Active Oxygen Pretreatment of Corn Stalk to Facilitate Biorefining: Structural Elucidation of Hemicelluloses in Yellow Liquor

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Biorefining is a potential pathway to cover the shortage of fuels, power, and chemicals from lignocellulosic biomass in the future. However, pretreatment of the biomass is recognized as a technological bottleneck for the cost-effective development of biorefineries, especially for the production of bio-fuels and chemicals. Active oxygen pretreatment is both an eco-friendly and efficient pretreatment process. To elucidate the effect of different chemicals on corn stalk and its hemicellulosic structure, five pretreatment processes were formed with MgO, H_2O_2, and O_2. Additionally, the MgO was also replaced by NaOH and $Mg(HO)_2$. Results show that MgO, which can be completely replaced by $Mg(OH)_2$, is an alkali source and a protective agent in preventing raw material from carbonizing and cellulose from degrading during pretreatment. High pressure oxygen is the main chemical for depolymerizing corn stalk. The removal degrees of lignin and hemicelluloses in the pretreatment processes with oxygen were 81.1 to 87.7% and 73.3 to 83.0%, respectively. Without oxygen, much lower removal degree were achieved (19.3 to 49.0% and 55.5 to 67.6%, respectively). Corn stalk hemicelluloses were composed of (1→4)-β-D-xylopyranose substituted with α-L-arabinofuranosyl residues and 4-*O*-methyl-α-D-glucuronic acid units. The molecular weight of hemicelluloses decreased from 22,000 g/mol to the range 3100 to 6400 g/mol.

Keywords: Active oxygen; Biomass pretreatment; Corn stalk; Hemicelluloses; Yellow liquor

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INTRODUCTION

Lignocellulosic biomass is mainly composed of three components: cellulose, lignin, and hemicelluloses. Deconstruction of lignocellulosic biomass into the three pure streams of chemical components is a promising alternative strategy in order to explore economically viable biorefining and avoid the cost restriction of single bioethanol production (Xu *et al.* 2014). Nevertheless, because of biomass' inherent recalcitrance, breakthrough technologies are still needed to overcome barriers for the development of cost-effective processes for converting biomass to fuels and chemicals. These needed

breakthroughs include improved pretreatment processes, low-cost cellulases, and microbial biocatalysts (Himmel 2008).

Pretreatment of lignocellulosic biomass has been considered as a technological bottleneck and is the "first-step" for the cost-effective development of bio-refineries (Vom Stein *et al.* 2011). Hemicelluloses and lignin can be removed from microfibrils in the pretreatment process, which can break down the macroscopic rigidity, decrease the physical barriers to mass transport, and boost the yield of fermentable sugars by exposing the crystalline cellulose core (Himmel *et al.* 2007). Previously, many pretreatment processes have been reported (Agbor *et al.* 2011), including eco-friendly green processing technology. This includes steam explosion (Wang *et al.* 2010), hydrothermal pretreatment (auto-hydrolysis) (Amendola *et al.* 2012), ionic liquids (Swatloski *et al.* 2002; Yuan *et al.* 2013), and wet oxidation (WO) (Klinke *et al.* 2002; Arvaniti *et al.* 2012).

WO has been reported to be a potentially effective pretreatment technique for turning lignocellulosic biomass into a hemicellulose fraction and a solid cellulose-rich fraction without generating potential inhibitors (Banerjee *et al.* 2009). This process is usually carried out under a high temperature (170 to 240 °C), oxygen pressure (0.8 to 3.2 MPa), and liquid-to-solid ratio (20 to 10:1, mL/g), in which oxygen or air is applied as an oxidative agent and Na_2CO_3 for the alkali source (Klinke *et al.* 2002; Arvaniti *et al.* 2012).

Currently, most of WO pretreatment processes focus on the versatility of cellulose for the production of biofuels (Martín *et al.* 2008; Banerjee *et al.* 2009; Arvaniti *et al.* 2012). However, less attention has been paid to the chemistry and application of hemicelluloses and lignin. The development of value-added products from the hemicelluloses and lignin can increase the opportunity for profitability in biorefining; however, their heterogeneity in composition and possible chemical changes during the pretreatment process impair their application as biopolymers. Consequently, examination of structure and chemical composition examinations is fundamental on a molecular-level for understanding how the various WO pretreatment processes affect the hemicelluloses and lignin. Furthermore, Na_2CO_3 in the pretreatment liquor is difficult to be recycled and may cause pollution.

An active oxygen pretreatment process was applied for the pretreatment of corn stalk to facilitate subsequent biorefining. Oxygen and peroxide were used as pretreatment chemicals (Shi *et al.* 2014). Slightly soluble MgO was applied as the alkali source. The previous study focused on the explanation of the structural characteristics of hemicelluloses during the pretreatment process in order to enhance biorefinery viability in the future (Shi *et al.* 2012, 2013; Xie *et al.* 2013). However, the role of chemicals in the pretreatment process and their effect on the corn stalk hemicellulosic structure was not completely clarified. Therefore, five pretreatment processes with MgO, H_2O_2, and O_2 were investigated in the work reported here. In addition, MgO was also replaced by the $Mg(HO)_2$ and NaOH. As a result, the main components and morphological characteristics of pretreatment corn stalk are described. In order to clarify the effects of various applied chemicals on the corn stalk hemicellulosic structure and to comprehensively understand the pretreatment mechanism, the hemicelluloses isolated from the corn stalk and different pretreatment processes yellow liquor are characterized by different methods.

EXPERIMENTAL

Materials

Corn stalk was supplied by China BBCA Group Corp., China. Magnesium oxide powder with a purity of over 98.0% was obtained from Tianjin Kermel Reagents Co. Ltd., China. All other reagents of analytical grade were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

Methods

Procedure of the active oxygen pretreatment processes

Seven pretreatment processes, which were formed with the combination of MgO (NaOH or $Mg(OH)_2$), H_2O_2, and O_2, were designed as follows; Process 1: $MgO/O_2/H_2O_2$; Process 2: MgO/O_2; Process 3: MgO/H_2O_2; Process 4: MgO; Process 5: $Mg(OH)_2/O_2/H_2O_2$; Process 6: $NaOH/O_2/H_2O_2$; Process 7: O_2/H_2O_2. For the pretreatment processes 50 g of corn stalk (oven-dried weight, o.d.) and the pretreatment chemicals (15% wt dosage of MgO, 3% wt dosage of H_2O_2, 15% wt dosage of NaOH, and 21.88% wt dosage of $Mg(OH)_2$, all values based on the o.d. of the corn stalk), were placed in a 2 L rotating stainless steel autoclave at a solid-to-water ratio of 1:6 (g/mL). After being sealed, the autoclave was filled with O_2 at 1.0 MPa. The pretreatment always was performed at 165 °C for 2 h. At the end of the pretreatments, the yellow liquor was separated from the raw stock and stored in a refrigerator. The resulting pulps were washed with 1000 mL deionized water three times and then air-dried. In Process 7, the raw material was carbonized; therefore, the pulps are not discussed in the current study.

Main components of the raw material and pulp

The main components of the corn stalk and of the air-dried pulp were evaluated according to the authors' previous work (Yang *et al.* 2012). The operations are given as follows; the acid insoluble Klason lignin was determined by a two-stage hydrolysis procedure (72% H_2SO_4/20 °C and 3% H_2SO_4/reflux). Lignin was left as insoluble residue recovered by filtration. The hydrolysis solution from the Klason lignin assay was determined by a spectrophotometer at 205 nm with an absorptivity of 110 L/g•cm for calculating the content of water-soluble lignin. The total amount of lignin was the sum of these two parts. The determination of holocellulose was according to Wises's sodium chlorite method. For cellulose determination, Kurschner-Hoffer's nitric acid method was used. The hemicellulose content was obtained by subtracting the cellulose content from the holocellulose content. The loss degree of celluloses, hemicelluloses, and lignin were calculated according to Eq. 1,

$$\text{Loss degree of main component (\%)} = 100\% - \frac{Y * C_P}{C_R} \quad (1)$$

where Y is the yield of the pulp at different pretreatment processes (%), C_P is the summed up content of celluloses, hemicelluloses, and lignin in the pulp obtained from different pretreatment processes (%), and C_R is the content of celluloses, hemicelluloses, and lignin in the raw material (%).

Isolation of hemicelluloses from the raw material and yellow liquor

Hemicelluloses in the raw material were extracted first with hot water and then

with 1% KOH, sequentially. In the first step, the ball-milled sample was suspended in water with a solid-to-liquid ratio (g/mL) of 1:20 at 55 °C for 3 h. The resulting mixture was centrifuged at 5000 rpm for 10 min. The supernatant was concentrated to 50 mL at a reduced pressure and poured into three volumes of 95% ethanol. The precipitated hemicelluloses were centrifuged, washed with 70% ethanol, and freeze-dried. This residue was then extracted in a second step at 50 °C for 3 h using 1% KOH with a solid-to-liquid ratio (g/mL) of 1:20. The resulting mixture was centrifuged at 5000 rpm for 10 min, and the pH of the supernatant was adjusted to approximately 5.5 with HCl. The rest of the process occurred according to the above description. The hemicelluloses extracted with hot water and alkali from raw material were named RW and RA, respectively.

The hemicelluloses in the yellow liquor could be directly precipitated by ethanol using the following procedure: 50 mL centrifuged yellow liquor was poured into three volumes of 95% ethanol; after centrifugation, the precipitated hemicelluloses were washed with 70% ethanol and freeze-dried. The pH value of pretreatment liquor from Process 6 was adjusted to 7.0 with HCl before further analysis, as outlined above. The hemicelluloses obtained from the yellow liquor were called Y-1, Y-2, Y-3, Y-4, Y-5, and Y-6.

Monosaccharides and uronic acids in hemicelluloses

The monosaccharides and uronic acids in the hemicelluloses were liberated by hydrolyzing an approximately 10 mg sample. This was done using 5.5 mL of 6.5% H_2SO_4 for 2.5 h at 105 °C according to the previous reported paper (Zhang *et al.* 2011). The hydrolyzates were diluted to 50-fold (after adjusting the pH value to 7.0), filtered, and injected into the high-performance anion-exchange chromatography (HPAEC) (Dionex ICS-3 000, USA). The HPAEC was completed with pulsed amperometric detection, a CarboPac^{TM} PA20 column (3×150 mm), and a CarboPac^{TM} Guard column (3×30 mm). The operations were run at 30 °C for 30 min and with the flow rate during the analysis process being 0.5 mL/min. For monosaccharide the eluent was 2 mM NaOH and 100 mM NaAc and 2 mM NaOH for the uronic acids.

Molecular weight analysis of hemicelluloses

The molecular weights of the hemicelluloses were determined by GPC. The GPC system comprised a Waters 1525 binary HPLC pump, a Waters 717 plus Auto-sampler, a Waters 2414 refractive index detector, and a Breeze (V3.3) GPC work station (Waters, USA). The samples were dissolved in the eluent and injected into the TSk-GELG-5000PW xL column (7.8×300 mm) and TSk-GELG-3000 PW xL column (7.8×300 mm) (TOSOH, Japan) in a series. The eluent flow (0.02 M pH 6.0 KH_2PO_4) was 0.6 mL/min. The GPC columns and injection system were maintained at 35 °C during the analysis. Various fractions of Pullulan were applied as the standards for molecular weight calibration.

FT-IR analysis of hemicellulose

FT-IR spectra of the hemicelluloses were obtained on a FT-IR spectrophotometer (Bruker Tensor 27, Germany). The samples were combined with KBr in slices containing 1% hemicelluloses. Thirty-two scans were taken for each sample recorded from 4000 to 400 cm^{-1} at a 2 cm^{-1} resolution in the transmission mode.

1H NMR analysis of hemicellulose

The ^{1}H NMR spectra of the hemicelluloses (80 mg in 1 mL D_2O) were obtained on the Bruker AV 600 instrument. The proton spectrum was recorded at 25 °C after 16 scans at 600 MHz. The acquisition time was 2.6477 s. The relaxation delay time was 1.00 s and the pulse width was 12.1 μs.

RESULTS AND DISCUSSION

Morphologic Characteristics of Corn Stalk under Active Oxygen Pretreatment Processes

Figure 1 show photographs of corn stalk as raw material and pulp originating from the different active oxygen pretreatment processes. Obviously, the corn stalk in processes without oxygen (Processes 3 and 4) was not treated well. Even the morphology of the corn stalk barely changed in Process 4. In the processes with oxygen (Processes 1 and 2) corn stalk was completely disintegrated into single fibers.

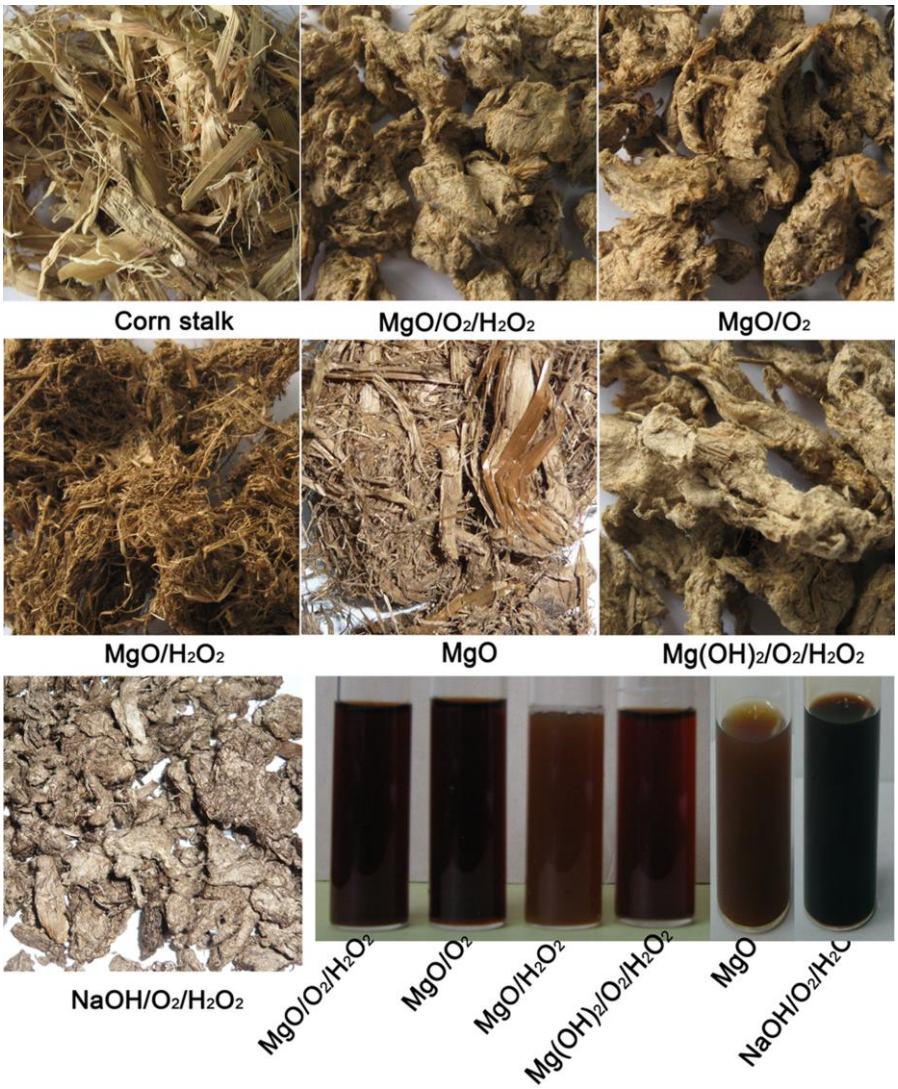

Fig. 1. Photographs of corn stalk and pulp from different pretreatment processes

Comparing Processes 1, 2, and 3, it can be seen that the morphological features of pulp from Processes 1 and 2 were similar; however, the differences between Processes 1 and 3 were obvious. These findings may indicate that oxygen plays a key role in the pretreatment process. When the MgO in Process 1 was replaced by $Mg(OH)_2$ (Process 5) and NaOH (Process 6), the corn stalk also can be disintegrated into single fibers, which meant that $Mg(OH)_2$ and NaOH played a similar role as MgO for the alkali source in the pretreatment process. The pictures of the various yellow liquor are also shown in Fig. 1. The colour of yellow liquor from the pretreatment processes with oxygen is deeper than that without oxygen.

The Effect of Active Oxygen Pretreatment Processes on the Main Components of Corn Stalk

The main components and yields of pulp from different active oxygen pretreatments are listed in Table 1. Processes 3 (MgO/H_2O_2) and 4 (MgO) achieved high yield, but had low removal degree of lignin when compared to Processes 1 ($MgO/O_2/H_2O_2$) and 2 (MgO/O_2). The above fact implies that both peroxide and MgO have an inferior ability of delignification in comparison with oxygen under the conditions of testing. The removal degree of lignin in Process 2 (MgO/O_2) was lower by 2.7% (as absolute percentage of composition) than that in Process 1 ($MgO/O_2/H_2O_2$), which suggested that high pressure oxygen plays a vital function for delignification, but the effect of oxygen in the air was very small for the delignification. Additionally, the removal degree of lignin in Process 3 (49.0%) and Processes 1 and 2 (85.5% and 82.8%, respectively) revealed that the synergistic benefits of peroxide and oxygen were limited from the point of view of delignification. It is very difficult for molecular oxygen to directly react with the solid raw material. Therefore, oxygen must be dissolved in the water, then formed in different active ions under the pretreatment conditions, which can break the cell wall matrix and remove the lignin and hemicelluloses.

Table 1. Main Components (%) Corn Stalk and Pulp from Different Active Oxygen Pretreatment Processes, and Their Loss Degree (%) During Active Oxygen Pretreatment Processes

Pretreatment Process		Yield of Pulp	Lignin			Holocellulose		
			Total Lignin	Klason Lignin	Acid soluble Lignin	Total Holocellulose	Cellulose	Hemicelluloses
-	Raw material	-	21.7	17.1	4.6	63.1	39.1	24.0
Process 1	$MgO/H_2O_2/O_2$	50.2	6.3(85.5)[a]	5.9	0.4	81.7	69.0(11.5)	12.7(73.4)
Process 2	MgO/O_2	52.2	7.2(82.8)	6.2	1.0	77.6	69.8(6.8)	7.8(83.1)
Process 3	MgO/H_2O_2	70.7	15.7(49.0)	14.8	0.9	66.1	55.0(0.6)	11.0(67.6)
Process 4	MgO	74.2	23.6(19.3)	22.5	1.1	61.2	46.8(11.3)	14.4(55.5)
Process 5	$Mg(OH)_2/H_2O_2/O_2$	52.2	4.1(87.7)	4.2	0.9	82.5	74.1(1.1)	8.3(81.8)
Process 6	$NaOH/H_2O_2/O_2$	40.4	10.2(81.1)	9.4	0.8	82.1	73.0(24.7)	10.9(81.7)
Process 7	H_2O_2/O_2 [b]	-	-	-	-	-	-	-

[a] Data in the bracket showed the loss degree of cellulose, hemicelluloses, and lignin, respectively.
[b] Data short for carbonization during the process.

The rate and quantity of mass transfer of oxygen are the major barriers during the pretreatment processes. High pressure oxygen can provide enough oxygen active ions as well as be the driving force for entering and breaking linkages in the cell wall. Hemicelluloses are amorphous, heterogenous, and branched polysaccharides of low molecular weight and can be easily extracted by hot water. The removal degree was 56% in Process 4 and 68% in Process 3, even without oxygen. In other pretreatment processes with oxygen, the removal of hemicelluloses was accelerated strongly. And the removal degree was 73.4 to 83.1%.

In comparison with Process 1, the higher removal degree of lignin and hemicelluloses in Process 5 ($Mg(OH)_2/H_2O_2/O_2$) indicates that $Mg(OH)_2$ can play the same role as the MgO. The higher removal degree of lignin and hemicelluloses is also given for Process 6 ($NaOH/H_2O_2/O_2$), but the high loss degree of cellulose (24.7%) and low yield of pretreated fiber (40.4%) indicated the serious degradation of cellulose. On the other hand, MgO can protect the cellulose from degradation. Carbonization appeared in the Process 7, caused by short of the alkali source provided by the MgO, $Mg(OH)_2$, or NaOH. Thus, another role of MgO is to prevent the raw material from carbonization.

Contents of Neutral Monosaccharides and Uronic Acids in Hemicellulose

To analyze the effect of active oxygen pretreatment processes on the hemicellulosic structures, the contents of neutral monosaccharides and uronic acid were investigated (Table 2). Xylose residual as monomeric component of backbone of xylans was the predominant sugar residual in the RW and RA. Additionally, noticeable amounts of arabinose (14.6%) were detected. However, hemicelluloses extracted with hot water contained more glucose (20.2%) and galactose (10.0%) residual than the hemicellulosic fraction extracted with alkali from raw material. Those data indicated that high-branched galactoarabinoxylans and some amounts of α-glucan were extracted by hot water from raw material (Peng *et al.* 2010a). Additionally, minor amounts of mannose (0 to 4.0%) and uronic acids (2.4 to 2.9%) residual, mainly glucuronic acid or 4-*O*-methyl-glucuronic acid, were also found in RW and RA.

The composition and content of monosaccharides in the hemicelluloses obtained from the yellow liquor was similar with the RA. Xylose residual (54.5 to 68.5%) was the dominant component in all of the hemicelluloses. Small amounts of arabinose (11.3 to 22.6%), galactose (7.5 to 11.9%), and glucose (5.8 to 7.7%) residual were detected. Additionally, there was minor mannose (2.0 to 2.8%) and uronic acid (3.0 to 3.5%) residual in all hemicelluloses. The ratio of xylose to arabinose (Xyl/Ara) is indicative of the degree of linearity or branching of hemicelluloses (Yuan *et al.* 2013). The lower ratio indicated a higher degree of branching of the xylan chains and also a higher solubility of the polymers (Sun *et al.* 2004).

In comparison to the process without oxygen, the higher Xyl/Ara value in hemicelluloses from yellow liquor with the oxygen process suggested that the oxygen can remove the side-chain from the backbone of hemicelluloses. Additionally, the Xyl/Ara value of Y-6 was higher than that of hemicelluloses obtained from the pretreament with oxygen. NaOH alone can be applied as the cooking chemical in soda pulping and efficiently breaks lingkages in cell walls and removes hemicelluloses and lignin. Therefore, the linkages between arabinose residuels and the main chain of hemicelluloses can be easily broken.

Table 2. The Monosaccharide Composition and Uronic Acid Content (%, W/W) of the Hemicelluloses Fractions from Corn Stalk, and Yellow Liquor of Different Active Oxygen Pretreatment Processes

Pretreatment Process	Hemi-celluloses	Neutral sugars and uronic acids						
		Arabinose	Galactose	Glucose	Xylose	Mannose	Uronic acid	Xyl/-Ara
Hot water	RW	14.6	10.0	20.2	48.3	4.0	2.9	3.3
Alkali	RA	14.6	3.8	6.3	73.0	Nd	2.4	5.0
$MgO/H_2O_2/O_2$	Y-1	15.7	8.5	6.8	63.6	2.1	3.3	4.1
MgO/O_2	Y-2	14.4	7.6	6.0	66.6	2.1	3.4	4.6
MgO/H_2O_2	Y-3	22.6	11.9	5.8	54.5	2.3	3.0	2.4
MgO	Y-4	19.7	10.1	7.4	56.8	2.8	3.3	2.9
$Mg(OH)_2/H_2O_2/O_2$	Y-5	14.3	7.5	5.2	67.5	2.0	3.5	4.7
$NaOH/H_2O_2/O_2$	Y-6	11.3	7.5	7.7	68.5	2.5	2.5	6.1

Average Molecular Weight of Hemicelluloses

The changes of molecular weight can show the effect of active oxygen on the hemicellulosic backbone during the pretreatment process. The weight-average molecular weights (M_W), number-average molecular weights (M_N), and polydispersities (M_W/M_N) of the hemicelluloses from the corn stalk and yellow liquor at different pretreatment processes are listed in Table 3. The M_W of hemicelluloses obtained from raw material with hot water and alkali was 20,089 and 22,755 g/mol, respectively. However, the M_W of hemicelluloses obtained from the yellow liquor (3,165 to 6,409 g/mol) was much lower than that of the raw material. Previous studies have shown that most of the lignin and hemicelluloses were removed during the heating-up period; also, the hemicelluloses were gradually degraded at the temperature' period (Shi *et al.* 2014). Subsequently, all of the hemicelluloses were seriously degraded during the pretreatment process. In comparison to the oxygen processes, the hemicelluloses obtained from yellow liquor of the pretreatment without oxygen (Process 3 and 4) showed lower M_W (3300 to 4300 g/mol). NaOH can break the backbones of hemicelluloses, and very low molecular weight (3100 g/mol) was obtained in the Process 6. The low index of polydispersity of hemicelluloses (1.2 to 1.8) illuminated the structural homogeneity of hemicelluloses in the yellow liquor.

Table 3. Weight-Average Molecular Weights (M_W), Number-Average Molecular Weights (M_N)(g/mol), and Polydispersity (M_W/M_N) of the Hemicelluloses from Corn Stalk and Yellow Liquor of Different Active Oxygen Pretreatment Processes

Pretreatment Process	Hemicelluloses	M_W	M_N	M_W/M_N
Hot water	RW	20 089	13 711	1.465
Alkali	RA	22 755	14 608	1.558
$MgO/H_2O_2/O_2$	Y-1	5 198	3 307	1.572
MgO/O_2	Y-2	5 010	3 219	1.556
MgO/H_2O_2	Y-3	4 314	2 682	1.609
MgO	Y-4	3 328	2 747	1.212
$Mg(OH)_2/H_2O_2/O_2$	Y-5	6 409	3 603	1.779
$NaOH/H_2O_2/O_2$	Y-6	3 165	1 760	1.798

FT-IR of Hemicelluloses

The FT-IR spectra of hemicelluloses obtained from corn stalk and the yellow liqour extracted are shown in Fig. 2. The similar profiles and relative intensities of most bands indicated a similar chemical structure. The absorbances of -OH stretching vibration appeared at about 3400 cm^{-1} (not shown in the spectra). Peaks at 2925 and 2873 cm^{-1} (not shown in the spectra) were attributed to the C-H stretching from the methylene and methyl units, respectively (Peng *et al.* 2012a). The bands at 1731 and 1248 cm^{-1} indicated the presence of acetyl or uronic ester groups in RW (Jin *et al.* 2009). The absence of those absorptions in the rest of hemicellulosic fractions implied that ester groups were completely cleaved during the pretreatment. The absorbances of absorbed water in the hemicellulose were at about 1647 cm^{-1} (Sun *et al.* 1998). However, the peaks of absorbed water drifting to low wavenumbers in Y-1, Y-2, and Y-5 were caused by the overlap of the $-COO^-$ groups and antisymmetric and symmetric stretching at 1607 and l419, respectively. This indicated that the hemicelluloses were oxidized in the process (Xu *et al.* 2013). After the pretreatment, the lignin was removed from the raw material. Therefore, the bands at 1508, 1460, and 1382 cm^{-1}, which were attributed to aromatic skeletal vibrations, $-CH_2$ symmetric bending, C-H asymmetric vibration, and C-H bending vibration of $-CH_3$ of lignin, respectively, were absent in the Y-1, Y-2, and Y-5 specimens.

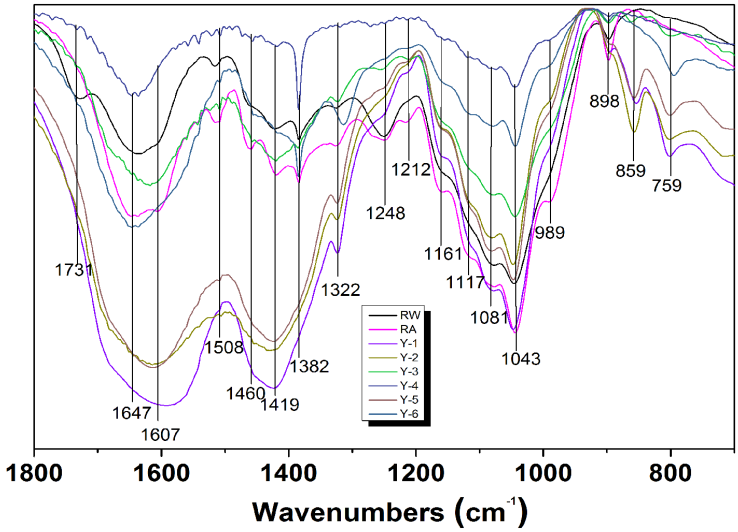

Fig. 2. The FT-IR spectra of the hemicelluloses obtained from corn stalk and yellow liquor

The peaks in the range 1200 to 800 cm^{-1} were the typical of xylans substituted by the arabinose. The strong peaks at 1043 cm^{-1} were assigned to the stretching and bending vibrations of C-O, C-C, C-OH, and glycosidic C-O-C. This indicated dominant xylans (Sun *et al.* 2012). The weak bands at 1161 cm^{-1} arose from C-O and C-O-C stretching with some contribution of -OH bending in arabinoxylans. The ring vibrations at 1081 and 989 cm^{-1} were affected by the degree of branching and hydration (Peng *et al.* 2010b). The C-C stretching gave the absorption at 1117 cm^{-1}. The sharps bands in RA and RW at 898 cm^{-1} were the glycosidic C_1-H deformation mode with ring vibration contributed to the *β*-anomer form of the pyranoid ring, which were characteristic of *β*-glycosidic linkages between xylose units of hemicelluloses (Sun *et al.* 2011). The signals at 859 and 759 cm^{-1} were indicative of α-glycosidic linkages, which showed that arabinose, glucose, and glucuronic acid were most likely linked by the α-glycosidic bonds. However, the

reduction of *β*-glycosidic linkage intensities and the increase of α-glycosidic linkage intensities of hemicelluloses from the yellow liquor, indicated that their backbones were seriously broken during the pretreatment process and that the hemicelluloses were high-side chain, which is consistent with the above analysis.

NMR Analysis

The ^{1}H NMR spectra of hemicelluloses from raw material and yellow liquor are shown in Fig. 3. In the RA spectrum, the relevant signals of the hemicellulosic fraction occurred in three regions, namely, the anomeric region (4.9 to 5.6 ppm for α-anomers and 4.3 to 4.9 ppm for β-anomers), ring proton region (4.5 to 3.0 ppm), and aliphatic region (3.0 to 0.5 ppm) (Wen *et al.* 2010).

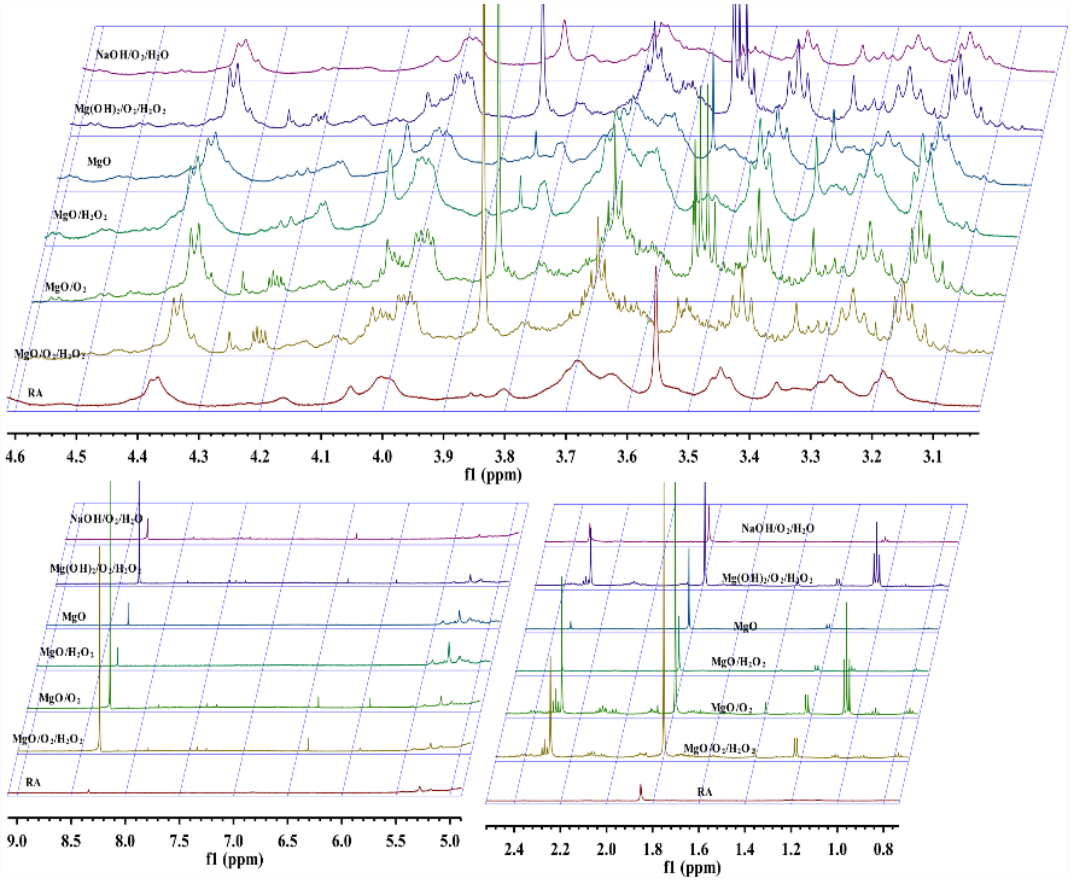

Fig. 3. NMR spectra of hemicelluloses from raw material and yellow liquor

In the anomeric region, the signals at 5.28 and 5.17 ppm were due to anomeric protons of L-arabinfuranosyl and 4-*O*-methyl-D-glucuronic acid residues that were linked to xylopyranosyl residues. The signals at 4.53, 4.42, and 4.37 ppm were indicative of anomeric protons of β-D-xylopyranosyl residues substituted at both C-2 and C-3 (di-substituted), only C-3 (mono-substituted), and unsubstituted residues, respectively (Peng *et al.* 2012b; Peng *et al*. 2012c). The other main chemical shifts of the H-5 equatorial, H-4, H-3, H-5 axial, and H-2 protons of the β-D-xylopyranosyl units in RA were detected at 4.00, 3.68, 3.45, 3.27, and 3.18 ppm, respectively. The chemical shifts at 4.16, 4.05, 3.80, 3.70, and 3.63 ppm were assigned to the H-4, H-3, H-2, H-5 equatorial, and H-5 axial protons of the a-L-arabinofuranosyl residue, respectively. The weak signals that arose

from the 4-*O*-methyl-D-glucuronic acid at 3.36, 8.42, 3.53, 3.12, 3.84, and 4.28 ppm were attributed to the proton of the methoxy groups, carboxy groups, and H-2, H-3, H-4, and H-5 protons, respectively. This was in good agreement with the sugar analysis (Wen *et al*. 2010). From the above analysis, it can be concluded that the hemicelluloses were composed of (1→4)-β-D-xylopyranose substituted with α-L-arabinofuranosyl residues and 4-*O*-methyl-α-D-glucuronic acid units.

From the spectra of hemicelluloses from yellow liquor, the main structure of hemicelluloses can also be defined as xylans. However, more signals appeared in the α-anomeric region of Y-3 and Y-4, indicating that the hemicelluloses from the yellow liquor of pretreatment without oxygen were highly branched. In contrast, the less and weak peaks in the α-anomeric region means that the hemicelluloses from the pretreatment process with oxygen were low-branching. The above conclusions are in agreement with the neutral monosaccharides analysis. In spectra Y-1, Y-2, and Y-5, the strong peaks at 8.42 and 3.84 indicated that the hemicelluloses from the pretreatment with oxygen were oxidized, which is consistent with the results of FR-IR spectra. In the aliphatic region, the peaks in 1.7-2.1 ppm were assigned to the acetate methyl groups; additionally, the peaks between 0.7 and 1.7 ppm were attributed to the protons of $-CH_3$ and $-CH_2-$.

CONCLUSIONS

1. MgO, which can be completely replaced by $Mg(OH)_2$, is an alkali source and a protective agent, which prevented the raw material from carbonization and cellulose from degrading during the pretreatment process.
2. Both high-pressure oxygen and peroxide are the main chemicals for the removal of lignin and hemicelluloses under pretreatment conditions. However, the former plays a vital function for the removal of lignin and hemicelluloses. Also, the synergistic benefits between oxygen and peroxide are limited.
3. The removal degrees of lignin and hemicelluloses in the pretreatment processes with oxygen (Processes 1, 2, and 5) were 81.1 to 87.7% and 73.4 to 83.1%, respectively. However, the corresponding values in the processes without oxygen (Process 3 and 4) were 19.3 to 49.0% and 55.5 to 67.6%, respectively. In the process with NaOH replaced by MgO, there was a highly degraded degree for cellulose.
4. Corn stalk hemicelluloses were composed of (1→4)-β-D-xylopyranose substituted with α-L-arabinofuranosyl residues, and 4-O-methyl-α-D-glucuronic acid units. The hemicelluloses in the yellow liquor were seriously degraded during the pretreatment oxidized in processes with oxygen (Processes 1, 2, and 5). The side chain of hemicelluloses was degraded in the processes with oxygen.

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