SEPARATION, PURIFICATION AND CHARACTERIZATION OF CORN STOVER HEMICELLULOSES

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Hemicelluloses were extracted from corn stover with potassium hydroxide and were further delignified with hydrogen peroxide. Physico-chemical properties, including chemical structure, composition, molecular weight and thermal stability, of the extracted hemicelluloses were investigated. As expected, xylan was found to occupy over 85.2% of the total hemicellulose, while the content of arabinan was of about 9.0%. Galactan and glucan were also detected in minor amounts. NMR and FT-IR analyses suggested that, by its structure, corn stover hemicellulose is an arabinoxylan consisting of (1→4)-β-D-xylan backbone substituted in O-2/O-3 by α-L-arabinose residue. After delignification, the lignin content remaining in hemicelluloses decreased from 4.6% to 1.5%. Ultrastructures analyzed by AFM revealed purified hemicelluloses as 40-nm diameter spheres connected to each other.

Keywords: hemicelluloses, corn stover, separation, HSQC NMR, AFM

INTRODUCTION

In recent years, considerable attention has been given to sustainable resources for the production of clean, renewable fuels and energy, due to the awareness of environmental risks of fossil fuels.[1,2] Lignocellulosic biomass, especially agricultural and forestry residues, has become the main focus of the biorefinery industry due to its abundant reserves and low price.[3] Cellulose, hemicelluloses and lignin are three main components of lignocellulosic biomass, in which hemicelluloses account for 20%-35% of the dry mass. Hemicelluloses are a class of alkali soluble amorphous heteropolysaccharides including hexosan (galactose, mannose, glucose) and pentosan (xylose, arabinose), which are often considered as the second most abundant polysaccharides in nature after cellulose.[4,5] Due to their excellent biocompatibility, biodegradability and bioactivity, hemicelluloses have been used for the preparation of biochemical, such as furfural, xylitol, acetone, and biopolymers.[6] They can also be used to produce films and hydrogels, which have potential application in tissue engineering.[7,8]

Fractionation of hemicelluloses is either necessary or helpful for full utilization of lignocellulosic biomass.[9-11] Generally, the methods for hemicellulose isolation can be divided into four categories: physical (mechanical comminution, pyrolysis), physico-chemical (steam explosion, microwave irradiation, autohydrolysis, *etc*.), chemical (alkali, dilute acid, oxidizing agents, organic solvents, *etc*.) and biological approaches.[12-15] Alkali treatment using NaOH, KOH or $Ca(OH)_2$ is the most frequently adopted chemical method. During alkali treatment, hydrogen bonds between cellulose and hemicelluloses are weakened, intermolecular ester bonds between lignin and hemicelluloses are saponified, which allows the dissolution of hemicelluloses and generally limits their degradation. Up to now, considerable studies have been done on alkali isolation and characterization of hemicelluloses.[16,17] However, hemicelluloses obtained from alkali treatment usually contain certain amounts of lignin. This associated lignin reduces the solubility and affects the applications of hemicelluloses, thus, it is of great importance to remove this part of lignin as much as possible without much degradation of hemicelluloses.[17] Hydrogen peroxide posttreatment is usually used to remove the aromatic residues and finally promote the quality of hemicelluloses products. Nevertheless, up to now,

the effects of hydrogen peroxide delignification on structural and morphological characteristics of hemicelluloses are not clear yet.

Corn stover, an agricultural by-product, is one of the most promising lignocellulosic raw materials for the production of bioenergy and biochemicals. However, most of it is underutilized up to now. In this study, hemicelluloses are extracted from corn stover with alkali. Since the potential applications of hemicelluloses highly depend on their composition and purity, we used alkaline H_2O_2 for further delignification. The effects of H_2O_2 treatment on e chemical composition, molecular weight, thermal stability and ultrastructure were investigated.

EXPERIMENTAL

Materials

Corn stover was collected from Shandong province, China. The materials were cut into 2-cm long pieces, depithed, washed and air-dried. The chemical composition of corn stover was determined previously[18] as follows: glucan 50.7 wt%, xylan 18.1 wt%, arabinan 2.2%, Klason lignin 15.5 wt%, acid soluble lignin 1.4 wt%, benzene-alcohol extractives 7.4 wt%, and ash 0.5 wt%.

Methods

Alkaline treatment

Fractionation of hemicelluloses was carried out in a 15 L electrothermal rotation digester (ZQS1 type, Machinery Factory of Shaanxi University of Technology). Corn stover (300 g, o.d.) was treated with 80 g/L potassium hydroxide at 75 °C for 2 h. The liquor-to-solid ratio was 4:1 (v/w). After the treatment, the extractives were collected, acidified to pH 5.5~6.0 and concentrated in a rotary evaporator. The crude hemicelluloses were then obtained by precipitating the extractives in absolute ethanol. After centrifugation, the precipitate was washed 3 times with 70% acidified ethanol (v/v). Hemicelluloses (H_1) were collected after freeze-drying.

Purification

Purification was carried out by stirring 1 g of crude hemicelluloses (H_1) in 20 mL alkaline H_2O_2 solution (10 g/L, pH 10.5) at 60 °C for 2 h. After H_2O_2 treatment, the purified hemicelluloses (H_2) were precipitated with absolute ethanol and freeze-dried.

Chemical composition

The identification of the sugar compositions of H_1 and H_2 was carried out by high performance liquid chromatography (HPLC) using a two-step acidic hydrolysis. Hemicelluloses were first pre-hydrolyzed by 72% (w/w) sulfuric acid at 20 °C for 1 h, followed by 4% sulfuric acid (w/w) catalyzed hydrolysis at 120 °C for 1 h.[19] The resulting solutions were diluted with ultra-pure water to the desired concentration and filtered through a 0.2 μm nylon filter before chromatography analysis. A Dionex ICS 3000 IC system equipped with a CarboPac PA 20 cartridge and an ED 50 detector was used for sugar detection. The lignin content of H_1 and H_2 was detected as Klason lignin according to TAPPI 222 om-02.[20] Residual lignin in hemicelluloses was determined using an Agilent 8453 UV spectrophotometer. 0.1 g of hemicellulose (H_1 and H_2) was dissolved in 100 mL NaOH solution (0.1 M) with the aid of ultrasonic treatment. The as-prepared solution was then centrifuged at 4000 r/min for 10 min. The supernant was collected for UV determination. NaOH solution (0.1 M) was used as a blank sample.

Molecular mass

Molecular weight was determined in an HPLC (Waters Ltd, US) system equipped with TSK-GEL G5000PWxl (7.8×300 mm) and TSK-GEL G3000PWxl (7.8×300 mm) linked in series to each other. The column temperature was maintained at 35 °C. A Waters 2414 refractive index detector was used for monitoring the peaks. The eluent system was 0.02 M KH_2PO_4 at a flow rate of 0.6 mL/min. The sample injection volume was 20 μL. Aqueous solutions of dextran standards (1 mg/mL) with molecular weights of 5200-668,000 g/mol were used as standard. Before analysis, hemicellulose samples were dissolved in 0.02 M KH_2PO_4 solution to a concentration of 1~2 mg/mL and filtered through a 0.45 μm nylon syringe filter.

NMR analysis

^1H NMR, ^{13}C NMR and HSQC 2D NMR spectra of H_1 and H_2 were obtained using a Bruck-DRX 400 NMR spectrometer at 298K. DMSO-d6 was used as solvent. FT-IR analysis was carried out with a Vector 33 spectrometer (Bruker, Germany), using a KBr disc containing 1% finely ground samples.

Thermal characterization

Thermogravimetric analysis was performed to understand the degradation characteristics of H_1 and H_2 using

a Q 500 series thermogravimetric analyzer (TA Instruments, USA). 10 mg (o.d.) samples were heated in a platinum crucible from ambient temperature to 650 °C at a heating rate of 10 °C/min^{-1}. Nitrogen was used as purge gas, and a positive pressure was maintained through the weighing chamber.

Ultrastructure analysis

Hemicellulose samples H_1 and H_2 were dissolved in deionized water to a concentration of 0.1 mg/L. After centrifuging at 10000 r/min for 3 min, the surpernant (10 μL) was dropped on freshly cleaved mica (1 cm × 1 cm). Then the mica was air-dried and sticked on a piece of stainless steel plate (Φ 1.0 cm). Samples were examined in a Multimode IIIa (Veeco, US) AFM in the tapping mode. The scanning rate was 1 Hz. All reported data were averaged from triplicate measurements.

RESULTS AND DISCUSSION

Composition of hemicelluloses

The chemical compositions of H_1 and H_2 are given in Table 1. Obviously, xylan is the predominant constituent in both H_1 and H_2, accounting for over 85.2% (mass fraction) in H_1. Arabinan (9.0%) and galactan (2.4%) were present in small amounts, while glucan was observed as a rather minor constituent. After washing repeatedly with 70% ethanol, there was still 4.6% Klason lignin remaining in H_1.

Owing to the delignification by hydrogen peroxide, the content of Klason lignin in H_2 was reduced to 1.1%. The proportion of xylan and arabinan increased from 85.2% and 9.0% to 89.5% and 9.6%, respectively. Galactan was further degraded or dissolved during alkaline peroxide purification. Negligible variation occurred in acid soluble lignin.

Ultraviolet spectra

The UV-Vis absorption spectra of H_1 and H_2 are shown in Figure 1. For the spectrum of H_1, there is an absorption peak at 280 nm, which is attributed to the dissociation of phenolic groups in alkaline solution. After the treatment by alkaline peroxide, the absorption peak at 280 nm (Fig. 1, H_2) almost disappeared. These results, together with the information in Table 1, suggest that alkaline peroxide had strong oxidizing capacities to break the linkages between lignin and hemicelluloses. Although not completely removed, hydrogen peroxide minimized the content of such residual lignin, which might exist in the form of lignin-carbohydrate complex (LCC), thus residual lignin was further dissolved.

Table 1
Chemical compositions of H_1 and H_2 (based on freeze-dried samples, wt%)

	Xylan	Arabinan	Galactan	Glucan	Mannan	Klason lignin	Acid soluble lignin
H_1	85.2	9.0	2.4	0.7	-	4.6	0.5
H_2	89.5	9.6	2.1	0.7	-	1.1	0.4

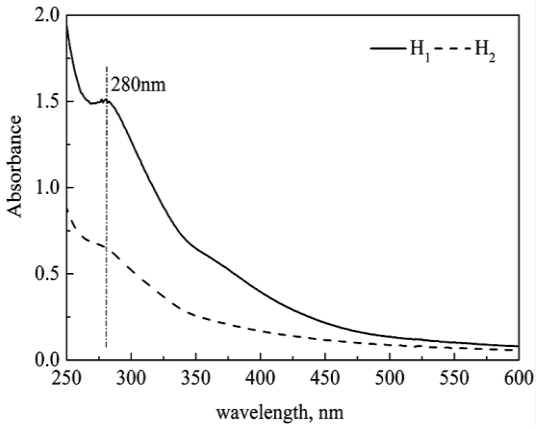

Figure 1: UV-Vis absorption spectra of H_1 and H_2

Table 2
Molecular weights and polydispersity ($\overline{Mw}/\overline{Mn}$) of H_1 and H_2

	H_1	H_2
Weight average ($\overline{Mw}$)	56431	52283
Number average ($\overline{Mn}$)	46911	45721
$\overline{Mw}/\overline{Mn}$ polydispersity	1.2	1.1

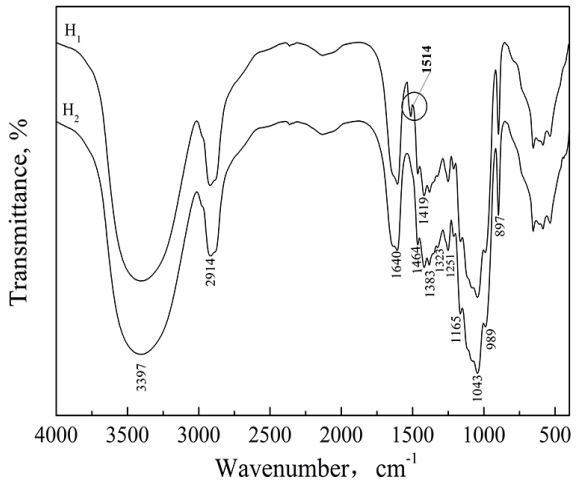

Figure 2: FT-IR spectra of H_1 and H_2

Molecular mass

The molecular weights of H_1 and H_2 are presented in Table 2. As can be seen, corn stover hemicelluloses had a high molecular weight, as compared with those isolated from other non-wood materials, such as wheat straw and sugarcane bagasse, under similar experimental conditions.[21,22] Besides, the polydispersities of both H_1 and H_2 were rather small, which suggested low-molecular-weight sugars were largely removed during the repeated ethanol washing and precipitation. Furthermore, it can also be concluded from Table 2 that hydrogen peroxide did not significantly affect the molecular weight under the conditions used.

FT-IR spectra

FT-IR spectra of H_1 and H_2 (Fig. 2) show a typical signal pattern of non-wood hemicelluloses. The prominent band around 3400 cm^{-1} represents the hydroxyl stretching vibrations of the hemicelluloses and water involved in hydrogen bonding. C-H stretching vibrations give a signal at 2914 cm^{-1}.[23] From the absence of the absorption at 1739 cm^{-1} in both spectra, it can be inferred that most of the acetyl or uronic acid groups were removed during the alkali treatment.[24] The band around 1640 cm^{-1} is attributed to the absorbed water.[25] Bands due to $-CH_2$ stretching vibrations were observed at 1464 and 1419 cm^{-1}. The absorption at 1383, 1323, and 1251 cm^{-1} are originated from C–H, OH, or CH_2 bending, corresponding to the C–O–C vibration in hemicelluloses.[26] The characteristic wavenumber for the distinction of typical xylan is 1043 cm^{-1}, which is assigned to the C-O and C-C stretching and the glycosidic linkage (C-O-C).[25] The presence of the arabinosyl side chains is documented by the two low-intensity shoulders at 1165 and 989 cm^{-1}, which has been reported to attach only at positions of the xylopyranosyl constituents.[26] The obvious absorption at 897 cm^{-1} corresponding to the C1 group frequency or ring frequency, is the characteristic peak of β-glycosidic linkages in sugar units. These results suggest the backbone of corn stover hemicelluloses is a β-linked xylose unit.[27]

There is no significant difference between H_1 and H_2, except a weak band at 1514 cm^{-1}. This band in H_1 is due to the aromatic skeleton vibrations in lignin, which confirms that hemicelluloses obtained by alkali extraction are connected with a small amount of lignin.[28] After the H_2O_2 treatment, this band disappeared, supporting that most of the associated lignin was removed.

NMR analysis

The structural features of the purified hemicelluloses sample, H_2, were studied using NMR. ^{1}H NMR together with HSQC show a typical signal pattern expected for hemicelluloses (Figs. 3 and 4). The 1→4 linked β-D-xylopyranosyl units were indicated by the signals at δ_C/δ_H 101.76/4.24 (C1-H), 72.62/3.03 (C2-H), 74.01/3.24 (C3-H), 5.41/3.49 (C4-H), 63.26/3.85, 3.15 (C5-H2) in the HSQC NMR spectrum. In Figure 3, the two chemical shifts at 3.85, 3.15 were assigned to the equatorial and axial protons linked at C5, respectively. The strong signal at 2.48 was attributed to the solvent, DMSO-d6. HDO in DMSO-d6 gave a signal at 3.3 in ^{1}H NMR spectrum, this signal was overlapped by the signal of C3-H. The anomeric protons of arabinofuranose linked to O-2 and O-3 of xylan were indicated by two weak signals at 5.32 and 5.26, respectively.[27] Chemical shifts at 5.10 and 4.98 were attributed to the anomeric protons of the reducing end terminal xylose and glucuronic acid, as well as hydroxyl groups.[29]

^{13}C NMR was used to confirm the structural features of the purified hemicellulose sample, H_2. As can be seen in Figure 5, five strong signals at 107.24, 83.7, 77.5, 75.7, and 57.8 were assigned to C-1, C-4, C-2, C-3, and C-5 of 1→4 linked β-D-xylan.[30] The signals at 107.20, 86.05, 80.26, 77.80 and 61.90 were originated from C-1, C-4, C-2, C-3 and C-5 of α-L-arabinofuranose linked to the β-D-xylose unit. Two signals at 76.2 and 69.4 were related to C-3 and C-5 of α-L-arabinofuranosyl residues (1→3) linked to β-D-xylan. The carbonyl resonances from uronic acids contributed to a weak signal at 173.09, which indicated C-6 in methyl uronic acids. Signal at 58.85 was due to 4-O-methyl residues of 4-O-methyl glucuronic acid at the side chains.[31] NMR spectra did not show signals related to acetyl groups. As reported, acetyl groups and a small amount of uronic acids were accessible to the action of strong alkali.[32] Therefore, most of the acetyl groups and uronic acids are expected to be removed during alkali extraction.

Thermal stability

Thermal gravimetric analysis (TGA) of H_1 and H_2 was performed and the results are shown in Figure 6. There seems to be no difference in the TGA graph between the two samples. The mass loss below 100 °C was due to moisture loss in both H_1 and H_2. The TGA curves showed a prominent effect at 190-350 °C with a maximum around 300 °C, representing 63.5% of the total weight loss. This part of weight loss was attributed to decarboxylation, dehydration and oxidation of carbohydrates and of the less condensed structures of the lignin macromolecules.[33] At a comparatively higher temperature, around 650 °C, a moderate weight loss (11.6% of H_1, 9.6% of H_2) was attributed to the destruction of the most resistant macromolecular moieties, such as condensed lignin.

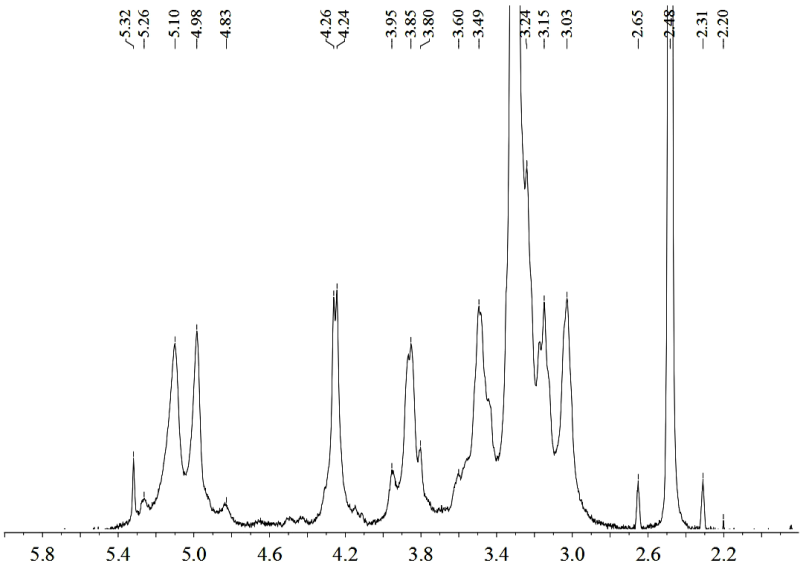

Figure 3: ^{1}H NMR spectrum of H_2

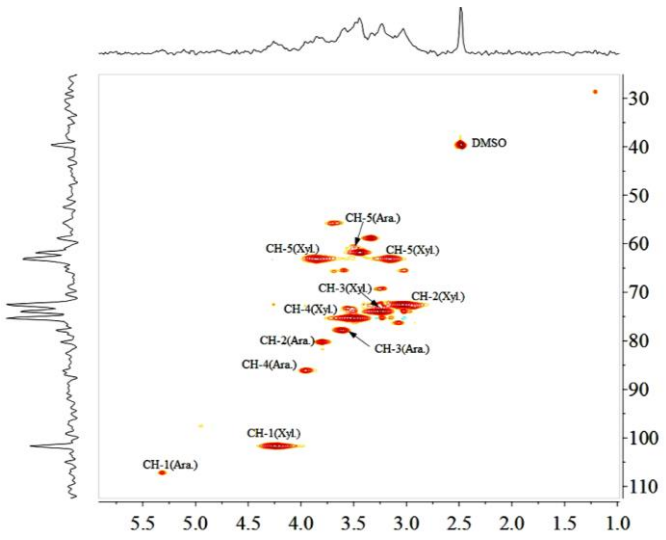

Figure 4: HSQC NMR spectrum of H_2

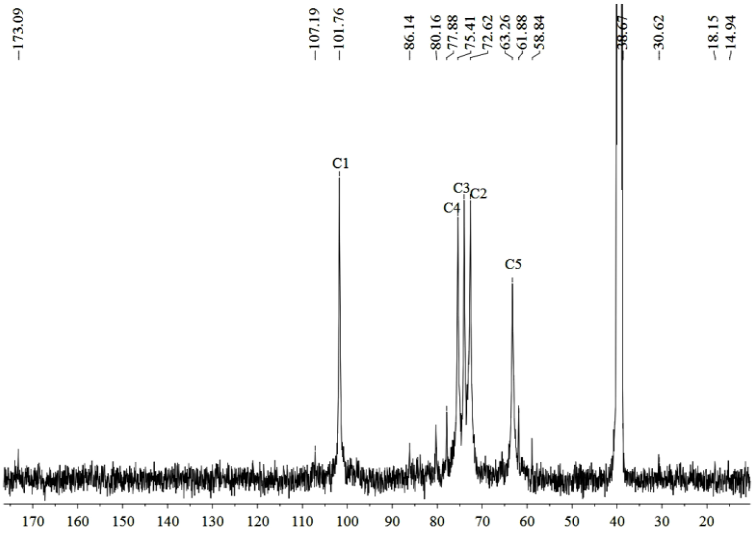

Figure 5: ^{13}C NMR spectrum of H_2

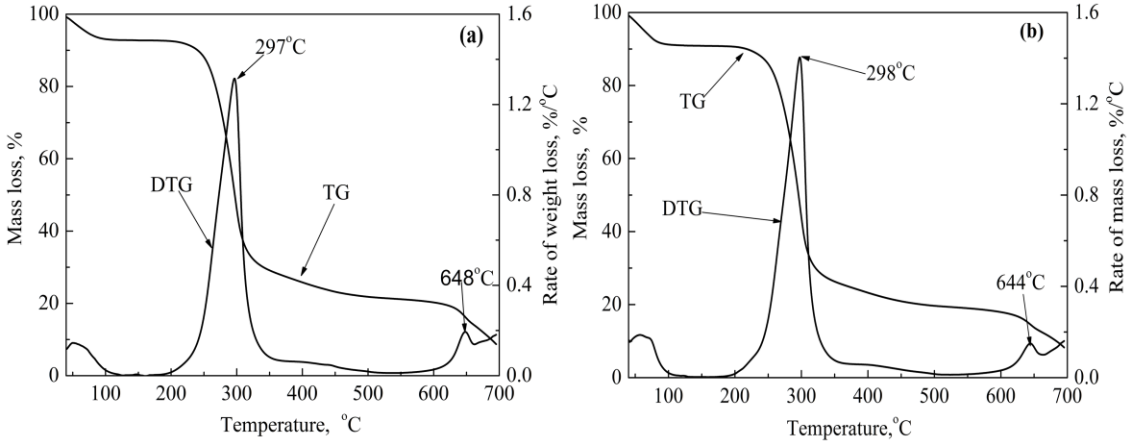

Figure 6: TGA and corresponding DTG curves of H_1 (a) and H_2 (b)

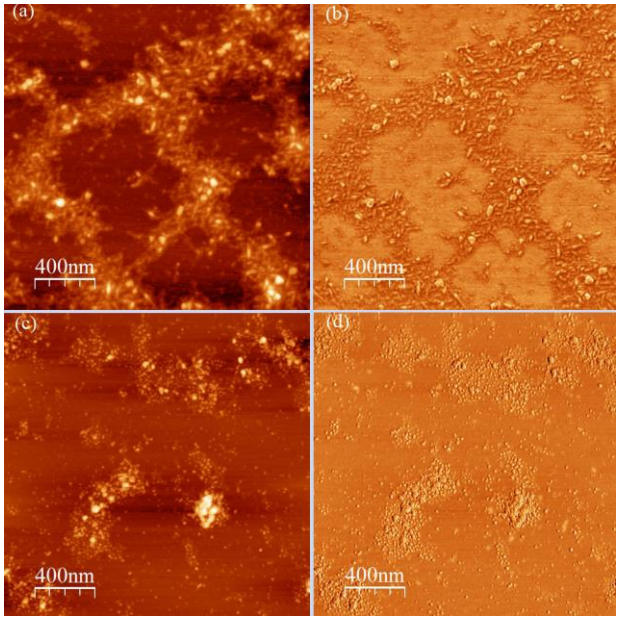

Figure 7: AFM images of H_1 and H_2 under tapping mode scanning (size: 2 μm × 2 μm, Z scale: 35 nm); (a), (c) height image of H_1 and H_2; (b), (d) phase image of H_1 and H_2

Atomic force microscopy

Morphological features of H_1 and H_2 were characterized by using tapping mode AFM, and the results are shown in Figure 7. Obviously, crude hemicellulose particles (H_1) with more lignin residues tended to aggregate, which was due to the hydrophobic interactions of lignin. Individual particles of H_1 had horizontal sizes of 40-60 nm and vertical sizes of 2-5 nm. On the contrary, H_2 particles dispersed well on the substrate without large aggregates. Individual H_2 particles were of spherical shape and could be attached one by one. The size of most particles was within 40 nm.

CONCLUSION

Hemicelluloses were extracted from corn stover and further purified with hydrogen proxide. After purification, the content of xylan and arabinan increased from 85.2% and 9.0% in H_1 to 89.5% and 9.59% in H_2, respectively, while the content of residual Klason lignin reduced from 4.6% in H_1 to 1.1% in H_2. GPC analysis showed the average molecular weight of H_1 and H_2 was 56431 g/mol, and 52283 g/mol, respectively. The molecular weight distribution was quite narrow in both samples. FT-IR together with NMR revealed the structure of purified corn stover hemicelluloses, which was an arabinoxylan with (1→4)-β-D-xylan as backbone and α-L-arabinose residue substituted in O-2 and/or O-3. The acetyl groups were removed during alkali extraction. The efficient decomposition temperature of hemicelluloses is 190-350 °C. TG and corresponding DTG curves of H_2 were similar to those of H_1, as confirmed by thermal analysis. Ultrastructure study by AFM suggested that crude hemicelluloses were aggregated spheres with sizes of 40-60 nm, while for purified hemicelluloses most particles were 40-nm diameter spheres attached together.

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