0.3	0.46±0.11
IL, 110°C	0.84	13.5±0.6	3.98	53.3±0.5	0.81±0.15
0.5% NaOH, 35°C	0.83	18.4±0.6	3.21	65.0±1.1	0.44±0.08
1.0% NaOH, 35°C	0.88	17.4±1.1	3.41	72.7±1.0	0.52±0.10
4.0% NaOH, 35°C	0.79	10.5±0.8	6.09	64.6±0.8	0.63±0.12
4.0% NaOH, 50°C	0.72	9.7±0.7	6.83	60.9±1.0	0.76±0.13

4. Discussion

Biomass pretreatment causes changes in relative content and interactions (covalent and non covalent) of biomass components, which are lumped together and reflected on both the TG-DTG data and enzymatic hydrolysis. In current work, the thermal degradation of corn stover samples is analyzed in terms of thermal degradation temperature, percentages of materials within certain temperature range and the activation energy. After IL and NaOH pretreatments, corn stover samples' thermal degradation temperatures decreased and percentages of materials decomposed at lower temperature ranges increased, suggesting deconstruction of plant cell walls. This is correlated with improved enzymatic hydrolysis efficiency. NaOH pretreatment may have resulted in more breaking of chemical bonds in corn stover than that of IL pretreatment, and in the interactions of cellulose with hemicelluloses and lignin, which are manifested by the significant decrease in thermal degradation temperatures.

However, as shown in Table 3, the activation energy is not always correlated with the enzymatic digestibility. All the samples pretreated by NaOH solutions showed higher or similar activation energy than that of untreated one, although they became easier to digest by enzymes. After IL pretreatment at 50, 70 and 90°C, the activation energy decreases and this is positively correlated with enhanced enzymatic digestion. After IL pretreatment at 110°C, the sample's activation energy increases due to an increase in cellulose crystallinity (cellulose II). Cellulose II has higher enzymatic digestibility than that of cellulose I[41], therefore the corn stover sample pretreated at 110°C exhibits the highest digestibility although its activation energy is not the lowest. The activation energy is mainly influenced by cellulose crystallinity, which explains the relation between activation energy and enzymatic digestibility.

5. Conclusions.

This study shows that biomass pretreatment leads to enrichment of polysaccharides, breaking of chemical linkages and subsequently weakening of inter molecular interactions, decrystallization and transformation of cellulose crystalline structure, which result in an improved enzymatic hydrolysis and a decrease in the thermal degradation temperatures. The activation energy is correlated with enzymatic digestibility through cellulose crystallinity. DTG data indicates NaOH pretreatment may have resulted in more structural changes in corn stover than that of IL pretreatment. In addition, IL molecules come into contact with cellulose microfibrils through their (101) and $(10\bar{1})$ lattice plans, suggesting a cellulose microfibril model which has a square cross section with (101) and $(10\bar{1})$ terminating surfaces. This

comparative study show the impact of the pretreatment on the structure and interactions of biomass components through analysis of enzymatic hydrolysis and thermal degradation processes, the results will aid in better understanding of biomass pretreatment and in the further development of each conversion process.

Acknowledgements

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