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Physicochemical Variation of the Main Components during Wild Pretreatment Process Based on the Concept of the Whole Utilization of Bamboo

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Abstract: Attempting to correlate the characteristics of the fractionated components from bamboo to its susceptibility to enzyme is often inconclusive depending on the parameters of pretreatment conditions. Based on the integrated analysis of chemical components, cellulose bioconversion, characteristic property of isolated hemicellulose, and lignin, the optimal mild pretreatment operation for Moso bamboo was 4% NaOH in 20% ethanol aqueous solution. A total of 91.9% mass was successfully recovered, and 66% bioconversion efficiency of the cellulosic sample was finally achieved. Meanwhile, over 25% hemicelluloses and 7% lignin were isolated, and the characteristic analysis indicated that the fractionated biomacromolecule maintained the original core structure, which is a benefit to be further utilized for the production of chemicals or polymers.

Keywords: bamboo; fractionation; whole utilization; hemicellulose; lignin

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1. Introduction

Facing the worsening of the world energy crisis, herbaceous and woody lignocellulosic plants are increasingly needed for large-scale fabrication of bioenergy and bioproducts since they are a universally renewable and available feedstock [1]. Increasing attentions have been paid to the utilization of lignocellulose, composed of three main polymers (cellulose, lignin, and hemicellulose), which are firmly linked together to form a complicated structure, resulting in an essential barrier for biotransformation processes [2]. The most significant purpose of pretreatment is to increase the bioconversion efficiency of the cellulosic substrates, and then to enhance the biotransformation efficiency and reduce the cost of utilization [3]. By breaking the lignin barrier, disrupting the cellulose crystal configuration and removing the non-cellulose components, the physicochemical and ultrastructural properties of lignocellulosic material could be destructed during the pretreatment process, improving the accessibility of cellulose [4]. In general, pretreatment is classified into chemical, physical, physicochemical, and biological processes, facing their own advantages and drawbacks, respectively [5].

Bamboo, which is a subfamily of Gramineae, is widespread in warm temperate, subtropical, and tropical regions from 46° N to 47° S, and a few species are distributed in temperate and cold regions, known as the "world's second largest forest.". As an important non-wood resource, the utilization scale of bamboo forest resources is increasing rapidly under the background of wood shortage and energy exhaustion [6]. China is the center

of bamboo distribution in the world, having the richest bamboo species and the widest distribution (43 genera and 857 species), accounting for about 1/2 of bamboo species worldwide [7]. The existing bamboo forest area is about 5.4 million hm2, accounting for 2.8% of the national forest area in China, and more than 3 million tons of miscellaneous bamboos are fell each year, equivalent to 10 million m^3 timber and almost 1/5 of Chinese annual timber harvest [8]. Bamboo is a versatile plant with numerous traditional utilizations, including chopsticks, toothpicks, implements, musical instruments and artwork, horticultural crop support tools, erosion control, fuel and soil protection, and house building materials. For the modern biological and food industries, the special characteristics of bamboo are very beneficial for extensive applications such as pulp and paper, dietary fiber food additives or cellulose in textiles, bioenergy, and biochemicals [9]. With the significant increment of labor and production costs, the overall profit level of the bamboo industry is declining, and one of the most important reasons is the lack of basic research and original innovation on bamboo science, thus, hardly supporting the further development of bamboo in modern industry [10]. Bamboo is a natural lignocellulosic material with a unique and complex multi-layered structure, which gives it excellent physical and mechanical properties. At the macroscopic level, bamboo has a typical reinforced structure with the matrix phase comprised of a thin-walled porous structure and the reinforcing phase composed of thick-walled bamboo fibers. At the microscopic level, the bamboo cell wall is a nanocomposite reinforced by microfibers and hemicellulose and lignin as the matrix.

The use of biomass is divided into direct utilization such as combustion, construction materials, etc., and indirect utilization such as the extraction of different components and their modification. Combustion of the biomass in question is important for direct use and has been widely investigated around the world. Bamboo has a low moisture and volatile matter content, high energy density, high heating value (HHV) and effective heat of combustion (EHC), low H/C and O/C ratios, and short time to ignition (TTI). During pyrolysis, the characteristic peaks of bamboo biomass shift to higher temperatures, indicating more stable combustion and higher combustion efficiency [11]. Bamboo has other important fuel properties, such as alkaline index and low ash content for bioenergy substances. The above-mentioned advantages of bamboo mean bamboo biomass is a suitable material for direct combustion [12]. However, due to its excellent mechanical property, the traditional utilization of bamboo is mainly concentrated on the production of various architectures, handicrafts and commodities with high value. In recent years, bamboo has received increasing attentions as an alternative raw material for the bioenergy production, facing excessive energy consumption and environmental pollution. Owing to the rapid growth property, a large amount of land and water is consumed during the biomass production of bamboo, requiring the cost process of vegetative propagation [13]. Biomass requires pretreatment techniques to break down the cell wall's resistance to degradation prior to indirect utilization. This paper focuses on a pre-treatment method that allows for better whole utilization of cellulose, lignin, and hemicellulose in bamboo biomass. The whole utilization of biomass greatly improves the use efficiency of biomass resources, lays the foundation for the follow-up of high-value materials, and relieves environmental pressure.

In recent years, researchers have studied the mechanism of various pretreatment techniques to break the anti-degradation barrier of cell wall, and made important progress. Dilute alkali pretreatment mainly works on saponification of ester bond between lignin and hemicelluloses, and weakening or trans-configuration of hydrogen bond in cellulose chains [14], resulting in the de-structure of cell wall, the increment of cellulose accessibility, and the dissociation of the main components [15,16]. There are few studies on the relationship between chemical components and morphological properties of cell wall in bamboo. In this paper, the distribution complexity and diversity of the ultrastructure and main components that lead to cell wall resistance, especially the change of ultrastructure during pretreatment and the mechanism of component dissolution difference in different morphological regions, were described, so as to lay the theoretical foundation of the sub-

sequent high-value processing and utilization of lignocellulosic fibers. In order to avoid the over usage of chemical and the following pollution problems, the mild alkali alcohol aqueous pretreatment at low temperature was employed.

2. Materials and Methods

2.1. Material

Moso bamboo was harvested from Anhui Province, China, and chipped using a custom-designed chipper. After being ground with pulverizer (TASITE FZ102, Tianjin, China), the portion passing 60 mesh was collected for the following pretreatment and chemical analysis. Meanwhile, the 1 × 1 cm^2 Moso bamboo slices were also prepared by cutting with microtome (YAMATO REM710, Tokyo, Japan), and the thickness was predesigned to be 20 μm, which is a benefit for the following process. Due to the hard texture, bamboo pieces were first boiled in water at 90 °C for about 3 weeks until the materials became soft enough to be sliced. All the powder and slice samples were stored in a 4 °C refrigerator.

2.2. Fractionation of Bamboo

The fractionation process for the powder sample was carried out in a sealed glass bottle. The raw material was mixed with a certain amount of NaOH alcohol aqueous solution and heated in a water batch for 120 min at 80 °C (solid-liquid ratio was 1:10 and the specific solution conditions are listed in the Supporting Materials, Table S1). Then, the solid cellulosic fractions were collected and thoroughly washed to neutral with distilled water. Meanwhile, the liquid fractions were further concentrated under vacuum and adjusted to pH 5–6 with 6 M hydrochloric acid. The hemicellulosic fractions were precipitated by adding three equivalent volumes of 95% ethanol and centrifuged at 5000 rap/min. The remaining supernatants were then concentrated and acidified with 6 M HCl to pH 2.0 to precipitate the lignin fractions. The obtained cellulosic, hemicellulosic, and lignin fractions were finally freeze-dried and labeled as P × C, P × H, and P × L, respectively, and the subscript × (1–5) was assigned to the different fractionation conditions (P_1–P_5) (Figure 1) [17]. The removal of hemicellulosic and lignin components in Moso bamboo was also proceeded on the slice with the same procedure as the sample P_2. (Specific solution proportioning conditions are shown in Table S1).

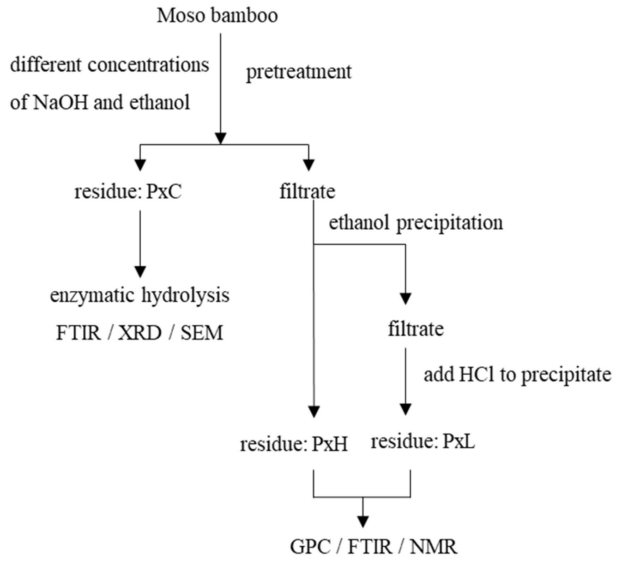


Figure 1. Experimental and analytical flow chart of the fractionation of Moso bamboo under mild conditions.

2.3. Characteristic Analysis

2.3.1. Compositional Analysis

The composition of the structural carbohydrate was determined according to National Renewable Energy Laboratory (NREL) protocol, and analyzed using high-performance liquid chromatography (HPLC) system on a Waters Alliance (Waters e2695, Milford, MA, USA) equipped a Bio-Rad Aminex HPX-87H analytical column (300 × 7.8 mm) with a detection temperature of 65 °C, and a refractive detector temperature of 35 °C with the 5 mM sulfuric acid used as the flow phase with a flow rate of 0.5 mL/min. The analysis process includes two-step acid hydrolysis to determine the content of carbohydrates, weighting process to calculate the acid-soluble lignin (ASL) content, and acid-insoluble lignin (AIL, Klason lignin) content [18]. Commonly, the sample was hydrolyzed by 72% H_2SO_4 (by weight) and then diluted to 4% sulfuric acid. After two steps of acid hydrolysis, the residue was filtered and dried until a constant weight, which was AIL content. Meanwhile, the ASL content was calculated from the absorbance of the UV photometer (Techcomp UV2310II, Shanghai, China) at 205 nm and is determined by the following formula:

$$B = A/110 \times D$$

where, B: Lignin content in the hydrolysate g/L; A: UV adsorption at 205 nm; D: Dilution Factor; 110: absorbance factor L/g cm.

2.3.2. Physicochemical Structure Analysis

The morphologic variation of the bamboo cell wall was observed by both optical and electrical technologies. After being washed with pure water, the treated bamboo slices were taken on the slide with tweezers and covered with cover glass to ensure the integrity of the slices as much as possible, and then observed under an optical microscope (Leica lmd6500, Weztlar, Germany). Meanwhile, raw and pretreated samples were aired and sputtered with gold and palladium for 120 s in a sputter coater on a HITACHI E-1010 equipment and then observed by scanning electron microscopy (SEM) system on a HITACHI S-3400 N equipment at 5 kV acceleration voltages.

Fourier-transform infrared (FT-IR) spectra of the selected samples were recorded on a Bruker Tensor II spectrophotometer by using KBr disc in the ratio of 100:1 and grinded to 74 μm to form a thin sheet. Spectra were obtained in the absorption mode over the scan range of 4000–600 cm^{-1} with a resolution of 4 cm^{-1}. The X-ray diffraction patterns of the cellulosic samples were recorded on a XRD instrument (Bruker D8 advance, Karlsruhe, Germany) from 5° to 35° with the 2θ scanning speed of 5°/min. The relative crystallinity of cellulosic fractions was calculated as the ratio between the diffracted portion of the crystalline domain of a sample and the total diffraction of the sample, according to the following formula [19]:

$$CrI = (I_{002} - I_{am})/I_{002} \times 100\%$$

where, CrI is the crystallinity index; I_{002} is maximum intensity of (002) plane diffraction; I_{am} is the diffraction intensity of amorphous region.

The molecular weight distribution of hemicellulosic and lignin fractions was determined by Gel Permeation chromatography (GPC) (Shimadzu LC-20AD, Kyoto, Japan) with diode-array detector (DAD) with PL aquagel-OH MIXED-M column and PLgeL 3 μm Mixed-E column, respectively. Hemicellulosic samples were dissolved and eluted with 5 mM sodium phosphate buffer (pH 7.5, containing 0.02 M sodium chloride) at a flow rate of 0.1 mL/min. The derivatization process can improve the average molecular weights of lignin, thereby lignin samples were directly dissolved in THF (0.2% *w*/*v*) and injected with 20 μL solution for isolation. The molecular weight was calculated according to the standard curve. The 1D ^1H NMR spectra of solution were required on a Bruker AVANCE 400 MHz spectrometer equipped with a z-gradient triple resonance probe at 298 K (25 °C). Sample concentrations of approximately 25 mg dissolved in 1.0 mL DMSO-d_6 were placed

in 5 mm i.d. glass tubes and operated at 25 °C for 2 h, separately. The central DMSO peak (d_H = 2.49 ppm) was used as an internal reference.

2.4. Enzymatic Hydrolysis of the Cellulosic Samples

The untreated material and cellulosic fractions were enzymatically hydrolyzed in a thermostatic water bath shaker with a rotating speed of 150 rpm at 30 °C for 72 h. In 100 mL conical flasks, the initial substrate concentration was set to be 10% (*w*/*v*) in 30 mL of sodium acetate buffer (pH 4.8), and an enzyme (Novozymes Biotechnology Co., Ltd., Copenhagen, Denmark) loading of 35 FPU/g substrate was used. The supernatant was periodically sampled for sugar testing after inactivating enzyme in the boiling water bath for 5 min [20]. Each sample was filtered with a 0.22 μm pore size hydrophilic membrane and diluted appropriately with deionized water. Quantitative analysis of glucose was measured using high-performance liquid chromatography (HPLC) system on a Waters Alliance (Waters e2695, Milford, MA, USA) equipped a Bio-Rad Aminex HPX-87H analytical column (300 × 7.8 mm) with the 5 mM dilute sulfuric acid was used as the flow phase with a flow rate of 0.6 mL/min. The enzymatic hydrolysis was calculated using following the equations:

$$\text{Bioconversion efficiency (\%)} = (\text{the amount of glucose released})/(\text{the amount of cellulose in substrate}) \times 100\%$$

3. Results and Discussion

3.1. Fractionation Efficiency and Morphologic Variation of Cell Wall during Pretreatment

In the present paper, the fractionation efficiency as the primary target for the whole utilization of bamboo is the emphasis of the pretreatment process. There were three parallel experiments for each condition, and the standard deviations of all data are less than 5%. The yields of all components obtained after fractionation was shown in Table S1, and the results of the compositional analysis of the three substances obtained were shown in Table 1. The data presented in Table 1 indicated that the increment of alkaline concentration improved either the carbohydrate degradation efficiency or the delignification, thereby, reducing the yields of the cellulosic fractions. However, the increased yield of total isolated products indicated that the higher efficient fractionation process was determined to be 4% NaOH in 60% EtOH aqueous solution. A similar phenomenon was also observed on the alkaline treatment of corn stalks [21]. The content of glucose was correspondingly increased from 40.7% (raw material) to around 50%, mainly due to the delignification process under alkaline condition. However, the slight decrement of cellulose in P_4C could probably be owing to the partial degradation of amorphous cellulose with 4% NaOH. Bai et al. also pretreated bamboo with increasing concentration and found that not only lignin and hemicellulose, but also cellulose was degraded to a certain extent [22]. Since only trace amount of hemicelluloses and a little amount (2.6%) of lignin were isolated with 1% NaOH, the slightly increased relative content of xylose in P_1C was not observed surprisingly. Next, the effect of EtOH concentration on the fractionation efficiency was investigated by changing from 20 to 80% with the fixed NaOH dosage (4%). Clearly, either the total yield of all fractions or the content of glucose in the cellulosic sample indicated that the most efficient condition for bamboo fractionation was 4% NaOH in 20% EtOH aqueous solution, especially for the extremely high yield of hemicellulosic fraction P_3H (25.6%). Although the concentration of NaOH in the aqueous solution could be significantly increased with higher EtOH content, expecting to enhance the fractionation efficiency, the hemicellulosic fraction could not be fully dissolved and then isolated in ethanol. As reported in previous literature, treated bamboo with higher ethanol concentration lost part of the cellulose and hemicellulose, mainly due to the oxidative polymerization or polycondensation between the furan monomers to form lignin-like aromaticity (pseudo-lignin) [23]. The degraded dextran and xylan could also form pseudo-lignin in ethanol, which is normally thought to be generated under hydrothermal or acid pretreatment. The increased content of lignin in P_5C sample further confirmed this conclusion.

Table 1. Yields and component analysis of the fractionated samples.

Samples		Yield [a] (%)	Chemical Components [b] (Relative wt%)			
			Glc	Xyl	AIL	ASL
Raw material		–	40.7	23.5	34.1	1.7
Cellulosic Fractions	P_1C	70.8	48.6	25.0	24.2	2.2
	P_2C	71.7	53.4	21.1	23.5	2.0
	P_3C	62.8	61.2	17.1	19.9	1.8
	P_4C	69.7	50.1	19.3	28.6	2.0
	P_5C	73.4	46.0	18.3	34.0	1.8
Hemicellulosic Fractions	P_1H	T [c]	–	–	–	–
	P_2H	0.5	0.5	62.3	34.6	2.6
	P_3H	25.6	0.7	60.5	36.2	2.6
	P_4H	7.8	2.0	69.0	26.7	2.4
	P_5H	8.3	0.8	65.4	31.3	2.5
Lignin Fractions	P_1L	2.6	0.6	T	96.4	0.2
	P_2L	6.8	1.0	0.9	95.6	0.2
	P_3L	3.6	0.7	T	94.6	0.1
	P_4L	5.8	0.4	0.2	97.0	0.3
	P_5L	7.2	0.5	T	96.6	0.3

[a] Values are weight % in oven-dried samples and the standard deviations are less than 5%. [b] Values are relative weight % and the standard deviations are less than 2%. Glc, Xyl, AIL, and ASL are assigned to glucose, xylose, acid-insoluble lignin, and acid-soluble lignin, respectively. [c] T = trace, <0.05%.

The morphologic property is commonly considered to be important for the accessibility and efficiency of the fractionation process. Obviously, the optical and SEM images substantiated that the mild alkaline pretreatment still resulted in the significant destruction of the intact cell structure (Figure 2), which paves the way for the following fractionation process. The raw material shows a compact and regular surface structure, and the fiber bundles were closely combined. Judging from the transverse section of the cell wall, lignin and cellulose were mainly assembled in the primary wall and secondary wall. Chu found that cellulose and lignin coexisted in the control group, and lignin hemicellulose structure existed in the cell wall [24]. After NaOH pretreatment, lignin was preferentially removed from the secondary cell wall. After the alkaline treatment in 60% ethanol aqueous solution, the variations on surface are observed apparently, containing the existence of gullies and gaps, the loss of cohesion within the fibers, and the exposure of inner cell wall. Fibril separation is clearly observed because the degraded fibers are split in the direction of the fiber axis, probably owing to the partial depolymerization and dissolution of hemicellulose and lignin fractions, and the partial degradation of the amorphous cellulose under alkaline condition [25]. In general, it could be speculated that the combination of polymer degradation and defibrillation of mild alkaline treatment released a large reactive area on the fiber surface, accordingly enhancing the subsequent fractionation efficiency. The extracted bamboo cellulose can be used in a number of applications. Other than its common role in pulp and paper production, the bamboo cellulose fraction could be used in a number of practical functions with energy and fiber composites such as plastic composites, food additives, and bioethanol [9].

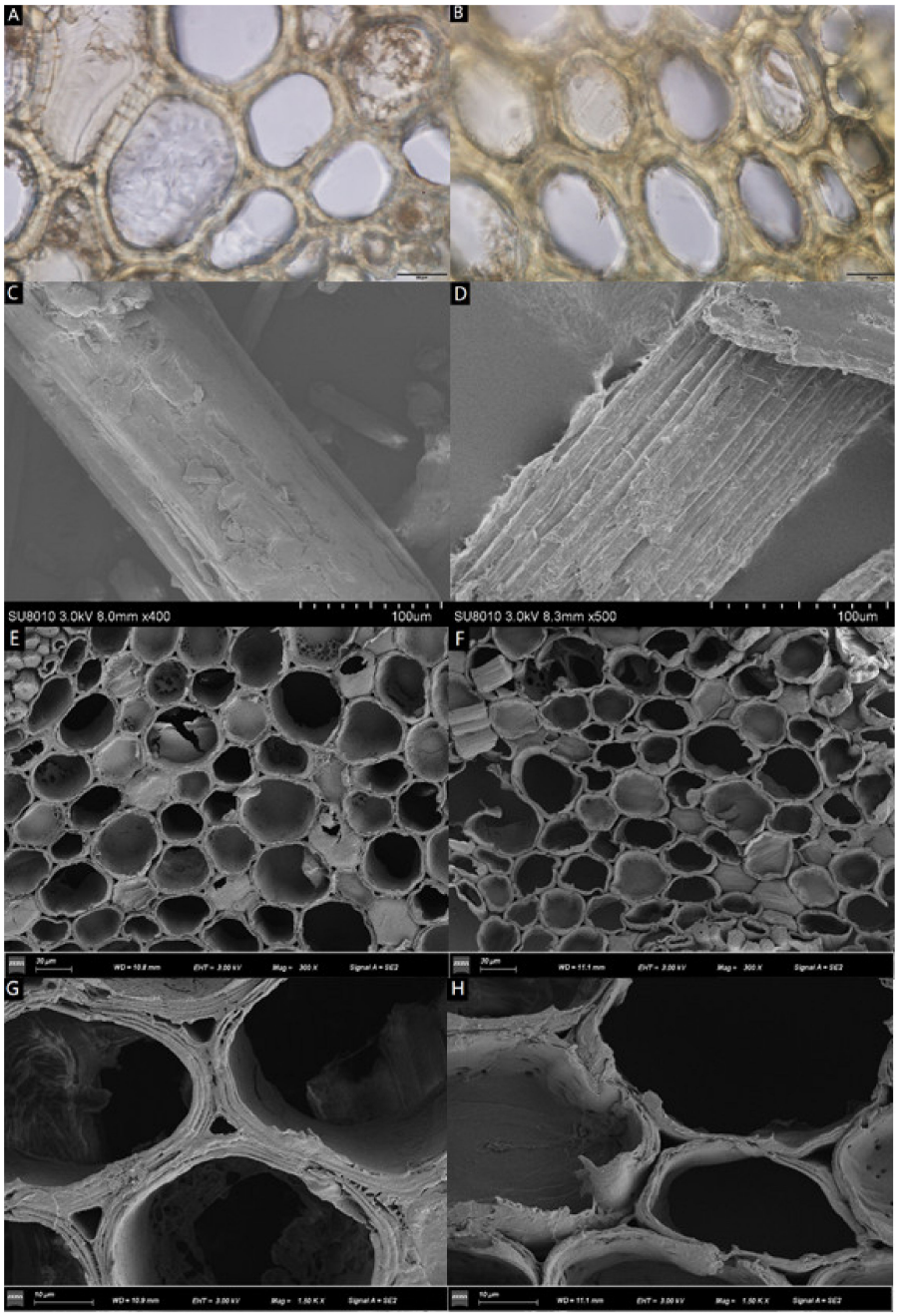
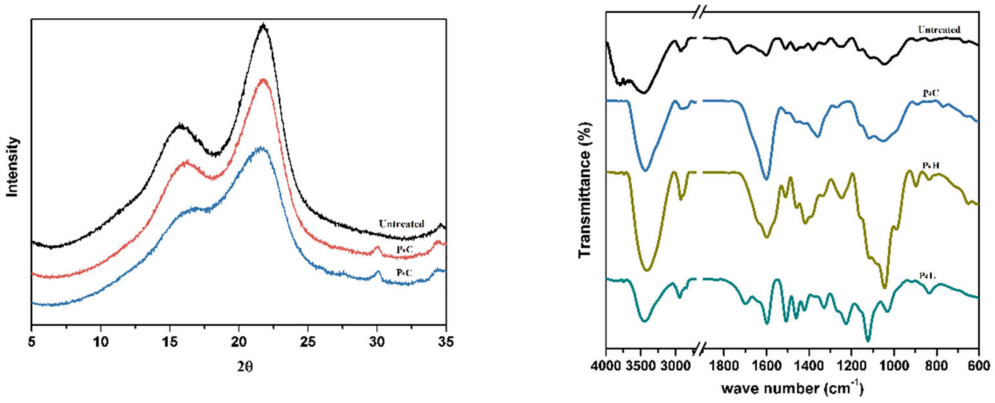


Figure 2. SEM images of bamboo biomass before and after pretreatment magnification ranging from 10 to 100 µm. (**A**,**E**,**G**) Untreated bamboo slices, (**C**) untreated bamboo powder; (**B**,**F**,**H**) 4%NaOH 20%EtOH treated bamboo slices, (**D**) cellulosic sample after pretreatment with 20% NaOH in 20% EtOH.

3.2. Physicochemical Analysis and Enzymatic Hydrolysis Efficiency of the Cellulosic Samples

The general physicochemical features of the cellulosic fractionations need to be sufficiently understood for analyzing the degradative capabilities of hydrolytic enzymes. Alkaline ethanol aqueous solution, as an efficient process for hemicellulose and lignin extraction, could be expected to achieve the partial fractionation of the main components, and distribution in the cell wall. XRD and FT-IR have been universally applied in the investigation of the supra-molecular order (crystal structure) of the cellulosic samples. The peak patterns of all spectra indicated typical cellulose (Figure 3), with the primary diffraction signals at 2θ values of 14.9°, 16.3°, and 22.5° in XRD curve [26], and the characteristic adsorptions at 3440, 1640, 1440, 1120, and 910 cm^{-1} in FT-IR spectroscope [27], respectively

(Supporting Materials, Table S2). By calculating, the crystallinity index of raw material was 24.5%, and it was significantly decreased to 18.8% (P_4C) after treating with 4% NaOH in 60% ethanol aqueous solution. A similar phenomenon was also presented in the previous literature, in which the crystallinity of bamboo chips were firstly increased and then decreased with increasing concentration of NaOH [15]. Meanwhile, the crystallinity index of the cellulosic samples fractionated under milder conditions (1 and 2% NaOH) were almost unchanged (26.2% for P_1C and 25.7% for P_2C), indicating that alkaline hydrolysis could occur with increasing alkaline concentration, leading to the swelling and dissolving of crystal cellulose. Furthermore, the concentration of ethanol also had obvious influence on the crystal configuration of cellulose, evidenced by the sudden decrement of crystallinity index (13.3% for P_5C). Since the ionization degree of ethanol is less than that of water, the concentration of H + in the solution is clearly decreased with adding alcohol, resulting in the significantly increased alkalinity of the solution. Therefore, serious alkaline hydrolysis could occur and the crystallinity of cellulose reduced.



(**a**) (**b**)

Figure 3. (**a**) X-ray diffractograms of the untreated and treated cellulosic fractions (untreated bamboo powder, P_4C, and P_5C); (**b**) FT-IR spectra of the raw and fractionated samples under wild conditions (untreated bamboo powder, P_3C, P_3H, and P_3L).

The majority of physicochemical modifications occurring at the cellulosic substrates could inevitably affect its accessibility to the hydrolytic enzymes. Removal of hemicellulose and lignin was thought to be effective for reducing the coating layers and linkages on cellulose, the non-productively binding of cellulase to matrix, as well as the inhibitory effect on the activity of commercial cellulase and β–glucosidase. Evidently, low bioconversion efficiency (around 30% yield for control) was obtained due to the limited accessibility and intact crystal structure of raw bamboo (Figure 4). After the mild alkaline fractionation, the efficiency of enzymatic hydrolysis significantly increased to around 70% after 72 h incubation, resulting from the partial removal of lignin and hemicellulose, and the improved accessibility of cellulose. Contrary to the expected effect, further increasing the alkaline concentration to 4% (P_4C) or the alcohol concentration to 80% (P_5C) lowered the bioconversion efficiency to the same level of raw material. One probable explanation could be the lignin-like substance was formed under these two conditions, responding to the high content of lignin in P_4C and P_5C, which reduces the efficiency of enzymatic hydrolysis [28]. Although the crystal structure of cellulose is known as the critical factor to inhibit the bioconversion process, the obvious decreased crystallinity indexes of these two samples did not positively affect the enzymatic hydrolysis. Therefore, the efficient removal of non-cellulose components could be the crucial element for enhancing bioconversion efficiency, according to the above physicochemical and structural analysis.

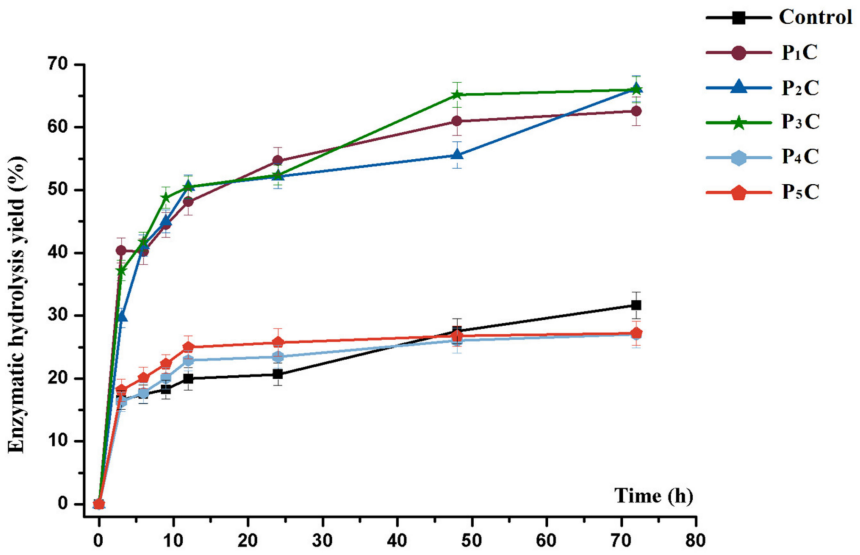


Figure 4. Effect of pretreatment conditions on enzymatic hydrolysis of the fractionated cellulosic samples.

3.3. Physicochemical Analysis of the Hemicellulosic Samples

Hemicellulosic components were extensively linked to lignin and cellulose by covalent bonds (ether and ester bonds) and non-covalent bond (hydrogen bond), respectively, forming the solid 3D network in the cell wall. The typical adsorptions of hemicelluloses are shown in the FT-IR spectrum (Figure 3). The stretching vibration of O-H hydroxyl group was detected at 3433 cm^{-1}, and the stretching vibration of C-H single bond was observed at 2922 cm^{-1} [29]. At 1614 cm^{-1}, glycoside bond and absorption of water-associated hydroxyl group were detected [30], and the C-H vibration of sugar end group was assigned between 1400–1200 cm^{-1} [31]. The characteristic peak for the stretching vibration of glycosidic bond was clearly exhibited from 1200 to 1000 cm^{-1}. Compared with the raw materials, the vibration of glycosidic bond was obviously enhanced in the relatively pure hemicellulosic fractions. Moreover, the stretching vibration of benzene ring at 1520 cm^{-1} and phenol hydroxyl OH vibration at 750 cm^{-1} are clearly seen in the FT-IR spectrum of P_4H, responding to the certain content of lignin from the component analysis above, indicating that current fractionation process was not adequately powerful to obtain the pure hemicellulosic polymer. The characteristic ^1H NMR spectrum of P_4H sample was further recorded to confirm the chemical property of hemicellulose (Figure 5). By the comparable analysis with the published paper, the peak at 1.69 ppm is assigned to the hydrogen atom of methyl or methylene [32], and the peaks in 3.81–4.26 ppm is ascribed to β-D-xylan. The signal at 4.95–5.12 ppm indicates that the glycosidic bond of hemicellulose is α–type [33]. The weak characteristic peaks of lignin were also found in the spectrum. The signal peaks at 6.0–6.3 ppm, 6.6–6.8 ppm, and 7.2–7.4 ppm were ascribed to the aromatic ring protons on syringic units, guaiacyl units, and para-hydroxy-phenyl units, respectively, and the peaks around 3.3–3.5 ppm are probably due to the hydrogen atoms at the side position of lignin.

The degree of polymerization is one of the most important characteristics of polymers, strongly affecting the direction and value of application. Therefore, the number average molecular weight (Mn) and weight average molecular weight (Mw) of the obtained three hemicellulosic samples were performed on gel permeation chromatography (GPC). There is not significant variation on the molecular weights of fractionated four hemicellulosic fractions, concentrating on a narrowly arrangement between 985 and 1032 Da. This phenomenon was also reflected from the slight shift of distribution curves in Figure 5. Under the mild conditions, the partial of hemicellulosic components were degraded and dissolved in alkaline aqueous solution and then precipitated in ethanol, and the left part still remained in the cell wall [34]. In this study, the value of Mw for all measured samples are much lower than the reported data, probably due to the location of growth and age of

bamboo. The Mw value of hemicellulose extracted from the hydrolysate of corn stalks was 2183 Da, and then decreased to 1267 Da after being precipitated in ethanol [35]. Because of its excellent biocompatibility, biodegradability, and physicochemical properties, the potential utilizations of hemicellulose have attracted an increasing amount of research over the last decade. Currently, research activities in the field of bamboo hemicellulose are focused on their utilization through conversion into biomaterials, biofuels, and foodstuffs. Peng also conducted studies on the biomaterials of bamboo hemicellulose in hydrogels, films, antioxidants, and stabilizers [36]. This study hopes to break the plant cell wall barrier to separate hemicellulose and to open new windows for the utilization of bamboo hemicellulose resources.

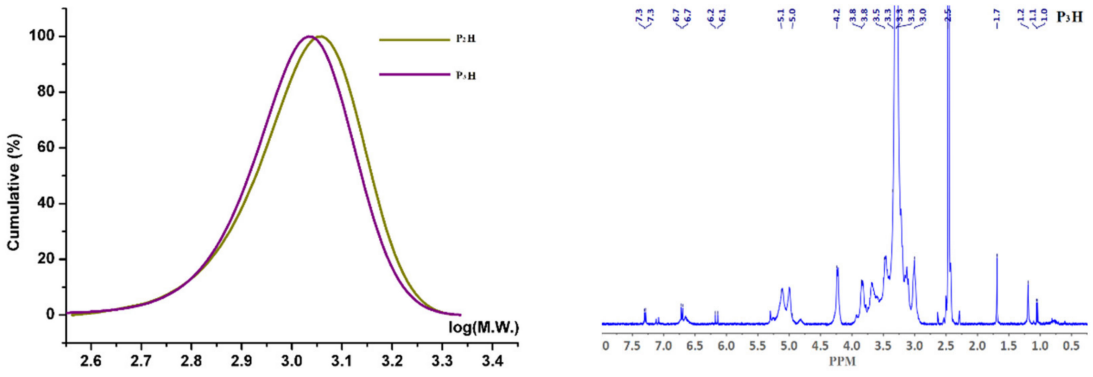
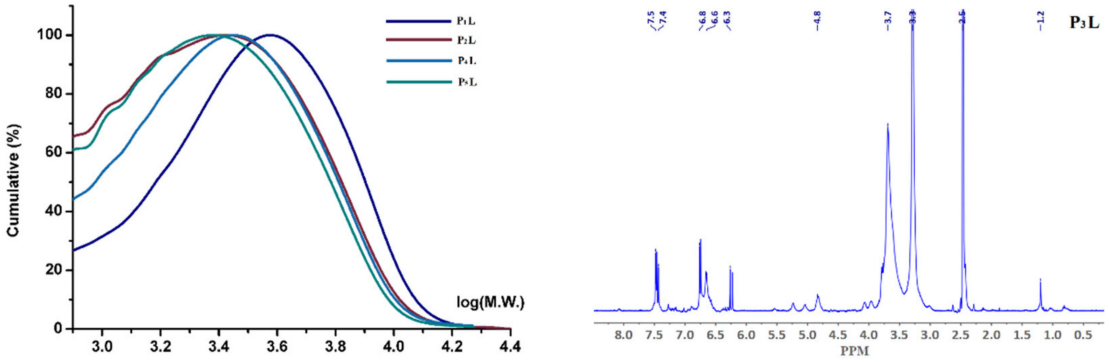


Figure 5. (**a**) Molecular weight distribution (P_2H and P_3H); (**b**) ^1H NMR spectrum of the selected hemicellulosic fractions (P_3H).

3.4. Physicochemical Analysis of the Lignin Samples

Fourier infrared spectroscopy is a supplementary and widely utilized instrument for acquiring information about hydrogen bonding patterns and molecular conformation. It was manifested from the spectra (Figure 3) that the stretching vibration of O-H and C-H are reflected at 3403 cm^{-1}, assigning to the aliphatic moieties in the lignin. The stretching vibration of C = O is reflected at 1720 cm^{-1} [37], and the aromatic hydrocarbon structure is shown at 1640 cm^{-1} [38]. The absorptions at 1605 and 1513 cm^{-1} manifest the aromatic skeleton vibrations, while the absorption at 1459 cm^{-1} is ascribed to the aromatic ring vibrations and C-H deformations. The sharp peak at 1114 cm^{-1} and the shoulder at 1033 cm^{-1} indicate the aromatic C-H in-plane deformation both in syringyl and guaiacyl type, separately. The structural characteristic of lignin fraction was further investigated with NMR technology (Figure 6). The signal peaks at 1.20 ppm are aliphatic hydrogen atoms, and the adsorption at 3.69 ppm is hydrogen atoms at the position of α, β, and γ on the side chain. The signal peaks at 6.26 ppm were side chain Hα (β-O-4 type), and at 6.65–6.76 ppm were aromatic ring protons on syringic units, side chain Hα (α, β conjugated double bond part) [39]. The signal peaks at 6.8–7.2 ppm were aromatic ring protons on guaiacyl units and at 7.2–7.6 ppm were aromatic ring protons on para-hydroxy-phenyl units [40]. The above FT-IR and NMR analysis exhibited the typical peaks from the functional groups in lignin. Since the pretreatment conditions in this study are not seriously changed, the structural variation and inter-connection breaking in the obtained lignin fractions was not obviously reflected. Therefore, the analysis on the molecular weight is important to verify the depolymerization of the lignin biomacromolecule (Figure 6). Clearly, the value of molecular weight of lignin fractions deceased from 3918 to 3017 Da with the increasing of the alkali and ethanol concentration, indicating the linkages break in the lignin substructure. It can be seen in the molecular weight distribution curves, the distinctive peak apparently became broader and shifted to the higher molecular weight region, representing a gradual increase of the polydisperisty and molecular weight. By increasing the concentration of ethanol in the extraction process, the portion in the lower

molecular weight (100–300 Da) was greatly decreased, and the portion in the higher molecular weight (300–10000 Da) was correspondingly increased [41].



(**a**) (**b**)

Figure 6. (**a**) Molecular weight distribution (P_1L, P_2L, P_4L, and P_5L); (**b**) ^1H NMR spectrum of the selected lignin fractions (P_3L).

4. Conclusions

In order to obtain a series of chemical products from bamboo, mild pretreatment and the following fractionation process were used to explore the efficient isolation of lignocellulosic materials in this study. Alkaline ethanol aqueous solution could obviously lose the compact structure of bamboo cell wall, resulting in the significant enhancement on the efficiency of component fractionation from bamboo. In respect to the enzymatic hydrolysis of cellulosic substrates, the optimum condition was determined to be 4% NaOH in 20% ethanol aqueous solution, 62.8% cellulosic fraction was separated, and 67% cellulose was biodegraded into glucose, which has a about 40% improvement in enzymatic efficiency compared to the untreated material. Meanwhile, over 25% hemicelluloses and 7% lignin were also isolated with the typical characteristics of a biomacromolecule, aiming to be further treated for the production of other chemicals or polymers. The molecular weight of the hemicellulose fraction was concentrated in a narrow arrangement between 985 and 1032, while the value of molecular weight of lignin fractions deceased from 3918 to 3017 Da. By combining the morphological variation of cell wall with the data from chemical and physical analysis, the critical factor on the fractionation process was explored and the further purification process on the obtained fraction should be focused on the following research. Although over 25% hemicelluloses were extracted, a certain amount of lignin still co-precipitated and decreased the purity of obtained hemicelluloses, which could have a negative effect on affect the following application of the polymer in different fields. Therefore, it is necessary to improve the separation efficiency of hemicellulose and lignin with high purity and original structure. The needed research is in progress. This study hopes to investigate an effective separation method for lignocellulosic materials that will allow all components in bamboo biomass to be better utilized for subsequent modification and research of high value materials to achieve whole utilization of bamboo biomass.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/en14216857/s1. Table S1: Summary of pretreatment conditions for separating main components of Moso bamboo, Table S2: Typical FTIR absorbance of functional groups in cellulose, hemicellulose and lignin.

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