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# Characteristics of Moso Bamboo with Chemical Pretreatment

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Zhijia Liu and Benhua Fei

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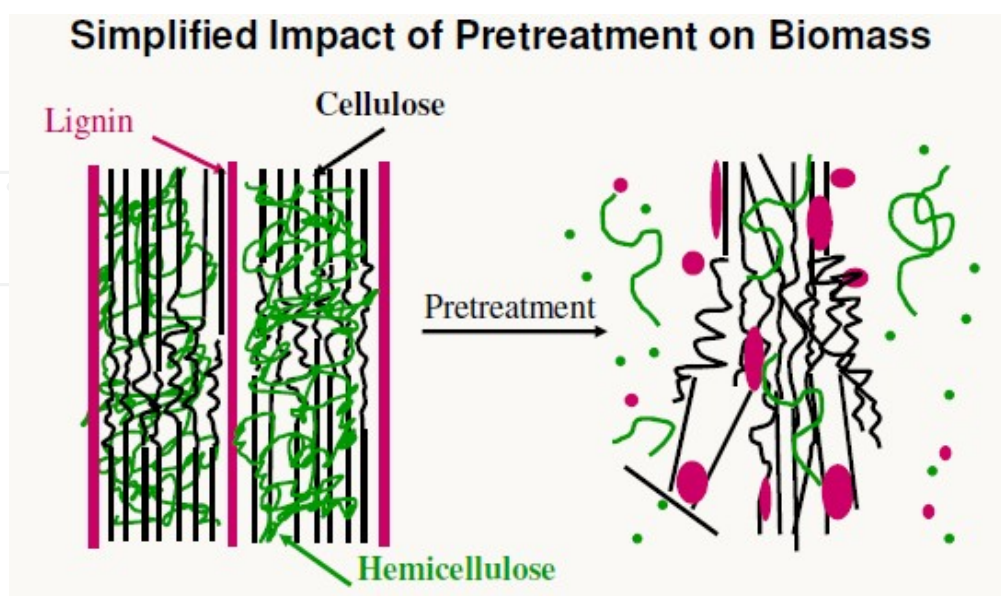
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## 1. Introduction

The world's present economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc [1]. There are several alternative energies which can replace fossil fuels in the future, such as hydro, solar, wind, biomass and ocean thermal energy. Among these energy sources, biomass is the only carbon-based sustainable energy and is utilized by most people around the world [2]. Furthermore, it is also confirmed that the petroleum-based fuels can be replaced by biomass fuels such as bioethanol, bio-diesel, bio-hydrogen, which derives from agricultural residues, forestry residues, municipal solid waste, manufacturing waste, vegetable oils, dedicated energy crops, etc [3]. Recently, it is a growing interest in manufacturing bioethanol using biomass materials. It is well known that production of ethanol from biomass includes three major processes, such as pretreatment, hydrolysis, and fermentation. One of the most important processes is biomass pretreatment in the production of biofuel. Biomass pretreatment can remove lignin and hemicelluloses, which significantly enhance the hydrolysis of cellulose. It is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars [4]. Figure 1 shows simplified impact of pretreatment on biomass [5].

Pretreatment technology contains physical pretreatment (mechanical size reduction, pyrolysis, microwave oven and electron beam irradiation pretreatment), physicochemical pretreatment (steam explosion or autohydrolysis, liquid hot water method, ammonia fiber explosion, CO<sub>2</sub> explosion), chemical pretreatment (acid pretreatment, alkaline pretreatment, wet oxidation, organosolv pretreatment), and biological pretreatment [1]. Recently, the information of various pretreatment methods is available. Lin et al. found that the yields of glucose and xylose were improved by adding any of the following dilute chemical reagents,

such as  $\text{H}_2\text{SO}_4$ ,  $\text{HCl}$ ,  $\text{HNO}_3$ ,  $\text{CH}_3\text{COOH}$ ,  $\text{HCOOH}$ ,  $\text{H}_3\text{PO}_4$ , and  $\text{NaOH}$ ,  $\text{KOH}$ ,  $\text{CaOH}_2$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$  in the ball milling pretreatment of corn stover [6]. Bjerre et al. studied the wet oxidation process of wheat straw as a pretreatment method. By using a specially constructed autoclave system, the wet oxidation process was optimized with respect to both reaction time and temperature (20 g/L straw,  $170^\circ\text{C}$ , 5 to 10 min) and gave about 85% w/w yield of converting cellulose to glucose [7]. Zhu and Pan evaluated the performances of three of the most promising pretreatment technologies, including steam explosion, organosolv, and sulfite pretreatment to overcome lignocelluloses recalcitrance (SPORL) for softwood pretreatment. SPORL was the most efficient process and produced highest sugar yield [8]. Keshwani et al. examined the feasibility of microwave pretreatment to enhance enzymatic hydrolysis of switchgrass. It was found that the application of microwave radiation for 10 minutes at 250 watts to switchgrass immersed in 3% sodium hydroxide solution (w/v) produced the highest yields of reducing sugar [9]. Yu et al. studied a two-step liquid hot water pretreatment (TSLHW) method. The first step of pretreatment was temperature range from  $180$  to  $200^\circ\text{C}$ , and the highest yield of total xylose achieved was 86.4% after 20 min at  $180^\circ\text{C}$ . The second-step of pretreatment was temperature range from  $180$  to  $240^\circ\text{C}$  for 0-60 min. The optimum reaction conditions of pretreatment with minimal degradation of sugars were  $200^\circ\text{C}$  for 20 min [10]. Sulfuric acid is widely used for acid pretreatment among various types of acid such as hydrochloric acid, nitric acid and phosphoric acid [11]. Maarten et al. compared the efficiencies of fumaric, maleic, and sulfuric acid in wheat straw pretreatment. At  $150^\circ\text{C}$  and 20-30% (w/w) dry wheat straw, the pretreatment with dilute fumaric or maleic acid could be a serious alternative to dilute sulfuric acid pretreatment [12]. Sun et al. studied the effectiveness of different alkaline solutions by analyzing the delignification and dissolution of hemicelluloses in wheat straw. The optimal process condition was 1.5%  $\text{NaOH}$  for 144h at  $20^\circ\text{C}$ , releasing 60% and 80% lignin and hemicelluloses, respectively [13].



**Figure 1.** Simplified impact of pretreatment on biomass modified from Mosier et al. [5]

Agricultural and forest residues represent a major fuel source for potential bio-energy projects in many developing countries [14]. Bamboo, like wood and agricultural residue, is mainly composed of hemicelluloses, cellulose and lignin, even though the contents of these compositions are different. The cellulose, hemicelluloses and lignin content in some agriculture, wood and bamboo wastes is showed in Table 1. Bamboo has been widely cultivated in the west and south of China. Currently, bamboo resources are very abundant. The total area of bamboo is about five million hectares and that of moso bamboo is about 3 million hectares in China [15]. Annual yield of moso bamboo is about eighteen million tons, and it is widely used to produce furniture, flooring and interior decoration materials. It has great potential as a bio-energy resource of the future in China. Despite these previous researches are very helpful in understanding the pretreatment of biomass materials, bamboo is a different type of material. To date, no research about bamboo biofuel is available. In this research, moso bamboo was therefore pretreated by 2% of sulfuric acid (w/w bamboo) and 10% sodium hydrate (w/w bamboo). Characteristics of pretreated bamboo were determined by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD), respectively. Pretreatment method was evaluated through comparing with characteristics of untreated and pretreated bamboo. The objective of this research is to investigate characteristics of bamboo pretreated by chemical methods and select a pretreatment method for exploring the biofuel using bamboo.

Biomass type	Species	Cellulose (%)	Hemicelluloses (%)	Lignin (%)	Ash (%)
Wood	Pine (hardwood)	40-45	25-30	26-34	----
	Maple (softwood)	45-50	21-36	22-30	----
Bamboo	Moso bamboo	42-50	24-28	24-26	1.3-2.0
Agriculture residues	Rice straw	41-57	33	8-19	8-38
	Rice husk	35-45	19-25	20	14-17
	Baggasse	40-46	25-29	12.5-20	1.5-2.4
	Cotton stalk	43-44	27	27	1.3

**Table 1.** Chemical composition of bamboo, wood and agriculture residue

## 2. Materials and methods

### 2.1. Material

Moso bamboo aging with 4 years was used in this study. They were taken from a bamboo plantation located in Anhui province, China. The initial moisture content of samples was about 6.13%, and the density was about 0.65g/cm<sup>3</sup>. Bamboo materials were cut off to sample size 40mm (longitudinal) by 3-8mm (radial) by 20-30mm (tangential). Then, they were broken down to particles with a Wiley mill and the size of bamboo particles used in the test was about

250-425  $\mu\text{m}$ . Finally, the particles were dried at  $105^\circ\text{C}$  until mass variability of samples was less than 0.2%.

## 2.2. Pretreatment

Bamboo particles were pretreated using a microwave accelerated reaction system. 2% of sulfuric acid (w/w of  $\text{H}_2\text{SO}_4$ /bamboo) and 10% sodium hydrate (w/w of  $\text{NaOH}$ /bamboo) were used in the pretreated process. About 3g bamboo particles were mixed with the solvent in a 100 mL vessel. The mass ratio of liquor-to-bamboo was about 8:1. The vessel with samples was positioned at the centre of a rotating circular ceramic plate in the microwave oven for pretreatment at the power level of 400 W. The temperature was raised to  $180^\circ\text{C}$  (target temperature) in about 10 min and maintained for an additional 30min. After the pretreatment, a few minutes were allowed for the temperature to drop down below  $50^\circ\text{C}$ , and then pretreated substrate and spent liquor were then separated by filtration. The substrate was washed using distilled water until pH value of washed liquor was about 7. Each experiment was carried out in three times and average results were reported.

## 2.3. Property test

### 1. FTIR test

The functional group difference of untreated and pretreated samples was analyzed by means of FTIR-spectrometer (Bruker, Bremen, Germany). The concentration of the sample in the tablets was constant of 1 mg/400 mg KBr. Scans were run at a resolution of  $4\text{cm}^{-1}$  and each sample consisted of 64 scans recorded in absorbance units from  $3800$  to  $750\text{cm}^{-1}$ . The spectra were ATR and baseline corrected and the spectra analyzed for carbonyl bands using relative indices. A minimum of two samples were tested, and data from the first run was used when it was shown to be in accordance with the second run. The experimental data could be directly obtained though FTIR-spectrometer, but they were analyzed by using Origin 8.0 software.

### 2. XRD test

XRD test of untreated and pretreated bamboo samples was carried out using an X-ray diffractometer (Diffraktometer D5000, Siemens, Germany) with an X-ray generator and a Co target ( $\lambda=0.1729\text{ nm}$ ) at a scanning speed of  $3^\circ/\text{min}$ , and the data were recorded every  $0.02^\circ$  ( $2\theta$ ) for the angle range of  $2\theta=5-45^\circ$ . The cellulose crystallinity (CrI) was calculated based on formula (1):

$$\text{CrI} = (I_{002} - I_{\text{am}}) / I_{002} \quad (1)$$

Where,  $I_{002}$  was the overall intensity of the peak at  $2\theta$  about  $22^\circ$  and  $I_{\text{am}}$  was the intensity of the baseline at  $2\theta$  about  $18^\circ$ .



### 3. Results and discussion

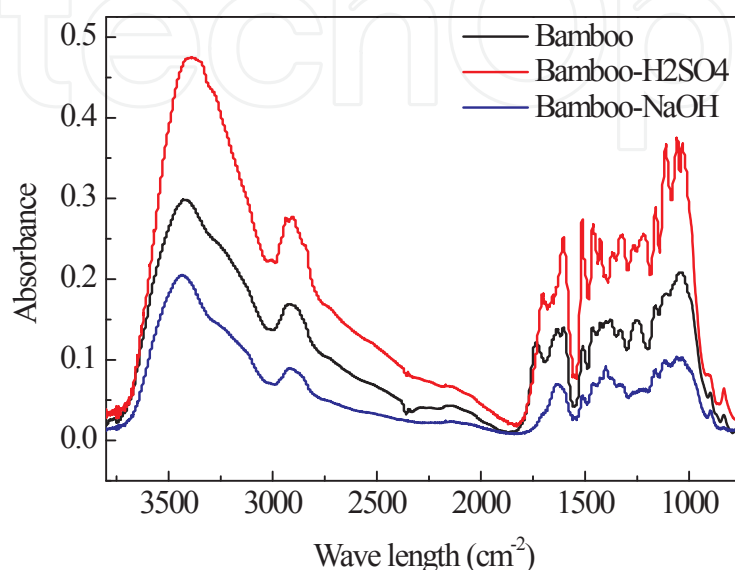
#### 3.1. FTIR analysis

The functional groups of untreated and pretreated bamboo were shown in the FTIR spectra presented in Figure 2. For untreated bamboo, there was a strong broad O-H stretching absorbance at  $3350\text{cm}^{-1}$ . The absorbance at  $2910\text{ cm}^{-1}$  was a prominent C-H stretching. In the fingerprint region (from  $1800$  to  $800\text{ cm}^{-1}$ ), some important information on various functional groups presented in untreated bamboo. The absorbance at  $1740\text{ cm}^{-1}$  was attributed to C=O stretching vibration in hemicelluloses. The bands from  $1600$  to  $1450\text{ cm}^{-1}$  was due to aromatic skeletal vibration in lignin. The absorbance at  $1370\text{cm}^{-1}$  was C-H bending of cellulose or hemicelluloses, and that of  $1230\text{cm}^{-1}$  was C-O stretching of phenolic hydroxyl group in the lignin. The absorbance at  $1160\text{cm}^{-1}$  and  $1030\text{ cm}^{-1}$  was respectively attributed to C-O-C stretching of cellulose or hemicelluloses and C-O stretching of cellulose, hemicelluloses or lignin [16].

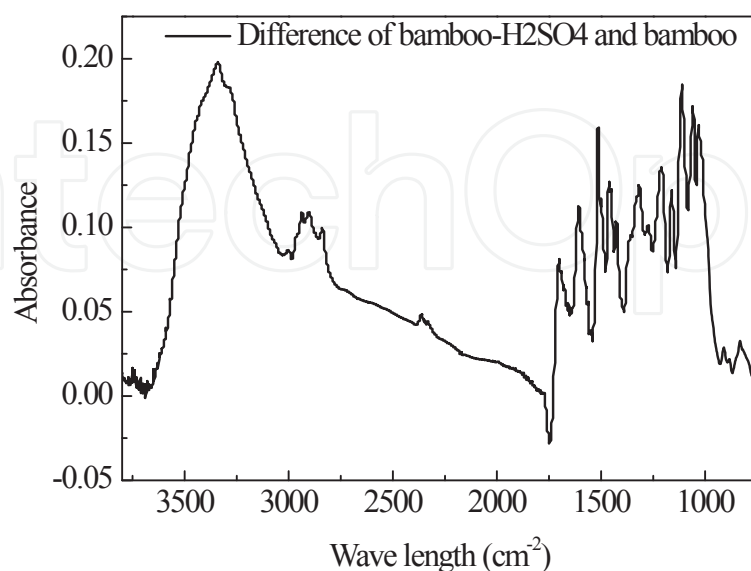
The chemical group difference of bamboo- $\text{H}_2\text{SO}_4$  and bamboo was showed in Figure 3. It was very obvious that the number of most chemical groups on the bamboo- $\text{H}_2\text{SO}_4$  sample surface was more than that of untreated bamboo surface, except for absorption of C=O stretching vibration. The information is very important, which confirms the removal of hemicelluloses of bamboo. The main feature of difference between hemicelluloses and cellulose is that hemicelluloses has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-omethylglucuronic, D-glucuronic, and D-galactouronic acids) [29]. Hemicelluloses is known to coat the cellulose microfibrils in the plant cell wall, forming a physical barrier to access by hydrolytic enzymes. Removal of hemicelluloses from the microfibrils is believed to expose the cellulose surface and to increase the enzymatic hydrolysis of cellulose [17, 18]. Dilute-acid pretreatment is a main method for the selective fractionation of hemicelluloses from biomass. Both cellulose and hemicelluloses components can also be hydrolysed using dilute-acid catalysed processes but in this case a two step-hydrolysis is required. The difference between two steps is mainly the operational temperature, which is high in the second step (generally around  $230\text{-}240\text{ }^\circ\text{C}$ ) [19, 20].

The chemical group difference of bamboo-NaOH and bamboo was showed in Figure 4. It was found that the number of all chemical groups on the bamboo-NaOH sample surface was less than that of bamboo surface. This indicated the removal of hemicelluloses and lignin of bamboo. Alkaline pre-treatments are very effective for lignin solubilisation exhibiting only minor cellulose and slightly higher hemicelluloses solubilisation [21]. Generally, alkaline pre-treatments include wet oxidation and the ammonia. The wet oxidation was used in this research. Wet oxidation is defined as pretreatment process including oxygen and water at elevated temperatures and pressure, promoting the oxidation of lignin and decomposing it to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and carboxylic acids [22, 23]. The hemicellulosic sugars remain mainly in the oligomeric form, and although there is a low formation of furan-aldehydes, a significant formation of carboxylic acids still exists [24]. It is well known that lignin confers integrity and structural rigidity on the plant cell wall. There is several information that cellulolytic enzymes

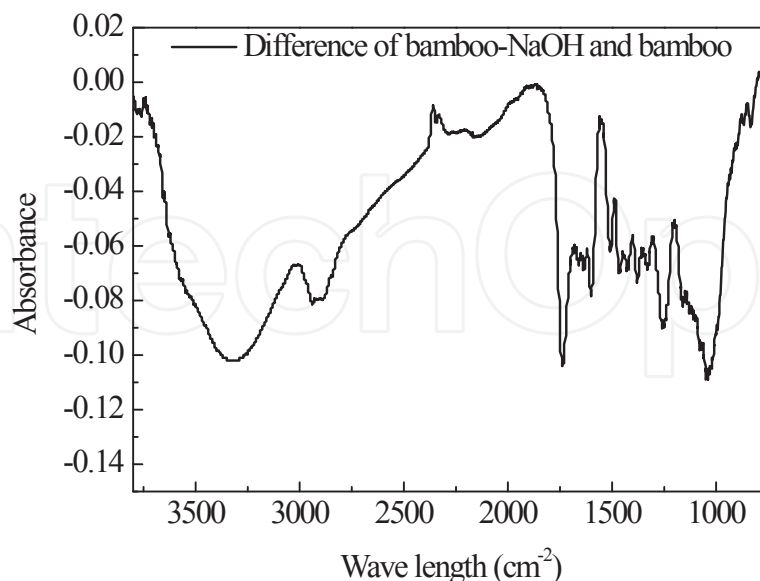
are adsorbed non-specifically on the lignin fraction of lignocelluloses even in the absence of a carbohydrate-binding module [25, 26, 27]. Therefore, the increase in the initial hydrolysis rate of cellulose and hemicelluloses should be due in part to the decreasing number of non-specific binding sites on lignin, making more enzyme available for hydrolysis [28].



**Figure 2.** The FTIR spectra of untreated and pretreated bamboo



**Figure 3.** The FTIR spectra difference of untreated and sulfuric acid pretreated bamboo



**Figure 4.** The FTIR spectra difference of untreated and sodium hydroxide pretreated bamboo

### 3.2. XRD analysis

The untreated and pretreated bamboo was determined using an X-ray generator in this study. The XRD results were shown in the Figure 5. The cellulose crystallinity was calculated according to formulas (1). The cellulose crystallinity of bamboo, bamboo-H<sub>2</sub>SO<sub>4</sub> and bamboo-NaOH samples were shown in Figure 6. It was obvious that the cellulose crystallinity of pretreated bamboo was greater than that of bamboo. Biomass materials exhibit two types of cellulose crystallinity: absolute and relative crystallinity. The relative crystallinity was used in this research. The relative crystallinity of bamboo, bamboo-H<sub>2</sub>SO<sub>4</sub> and bamboo-NaOH samples was 44.4%, 49.8% and 55.2%, respectively. This phenomenon further confirmed that some compositions of bamboo were removed in the pretreatment process. It also expressed that the NaOH pretreatment was better than H<sub>2</sub>SO<sub>4</sub> pretreatment. This can be explained based on substrate content of different pretreatment. It was found from Table 2 that the substrate content of NaOH pretreatment (64.1%) was lower than that of H<sub>2</sub>SO<sub>4</sub> pretreatment (70.3%).

Bamboo is composed of cellulose, hemicelluloses, lignin and a remaining smaller part comprising extractives and minerals. The cellulose and hemicelluloses typically comprise up to 70% of the bamboo and are the substrates for second generation ethanol production. The microbial conversion of the hemicelluloses fraction, either in the monomeric form or in the oligomeric form, is essential for increasing fuel ethanol yields from bamboo. Unlike cellulose, hemicelluloses are not chemically homogeneous and different hydrolytic technologies and various biological and non-biological pre-treatment options are available both for fractionation or solubilisation of hemicelluloses from lignocellulosic materials [21]. Depending on the process and conditions used during pre-treatment, hemicelluloses sugars may be degraded to weak acids and furan derivatives which potentially act as microbial inhibitors during the



fermentation step to ethanol. For fuel ethanol production, hemicelluloses are commonly removed during the initial stage of biomass processing aiming to reduce structural constraints for further enzymatic cellulose hydrolysis. Lignin affects the enzymatic hydrolysis of lignocellulosic biomass because it forms a physical barrier to attack by enzymes. The removal of hemicelluloses and lignin through pretreatment is very helpful to make the cellulose accessible to hydrolysis for conversion to fuels.

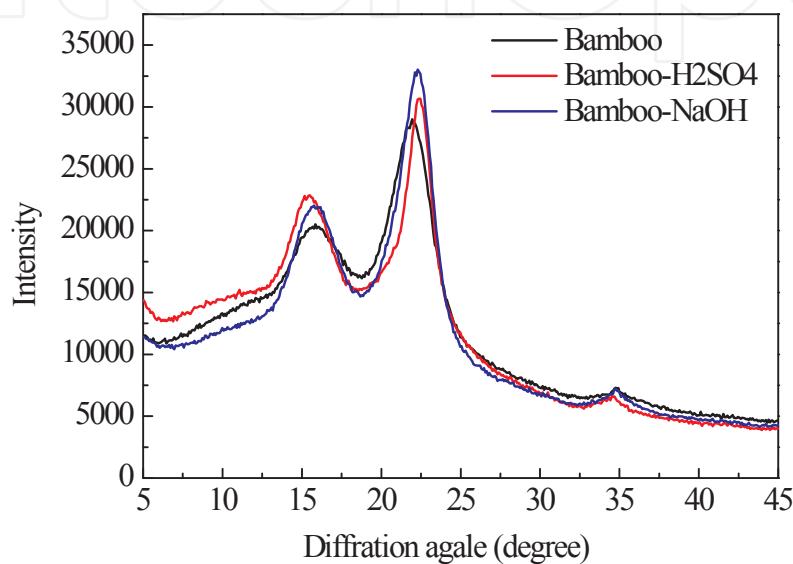


Figure 5. XRD curve of untreated and pretreated bamboo

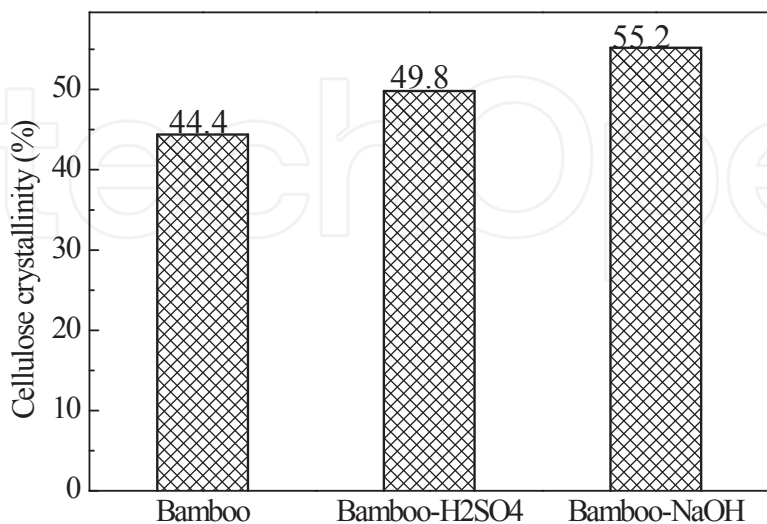


Figure 6. The cellulose crystallinity of untreated and pretreated bamboo

Pretreated method	Bamboo mass (g)		Substrate content (%)
	Untreated	Pretreated	
H <sub>2</sub> SO <sub>4</sub>	3.015	2.119	70.3
NaOH	3.017	1.934	64.1

**Table 2.** The substrate content of pretreated bamboo

## 4. Conclusions

It can be concluded from this research that hemicelluloses and lignin of bamboo are removed by H<sub>2</sub>SO<sub>4</sub> and NaOH pretreatment. The number of most chemical groups on the bamboo-H<sub>2</sub>SO<sub>4</sub> sample surface is more than that of bamboo surface, except for absorption of C=O stretching vibration, which indicates hemicelluloses removal of bamboo through H<sub>2</sub>SO<sub>4</sub> pretreatment. The number of all chemical groups on the bamboo-NaOH sample surface is less than that of bamboo surface, which expresses hemicelluloses and lignin removal of bamboo through NaOH pretreatment. The relative crystallinity of bamboo-H<sub>2</sub>SO<sub>4</sub> and bamboo-NaOH samples is 49.8% and 55.2%, respectively. The substrate content of NaOH pretreatment (64.1%) is lower than that of H<sub>2</sub>SO<sub>4</sub> pretreatment (70.3%). This phenomenon further confirms that NaOH pretreatment is better than H<sub>2</sub>SO<sub>4</sub> pretreatment. This research represents an initial stage in the study of bamboo bioethanol and may provide guidelines for further research, such as substrate hydrolysis, biofuels synthesis, etc.

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