

Physicochemical Characterization of Rice Straw Pretreated with Sodium Hydroxide in the Solid State for Enhancing Biogas Production

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Received February 10, 2008. Revised Manuscript Received March 29, 2008

The biogas yield of rice straw during anaerobic digestion can be substantially increased through solid-state sodium hydroxide (NaOH) pretreatment. This study was conducted to explore the mechanisms of biogas yield enhancement. The chemical compositions of the pretreated rice straw were first analyzed. Fourier transform infrared (FTIR), hydrogen-1 nuclear magnetic resonance spectroscopy (¹H NMR), X-ray diffraction (XRD), and gas permeation chromatography (GPC) were then used to investigate the changes of chemical structures and physical characteristics of lignin, hemicellulose, and cellulose. The results showed that the biogas yield of 6% NaOH-treated rice straw was increased by 27.3–64.5%. The enhancement of the biogas yield was attributed to the improvement of biodegradability of the rice straw through NaOH pretreatment. Degradation of 16.4% cellulose, 36.8% hemicellulose, and 28.4% lignin was observed, while water-soluble substances were increased by 122.5%. The ester bond of lignin–carbohydrate complexes (LCCs) was destroyed through the hydrolysis reaction, releasing more cellulose for biogas production. The linkages of interunits and the functional groups of lignin, cellulose, and hemicellulose were either broken down or destroyed, leading to significant changes of chemical structures. The original lignin with a large molecular weight and three-dimensional network structure became one with a small molecular weight and linear structure after NaOH pretreatment. The cellulosic crystal style was not obviously changed, but the crystallinity of cellulose increased. The changes of chemical compositions, chemical structures, and physical characteristics made rice straw become more available and biodegradable and thus were responsible for the enhancement of the biogas yield.

1. Introduction

Rice is one of the major crops of central and southern China, and 230 million tons of rice straws are generated annually.¹ Although there are a few methods available for rice straw reuse, such as animal feed, fuels for cooking, house heating, and paper-making, a significant amount of rice straw remains unused and burned in open fields, causing serious environmental and safety problems, such as air pollution and fire disaster. On the other hand, rice straw is an organic material and can be used to produce biogas through anaerobic digestion, thus providing an alternative for rice straw use and mitigating the pollution.

Anaerobic technology has been widely applied for conversion of various organic wastes, such as sewage, animal manure, food wastes, and municipal solid wastes, to biogas.^{2–4} During the process, the biomass is transformed into biogas, a mixture of methane and carbon dioxide, which is a clean fuel for energy generation. However, very few attempts have been made to investigate the potential of using rice straw as the sole feedstock

to produce biogas. The main reason is that rice straw contains a high percentage of polysaccharides and lignin. Cellulose, a semicrystalline biopolymer with ordered crystalline and disordered amorphous regions, is a linear polymer of anhydroglucose units linked at the first and fourth carbon atom by a β -glycoside bond.⁵ Hemicellulose is a large group of polysaccharides found in the primary and secondary cell walls. Lignin is a polymer of phenylpropane units, which form a three-dimensional network inside the cell wall. The major interunit linkage is an aryl–aryl ether type. Moreover, lignin has association with other cell-wall polysaccharides. The former two components are hydrophilic, and the latter is hydrophobic. They are sparingly insoluble in water and partially in organic solvent because of the hydrogen bonds between polysaccharides and adhesion of lignin to the polysaccharides. Such complex structures make rice straw hard to be biodegraded and used by anaerobic microorganisms and cause low digestion rate and biogas production.

Pretreatment prior to anaerobic digestion has been proven to be one of simple and effective methods to improve biodegradability and biogas production of lignocellulosic materials.^{6–8} The physical structures and chemical compositions of lignocellulosic

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materials could be altered through various pretreatments, making the compositions in lignocellulosic materials more accessible and more readily biodegradable to anaerobic microorganisms, thus increasing digestion efficiency and biogas production. Various physical, chemical, and biological pretreatment methods, such as size reduction, steam explosion, ammonization, and fungi biodegradation, have been investigated.^{9,10} Alkali pretreatment has been proven to be a promising one,^{11–13} which has practical advantages when such pretreatment is to be followed by anaerobic digestion. During the process of alkaline treatment, degradability remains mainly in the solid phase and the subsequent solids separation is easy. Additionally, any alkali remaining with the treated solids is useful in subsequent digestion, because anaerobic treatment of lignocellulosic materials generally requires alkalinity addition for pH control. Thus, alkaline treatment is more compatible with subsequent anaerobic digestion than other treatments. The most commonly used alkali is sodium hydroxide. Wu et al.¹⁴ reported that the biogas yield of wheat straw could be increased by 38–119% through anaerobic digestion after pretreated by NaOH solution. Dar and Tandon¹⁵ found that the addition of the wheat straw pretreated by 1% NaOH for 7 days to cattle dung improved microbial digestibility and biodegradability during anaerobic fermentation at ambient temperature.

However, currently, most alkali pretreatments use a large amount of chemical solution and water to soak substrates, which need recycling of chemicals, disposal of waste solution, and sometimes high temperature, and thus, could result in high facility investment, high treatment cost, and potential environmental pollution.^{14,16,17} In our study, NaOH was applied as a chemical reagent for rice straw pretreatment in the solid state and at ambient temperature. A limited amount of water was used to maintain rice straw in the saturated state but no extra water existing inside the material. This is why the process was called “solid state”. The pretreatment process was operated at ambient temperature all the time without any extra energy input. This could bring a number of advantages over conventional chemical solution pretreatment, such as less water used, no waste solution generated, no waste solution treatment and disposal needed, and no energy input required. Our studies have proven that NaOH pretreatment could improve biodegradability and enhance biogas production significantly.¹⁸ However, the mechanisms for such improvement are yet unclear. This study was conducted to explore the reasons for the changes of chemical compositions, chemical structures, and physical characteristics during NaOH pretreatment and analyze the effects of such changes on biodegradation and biogas production of rice straw.

2. Experimental Section

2.1. Solid-State NaOH Pretreatment. The rice straw used in this study was collected from Tong County of Beijing City, China.

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The rice straw was chopped by a paper chopper (PC500, Staida Co., Tianjing, China) and then ground into 5–10 mm particles by a hammer mill (FE130, Staida Co., Tianjing, China). Solid NaOH of 6% of the dry rice straw was used in the pretreatment in 10 L-beakers. The amount of NaOH amount was determined in our previous study and considered to be the optimal dose.¹⁹ First, 100 g of dry rice straw was placed into each beaker, and then 6 g of NaOH was added into each beaker, followed by the addition of 80 g of distilled water to make the moisture content at 80% on a dry basis. This moisture content was determined by previous tests conducted in our laboratory, which showed that such moisture content could maintain rice straw in the saturated state, while no extra water existed in the material, providing minimal water just enough for chemical reaction. No waste chemical solution was generated in the pretreatment process; hence, no waste solution treatment and disposal were needed. This would avoid possible pollution associated with chemical pretreatment as well as the costs for the treatment and disposal of the waste chemical solution. Then, all of the prepared beakers were covered with plastic films, closed with plastic rings, and placed in the laboratory at ambient temperature (20 ± 2 °C) for 3 weeks. By the end of the chemical pretreatment, the NaOH-pretreated rice straw was dried in an oven and then kept in a refrigerator for chemical analyses and anaerobic digestion experiments.

2.2. Anaerobic Digestion. The untreated (raw) and NaOH-treated rice straws were digested in batch anaerobic digesters. The volume of each digester was 2 L, with a working volume of 1.5 L. Four loading rates (LRs) of 35, 50, 65, and 80 g L⁻¹ were applied for both untreated and 6% NaOH-treated rice straws. Each digester was seeded with the activated sludge taken from a mesophilic anaerobic digester in Gaobeidian Wastewater Treatment Work in Beijing, China. The sludge contained 21.9 g L⁻¹ total solids (TSs), 10.4 g L⁻¹ volatile solids (VSs), and 20.7 g L⁻¹ mixed liquor suspended solids (MLSSs). Each digester was seeded to maintain the activated sludge MLSS in the digester at 15 g L⁻¹, which was based on the research result from Zhang.¹⁶ The amount of ammonia chloride (NH₄Cl) was added to each digester to adjust the carbon/nitrogen ratio (C/N) to 25, which is believed to be optimal for anaerobic bacteria growth. The prepared digesters were then placed in the shakers for anaerobic digestion tests at mesophilic temperature (35 °C) and 120 rpm shaking speed.

2.3. Analytical Methods. **2.3.1. Chemical Composition Analyses.** The untreated and NaOH-treated rice straw samples were analyzed for TS and VS according to the APHA standard methods.²⁰ The total carbon (TC) and total nitrogen (TN) were analyzed with the TC analyzer (Skalar Primacsslc, The Netherlands) and the total Kjeldahl nitrogen analyzer (Model KDN-2C, Shanghai, China). The content of cellulose, hemicellulose, and lignin was analyzed according to the procedure of Van Soest.²¹ The extractives in cold water, hot water, and benzene–ethanol solvent were determined according to the standard methods.²²

2.3.2. Isolation and Purification. The separation of lignin, cellulose, and hemicellulose from rice straw was conducted according to the procedure given by Björkman,²³ Krschner and Hoffer,²⁴ and Zhan,²⁵ respectively. Acetylation of lignin was measured according to Qin.²⁶

2.3.3. Spectroscopy. Fourier transform infrared (FTIR) spectra were obtained on a FTIR spectrophotometer (Nicolet 5DXC) using

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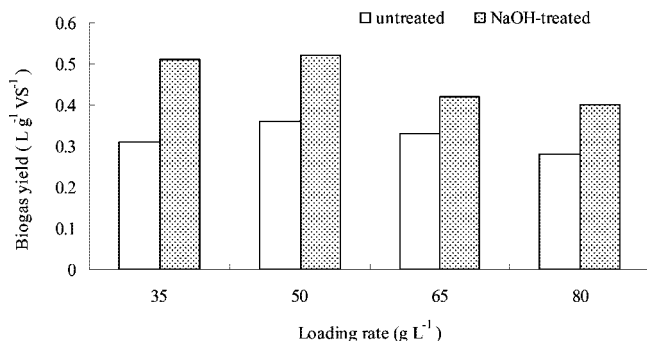


Figure 1. Comparison on the biogas production based on VS loaded of the untreated and 6% NaOH-treated rice straws.

a KBr disk containing 1% finely ground samples. A total of 30 scans were taken, with a resolution of 4 cm⁻¹. ¹H nuclear magnetic resonance (NMR) spectra were recorded with a Bruker AV 600 spectrometer at 600 MHz from 200 mg of acetylated sample dissolved in 1.0 mL of CDCl₃, following 64 scans. A 5.7 μs pulse width and a 2 s delay time between scans were used.

2.3.4. Molecular Weight. Average molecular weights and their distribution of acetylated lignin samples were estimated by gel permeation chromatography (GPC) on a high-performance liquid chromatography (HPLC) system (Waters) with Waters Styragel HT3, HT5, and HT6 columns. The columns were calibrated with authentic polystyrene. The samples were dissolved in tetrahydrofuran (THF), and 100 μL was injected into the columns. The column was operated at 30 °C and eluted with THF at a flow rate of 1 mL/min.

2.3.5. X-ray Diffractometry. X-ray diffraction was performed with a Rigaku D/Max 2500 diffractometer. The radiation was Ni-filtered Cu Kα radiation at a wavelength of 0.1541 nm. The X-ray unit was operated at 40 kV and 200 mA. Samples were scanned over the angular range 10–50°, 2θ.

Crystallinity of cellulose was calculated according to the empirical method proposed by Segal²⁷ for native cellulose

$$\text{CrI} (\%) = [(I_{002} - I_{18.0^\circ})/I_{002}] \times 100$$

where CrI is the crystalline index, I_{002} is the maximum intensity of the (002) lattice diffraction, and $I_{18.0^\circ}$ is the intensity diffraction at 18.0°, 2θ degrees.

3. Results and Discussion

3.1. Biogas Production. The untreated and 6% NaOH-treated rice straws were anaerobically digested, and the daily biogas production for each run was recorded. The biogas yield, which was defined as the biogas production per gram of VS loaded, was calculated and used to evaluate the effectiveness of NaOH pretreatment. The results in Figure 1 showed that, in comparison to the untreated rice straw, the NaOH-treated one achieved 64.5, 44.4, 27.3, and 42.9% more biogas yield at the loading rate of 35, 50, 65, and 80 g L⁻¹, respectively. The highest biogas yield of 0.52 L g⁻¹ was obtained at the loading rate of 50 g L⁻¹ for the NaOH-treated rice straw, which was significantly higher than 0.36 L g⁻¹ for the untreated one. The results proved that NaOH pretreatment was one of the efficient approaches to enhance biogas production from rice straw. The increase of biogas yield was attributed to the improved biodegradability of rice straw after NaOH pretreatment, which made more substrates available to be digested by anaerobic microorganisms. Research therefore needs to be conducted to explore the mechanism of such improvement resulting from NaOH pretreatment.

3.2. Changes of Chemical Compositions. The changes of chemical compositions and structures as well as physical

Table 1. Changes of Main Compositions of Rice Straw after NaOH Pretreatment

parameters	content (% DM)	
	untreated (raw)	NaOH-treated
cellulose	33.4	29.9
hemicellulose	28.2	19.1
lignin	7.4	5.7
LCH	69.0	54.7
DM loss		6.4
cold-water extractives	15.6	35.0
hot-water extractives	17.8	39.6
benzene–ethanol extractives	10.4	5.1
TS	94.0	91.3
VS	87.2	79.2
TKN	0.8	0.9
TC	41.5	37.2
ash	12.8	20.8

characteristics of lignocellulosic biomass occurred during the alkali pretreatment process because of the physical and chemical reactions of alkali. The changes include fiber swelling, decrease of the association of lignin with carbohydrate, and some degradation and solubilization of lignin and carbohydrate, which result in the improvement on biodegradability.¹⁷ Lignin, cellulose, and hemicellulose (LCH) are the main compositions of rice straw, which account for 69.0% of the total dry matter of rice straw and provide the main carbon source for anaerobic microorganisms. Biogas production is greatly affected by the availability and digestibility of cellulose and hemicellulose as well as the association of lignin with the carbohydrates. The main compositions were analyzed to investigate their changes during NaOH pretreatment and the effects of the changes on biodegradability and biogas production of rice straw (Table 1).

After NaOH pretreatment, total LCH, cellulose, hemicellulose, and lignin were reduced by 25.8, 16.4, 36.8, and 28.4% (dry basis), respectively, and the corresponding contents were decreased from 69.0, 33.4, 28.2, and 7.4% to 54.7, 29.9, 19.1, and 5.7%, respectively. It appears that considerable lignocelluloses were degraded. However, the degradation rates were different; more hemicellulose was degraded as compared to lignin and cellulose. This might be due to the chemical reaction of more hemicellulose with NaOH. Meanwhile, it was observed that the content of cold- and hot-water extractives was increased significantly after NaOH pretreatment. The newly generated extractives in the cold and hot water were inferred to be byproducts mainly from the degradation of hemicellulose and partially from the degradation of lignin and cellulose. The cold- and hot-water extractives were believed to be the compounds with simpler chemical structures and smaller molecular weights, which could be more readily biodegradable than lignin, cellulose, and hemicellulose. The increased amount of the cold- and hot-water extractives would generally help improve the biodegradability and contribute to the increase of biogas production from rice straw. The benzene–ethanol extractives mainly contain resins, waxes, fattinesses, tannins, and pigments,²⁸ which are normally not readily biodegradable. The decreased content after pretreatment was favorable for improving biodegradability and biogas production.

3.3. Changes of Chemical Structures. **3.3.1. Lignin–Carbohydrate Complexes.** In the structure of straw, lignin does not simply deposit in the polysaccharides of the cell wall but is associated with carbohydrate (cellulose and hemicellulose) by certain chemical bonds, such as α-ether bonds, phenyl glycosidic

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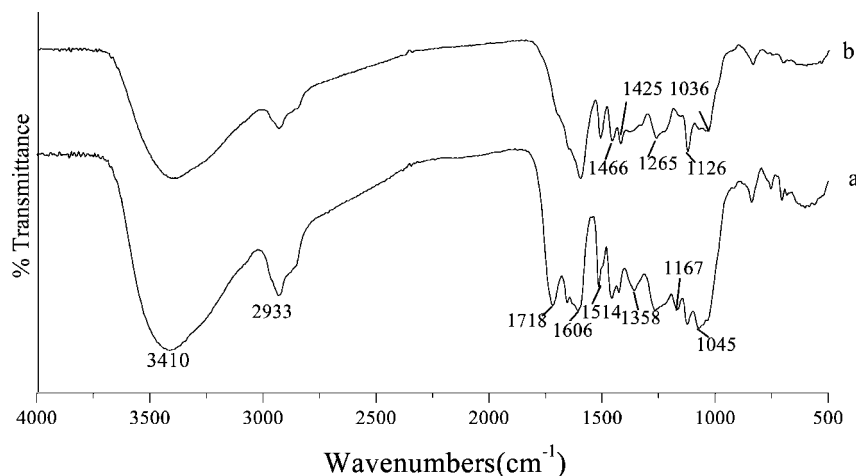


Figure 2. FTIR spectra of lignin preparations extracted from untreated (spectrum a) and 6% NaOH-pretreated (spectrum b) rice straws.

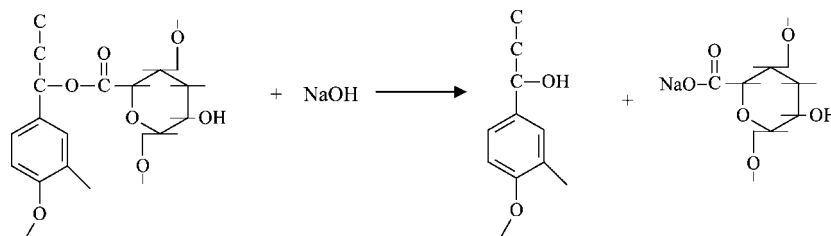


Figure 3. Scheme of lignin carbohydrate complexes reacting with NaOH.

linkages, acetal linkages, and ester bonds. The bonds form lignin-carbohydrate complexes (LCCs),²⁹ which are obstacles for efficient bioconversion of straw into biogas. Alkali treatment can release some cellulose and hemicellulose by hydrolyzing ester bonds between lignin and cellulose or hemicellulose³⁰ and, thus, can improve the biodegradability of rice straw. FTIR was used to analyze the spectra of lignin preparations extracted from untreated (spectrum a) and 6% NaOH-pretreated (spectrum b) rice straws to investigate the changes of LCCs (Figure 2). It was observed that the absorption at 1167 cm^{-1} , which is indicative of ester bond stretching, disappeared after NaOH treatment. The finding implied that the saponification reaction was inferred to occur during pretreatment as shown in Figure 3. Such a reaction damaged the ester bond linkage between lignin and carbohydrate and released cellulose from the encapsulation of lignin, making more cellulose exposed and available for anaerobic microorganisms.

3.3.2. Lignin. Lignin is a multifunctional natural polymer. It is built up by oxidative coupling of three major $\text{C}_6\text{-C}_3$ (phenylpropanoid) units, which form a randomized structure in a tridimensional network by certain interunit linkages, such as $\beta\text{-O-4}$, $\beta\text{-5}$, and $\beta\text{-}\beta$. The important functional groups of lignin units include carbonyls, phenol hydroxyls, aromatic rings, and methoxyls.³¹ ^1H NMR spectroscopy and FTIR spectroscopy were used to investigate the changes of the linkages of lignin units and the functional groups in rice straw samples.

The ^1H NMR spectra of lignin fractions extracted from the untreated and NaOH-treated rice straws were shown in Figures 4 and 5. The signals at δ 4.5 and 3.5 ppm represent $\text{H}\alpha$ and $\text{H}\beta$ of $\beta\text{-O-4}$ structures and $\text{H}\gamma$ of $\beta\text{-5}$ and $\beta\text{-}\beta$ structures, respectively. Both signals were found to disappear after NaOH

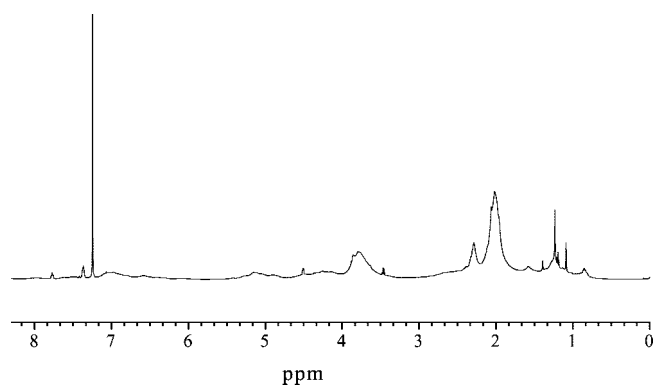


Figure 4. ^1H NMR spectrum of lignin extracted from untreated rice straw.

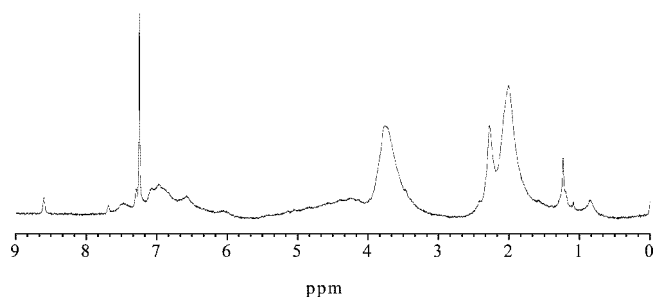


Figure 5. ^1H NMR spectrum of lignin extracted from 6% NaOH-pretreated rice straw.

treatment, indicating that the cleavage of $\beta\text{-O-4}$, $\beta\text{-5}$, and $\beta\text{-}\beta$ bonds³² occurred as shown in Figure 6. The depolymerization caused partial degradation of lignin into other substances and the decrease of lignin content as shown in Table 1.

FTIR spectroscopy analyses showed that the functional groups of lignin were also changed obviously during NaOH pretreat-

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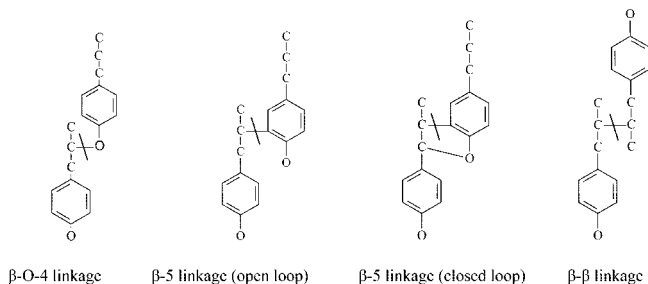


Figure 6. Lignin structure linkages.

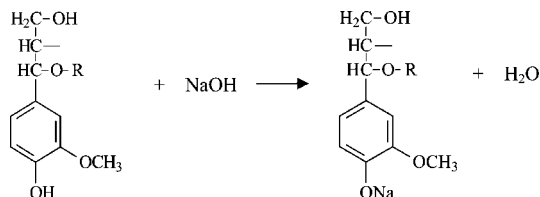


Figure 7. Scheme of lignin reacting with NaOH.

ment (Figure 2). The changes could be classified into three types: (1) Disappearance of bands. The band at 1718 cm^{-1} is assigned to the carbonyl ($\text{C}=\text{O}$) stretching unconjugated ketones.³³ Carbonyls mainly exist in the side chains of lignin structural units and are also an important functional group in the side chains, which are either aldehyde groups lying in $\text{C}-\gamma$ or keto lying in $\text{C}-\beta$. The disappearance of such bands indicated that the side chain of lignin was broken down during NaOH treatment. The band at 1358 cm^{-1} is attributed to phenol hydroxyl stretching. The band disappeared after NaOH treatment because of the reaction of phenol hydroxyl with NaOH (Figure 7). (2) Decrease of functional group contents. The prominent band at 1265 cm^{-1} corresponds to methoxyl stretching. It was observed that, as compared to the untreated rice straw, the content of methoxyl in the lignin of the NaOH-treated one was decreased. This was mainly attributed to the nucleophilic reaction of methoxyl with NaOH (Figure 8). The band at 1466 cm^{-1} represents $\text{C}-\text{H}$ deformations (asymmetric in methyl, methylene, and methoxyl groups). Aromatic skeletal vibrations are assigned at 1606 , 1514 , and 1425 cm^{-1} .³⁴ The band at 1126 cm^{-1} appears from the aromatic ring stretching for the syringyl type. The contents of such bands all decreased after NaOH treatment. (3) Appearance of bands. Some bands such as the band at 1036 cm^{-1} appeared after NaOH pretreatment. This band represents aromatic ring deformation in the $\text{C}-\text{H}$ plane. Its appearance implied that the content of aromatic compounds was increased.³⁵ The results from ^1H NMR and FTIR spectroscopies showed that NaOH pretreatment could not only break down the interunit linkages but also change the functional groups of lignin. The changes of lignin structure would have certain impacts on the biodegradability of rice straw.

3.3.3. Cellulose. Cellulose is a linear polymeric compound, which is built up by coupling β -D-glucose using 1,4-glycosidic bonds. Hydrogen bonds, methyls, methylenes, and $\text{C}-\text{O}-\text{C}$ are some important functional groups of cellulose units. FTIR spectroscopy was used to investigate the changes of cellulose structures during NaOH treatment. The results showed that the spectra of celluloses extracted from the untreated and 6% NaOH-treated rice straws had similar profiles but different intensities

of the absorption bands (Figure 9). The difference indicated that the structure of cellulose was changed after NaOH treatment, and the changes were both intra- and intermolecular.

The intramolecular changes were represented by the decreases of functional group contents. Each glucose group of cellulose has three alcoholic hydroxyl groups. Hydrogen atoms and nearby oxygen atoms can form hydrogen bonds if their distance is less than $0.28\text{--}0.30\text{ nm}$. Hydrogen bonds prevent anaerobic microorganisms or degradation enzymes from reacting with cellulose. The absorption at 3421 cm^{-1} represents the stretching of $-\text{OH}$ groups, which was reduced after NaOH treatment. It specified that partial hydrogen bonds of cellulose were destroyed, leading to enhance accessibility of cellulose to reagents.³⁶ The band at 2900 cm^{-1} represents the $\text{C}-\text{H}$ stretching, the decrease of which content indicated that methyl and methylene of cellulose had some rupture.³⁷ The absorption at 1637 cm^{-1} is principally associated with deformation vibrations of $\text{H}-\text{OH}$ in absorbed water. The symmetric $-\text{CH}_2$ bending occurs at 1433 cm^{-1} . The band near 1319 cm^{-1} can be ascribed to CH_2 wagging vibrations in cellulose and hemicellulose, while the band near 1163 cm^{-1} is representative of the antisymmetric bridge stretching of $\text{C}-\text{O}-\text{C}$ groups in cellulose and hemicellulose. The contents of the functional groups mentioned above were all decreased after NaOH treatment. However, slight changes were found for some functional groups. For instance, the band near 1383 cm^{-1} can be ascribed to $\text{C}-\text{H}$ bending in cellulose and hemicellulose. The in-plane ring stretching gives a slight shoulder at 1103 cm^{-1} . The peak at 1064 cm^{-1} is indicative of $\text{C}-\text{O}$ stretching at $\text{C}-3$, $\text{C}-\text{O}$ stretching at $\text{C}-6$, and $\text{C}-\text{C}$ stretching.³⁸

The intermolecular change was also observed in the band of 898 cm^{-1} , which represents β -(1 \rightarrow 4)-glycosidic linkages and breakage degree of intramolecular hydrogen bonds. The content was obviously reduced after NaOH treatment, indicating the breakage of the linkages. This proved that NaOH was capable of breaking some intermolecular hydrogen bonds through complex chemical reactions. This would lead to the degradation of cellulose and make cellulose easier to be attacked by anaerobic microorganisms, thus improving the biodegradability.

3.3.4. Hemicellulose. Hemicelluloses are made of a relatively limited number of sugar residues. The general formulas of hemicelluloses are $(\text{C}_5\text{H}_8\text{O}_4)_n$ and $(\text{C}_6\text{H}_{10}\text{O}_5)_n$, which are called pentosans and hexosans, respectively. There are β -glycosidic linkages between the sugar units.³⁹ FTIR spectroscopy analyses showed that the spectral profiles of the bands in hemicelluloses of the untreated and NaOH-treated rice straws were rather similar but the relative intensities of the bands were different (Figure 10). This indicated that the two hemicelluloses had similar structures but different content of functional groups and linkages. The differences were the results from the intra- and intermolecular degradation of hemicellulose during NaOH pretreatment.

The intramolecular degradation of hemicelluloses was represented by the decreased contents of functional groups and the disappearance of some bonds after NaOH pretreatment. A strong broadband at 3422 cm^{-1} was found, which is attributed to the hydroxyl groups in the hemicelluloses from both the untreated and NaOH-treated rice straws. The intensity of the band decreased after NaOH treatment, because of the disruption and

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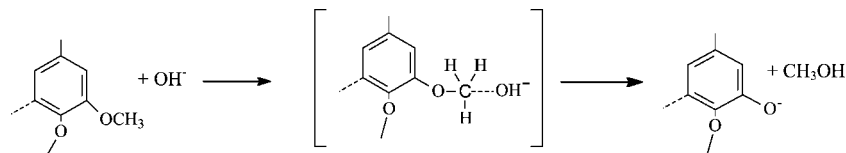


Figure 8. Scheme of methoxyl separating from the lignin structure.

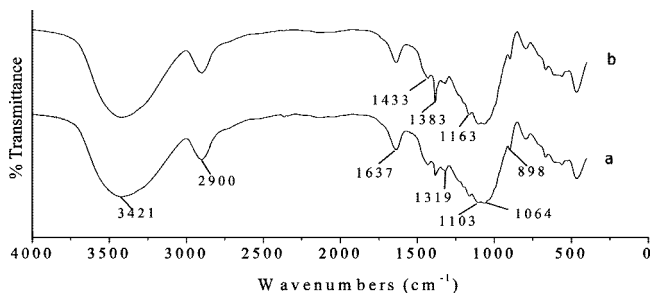


Figure 9. FTIR spectra of cellulose preparations extracted from untreated (spectrum a) and 6% NaOH-pretreated (spectrum b) rice straws.

breakage of hydrogen bonds.⁴⁰ In the carbonyl stretching region, the absorption at 1641 cm^{-1} is principally associated with absorbed water. The bands between 1125 and 1000 cm^{-1} are of typical xylans. The linear and branched (1 → 4)- β -xylans, such as glucuronoxylan and arabinoxylans, show the main band maximum at about 1044 cm^{-1} .⁴¹ A small band at 1512 cm^{-1} was found in two spectra, which is mainly due to the presence of a small amount of associated lignin in hemicelluloses. The band at 1082 cm^{-1} corresponds to the C–OH bending, which is strongly influenced by the degree of branching.⁴² The contents of the functional groups mentioned above all decreased after alkali treatment. However, some bands totally disappeared after NaOH treatment. The band at 1252 cm^{-1} is indicative of C–O stretching of syringyl units. The band at 1155 cm^{-1} is characterized by the C–O–C vibrations in the anomeric region of hemicelluloses. The two bands were found disappeared, elucidating that the structure of hemicellulose was changed after NaOH treatment; the hemicellulose was thus degraded.

Intramolecular degradation was represented by the changes of the functional groups occurring in the hemicellulose structure as mentioned above. While, in the anomeric region (950 – 700 cm^{-1}), a small sharp band at 898 cm^{-1} was observed. This band corresponds to the C₁ group frequency or ring frequency and is indicative of β -glycosidic linkages between the sugar units.⁴³ It was obviously reduced after NaOH treatment, indicating that the linkages between the sugar units were changed and intermolecular degradation occurred in the hemicellulose structure. According to Mansfield et al.,⁴⁴ the lignocellulosic materials in the original form are relatively resistant to microorganism attack but the hemicellulose and lignin removal causes extensive changes in the structure and accessibility of cellulose that becomes more accessible and more open to swelling upon contact with microorganisms. Therefore, both intra- and intramolecular degradations of hemicelluloses were beneficial for biogas production.

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3.4. Physical Characteristics. 3.4.1. Molecular Weight.

Lignin is a multifunctional natural polymer with high molecular weight. The changes of chemical structure of lignin, as discussed above, would lead to the change of molecular weight. The average molecular-weight distributions of acetylated lignin extracted from the untreated and NaOH-treated rice straws are shown in Figure 11. It was apparent that the lignin of the untreated rice straw had one molecular-weight distribution, while the lignin of the NaOH-treated one had three molecular-weight distributions. In addition, the peak molecular weight (M_p) of the former was higher than that of the latter. The findings indicated that the original lignin with a large molecular weight was degraded into the lignin with a small molecular weight. Meanwhile, polydispersity was also calculated. Polydispersity is defined as the ratio of weight-average (M_w) to number-average (M_n) molecular weight,⁴⁵ which is an important characteristic parameter of natural high polymers. It was found that the polydispersity was decreased from 3.53 with the untreated rice straw to 1.62, 1.09, and 1.03 with the NaOH-treated one. According to Jiang,⁴⁶ one molecule would have a three-dimensional network structure if the polydispersity is greater than 2 but a linear structure if the polydispersity is less than 2. The polydispersities of the lignin in the NaOH-treated rice straw were all less than 2, implying that the lignin was changed from a three-dimensional network structure to a linear structure. The change was due to the breakage of the linkages between lignin units. The above results proved that NaOH pretreatment was able to degrade original lignin with a large molecular weight and three-dimensional network structure to one with a small molecular weight and linear structure. The changes would make the pretreated rice straw more widely to be attacked and more easily to be digested by anaerobic microorganisms and thus have positive impacts on the improvement of biodegradability of rice straw.

3.4.2. X-ray Diffraction and Crystallinity. Cellulose is a complex polymer with crystalline and amorphous areas. Materials with crystalline structures can diffract and form specific patterns if X-ray is used to irradiate samples, which can be used to study the inner microstructures of cellulose.

As Figure 12 shows, the two rice straws showed similar X-ray diffraction patterns. Two peaks appeared at 2θ of 22.0° and 18.0° for the untreated and NaOH-treated rice straws, respectively. The bigger one was the main peak, representing the presence of a highly organized “crystalline” cellulose structure. The smaller one was a secondary peak, representing a less organized “amorphous” cellulose structure.⁴⁷ Sodium hydroxide is a swelling impregnant of cellulose. It is apparent that the X-ray diffraction pattern did not change after NaOH pretreatment, indicating that sodium hydroxide only reached the surfaces of amorphism and crystal areas and the swelling only took place between the crystal areas. As a result, the cellulosic crystal style in NaOH-treated straw was not obviously changed and was still maintained in the coexistence of crystal and noncrystal areas.

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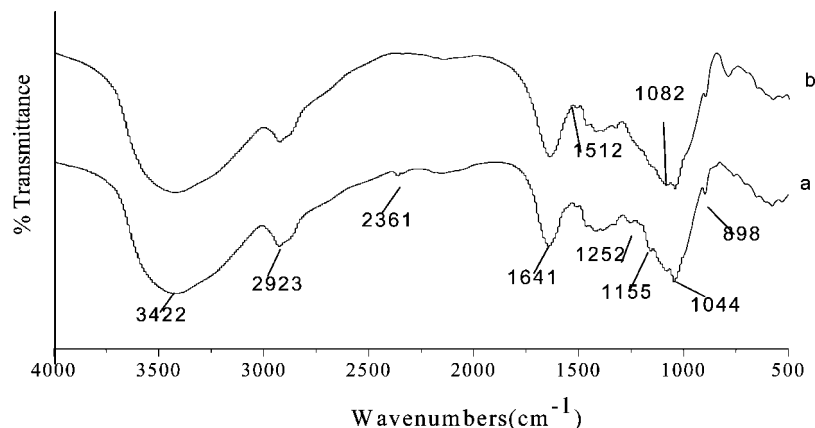


Figure 10. FTIR spectra of hemicellulose preparations extracted from untreated (spectrum a) and 6% NaOH-pretreated (spectrum b) rice straws.

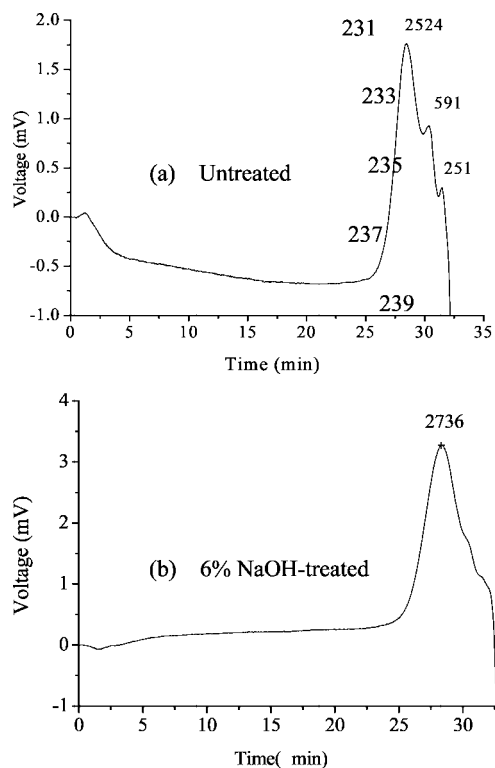


Figure 11. Molecular-weight distribution curve of MWL extracted from untreated (a) and 6% NaOH-treated (b) rice straws.

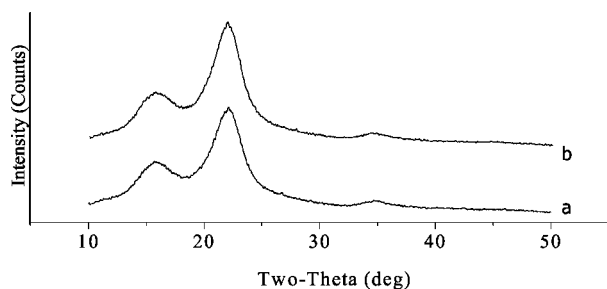


Figure 12. X-ray diffraction of cellulose preparations extracted from untreated (spectrum a) and 6% NaOH-pretreated (spectrum b) rice straws.

Crystallinity is defined as the ratio of the amount of crystalline cellulose to the total amount of sample material, including crystalline and amorphous cellulose.³⁸ It was found that the crystallinity of cellulose increased from 60.26% with the

untreated rice straw to 64.33% with the NaOH-treated one. This could be attributed to the greater hydrolyzation of the amorphous areas than crystalline areas and the occurrence of the peeling reaction in the amorphous areas, which caused the calculated relative crystallinity after NaOH treatment increased. Actually, the absolute crystallinity after NaOH treatment decreased. Sodium hydroxide can destroy cellulosic crystalline areas and enlarge the pore ratio and inner surface areas; therefore, it is advantageous to later anaerobic digestion.

4. Conclusions

In comparison to the untreated rice straw, the biogas yield of the 6% NaOH-treated one increased by 64.5, 44.4, 27.3, and 42.9% at the loading rate of 35, 50, 65, and 80 g L⁻¹, respectively. The enhancement of the biogas yield was attributed to the improvement of biodegradability through NaOH pretreatment. Cellulose (16.4%), hemicellulose (36.8%), and lignin (28.4%) were degraded, considerable proportion of which were converted to soluble substances as indicated by a 122.5% increase of water-soluble extractives in the NaOH-treated rice straw. The decrease of LCH, cellulose, hemicellulose, and lignin contents as well as the increase of water-soluble substances made the NaOH-treated rice straw more digestible. The ester bond of LCC was destroyed by hydrolysis reactions, resulting in the release of more cellulose for biogas production. The linkages of interunits and the functional groups of lignin, cellulose, and hemicellulose were either broken down or destroyed, causing intra- and intermolecular changes of chemical structures. The analyses on molecular-weight distributions and the polydispersity showed that the original lignin with a large molecular weight and three-dimensional network structure became one with a small molecular weight and linear structure after NaOH pretreatment. The cellulosic crystal style was not obviously changed, but the crystallinity of cellulose increased in the NaOH-treated rice straw. The changes of chemical compositions, chemical structure, and physical characteristics made the NaOH-treated rice straw become more available and biodegradable and thus were responsible for the improved biodegradability and enhanced biogas production.

Acknowledgment. The authors are grateful for the financial support of this research from the Hi-tech Research and Development Program of China (2006AA10Z425).

EF8000967