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Coproduction of Furfural, Phenolated Lignin and Fermentable Sugars from Bamboo with One-Pot Fractionation Using Phenol-Acidic 1,4-Dioxane

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Abstract: A one-pot fractionation method of Moso bamboo into hemicellulose, lignin, and cellulose streams was used to produce furfural, phenolated lignin, and fermentable sugars in the acidic 1,4-dioxane system. Xylan was depolymerized to furfural at a yield of 93.81% of the theoretical value; however, the prolonged processing time (5 h) led to a high removal ratio of glucan (37.21%) in the absence of phenol. The optimum moderate condition (80 °C for 2 h with 2.5% phenol) was determined through the high fractionation efficiency. Consequently, 77.28% of xylan and 84.83% of lignin were removed and presented in the hydrolysate, while 91.08% of glucan was reserved in the solid portion. The formation of furfural from xylan remained high, with a yield of 92.92%. The extracted lignin was phenolated with an increasing content of phenolic hydroxyl. The fractionated lignin yield was 51.88%, which suggested this could be a low-cost raw material to product the activated carbon fiber precursor. The delignified pulp was subjected to enzymatic hydrolysis and the glucose yield reached up to 99.03% of the theoretical.

Keywords: one-pot fractionation with acidic 1,4-dioxane; Moso bamboo; furfural; phenolated lignin; enzymatic hydrolysis; high-efficiency fractionation

1. Introduction

Lignocellulosic biomass is a resource with great capacity and huge development potential of becoming a biorenewable feedstock for the bioeconomy [1,2]. It could be utilized as a renewable resource for producing materials, chemicals, and fuels [3,4]. However, for these purposes, it is essential to break down the crosslinking of the plant cell walls for the selective conversion of various chemicals [5,6]. For instance, hexose and pentose sugars can be transformed from the cellulose and hemicellulose fractions, the intermediate products for ethanol, furfural, 5-hydroxymethylfurfural, levulinic acid, and other chemicals productions [7]. However, the irreversibility of condensation tends to occur during the isolation process, which hinders the potential use of lignin as a renewable

resource [8]. Consequently, reducing side reactions (e.g., condensation) during early-stage biomass fractionation is essential for establishing a lignocellulose-to-chemicals value chain with competitive advantages [9,10].

An alternative strategy, called "lignin first process" (LFP), was introduced in order to maximize lignin valorization through a combination of extracting and depolymerizing lignin with a stabilized intermediate product [2]. The LFP concept has been well summarized in recent articles [11]. The lignin oil that was obtained through LFP has a low molecular weight and it illustrates a sharp contrast from traditional pulping methods (e.g., kraft, soda), which produce lignin with more condensation and less functionality [11,12]. There are still drawbacks despite the widespread usage of this process. The occurrence of repolymerization promotes a lower monomer yield and selectivity under the harsh processing conditions. The upgrading of obtained products has created a bottleneck, requiring further biorefinery for bulk chemicals (e.g., phenol and propylene) [13]. The efficiency of biomass refining can further increase if appropriate modified synthetic materials were directly obtained in the reaction system.

The principle way for lignin to overcome the constraint in LFP is likely by directly converting lignin into a polymeric matrix for material applications. It is known that activated carbon fibers (ACFs) have a highly developed pore structure with a specific surface area and it is uniformly microporous; therefore, they are superior to commercial activated carbons [14]. However, the high cost and limited supply of this material are obstacles for high quality production on a large scale. Recently, the development of products from the phenol-formaldehyde reaction has attracted more attention. As products of the lignin-to-materials value chain, there is a promising future for these products in the fields of biology, environment, and energy [15–17]. Phenolated lignin is a desired product of phenol-formaldehyde reaction that is created by controlling the reaction conditions. Phenolated lignin is a raw material for ACFs production that has a low price due to its high accessibility and renewability [16]. Phenolation treatment was an effective method for improving lignin reactivity under basic and acidic conditions. Hence, phenolated lignin constitutes a better product for macromolecular application (i.e., lignin-to-materials).

Recently, targeting the one-pot fractionation has been reported to extract and depolymerize both lignin and hemicellulose for stable target products (phenolics and polyols, respectively) [18]. Relative studies have shown that a disadvantage of LFP is the irreversible modification of the polysaccharide fraction, in addition to bulk chemical refining issues [13]. The accurate control of the reaction conditions is required to exploit hemicellulose for stable and soluble platform chemicals [3,19]. One possible solution has been demonstrated in the dioxane solvent system with acid catalytic to convert solubilized hemicellulose into high-yield furfural via dehydration [20]. No relevant literature is available on simultaneously depolymerizing lignin and hemicellulose into a polymeric matrix and furfural through one-pot fractionation. Precise tuning of process conditions is required in order to further optimize the fractionation efficiency.

Here, a creative biorefinery concept was investigated, aiming at the one-pot fractionation of raw lignocellulose into three streams: (i) hemicellulose-derived furfural, (ii) lignin-derived phenolated lignin, and (iii) cellulosic pulp. Fractionation was performed in the mixture of 1,4-dioxane and water, hydrochloric acid (HCl) as a catalyst with phenol addition. After the reaction, the three main components of lignocellulose were separated, and an efficient method of transformation from those into the corresponding products was proposed. This work highlighted the potential benefits of phenol as an increasingly efficient promoter of the preferred process of fractionation and it provided a foundation for further development of integrated biorefinery with acidic 1,4-dioxane system.

2. Materials and Methods

2.1. Materials

Moso bamboo, from the International Center of Bamboo and Rattan (Beijing, China), was air dried and screened through 40 mesh. The water content of the Moso bamboo material was approximately 10.03%. The utilized chemicals were analytical grade.

2.2. Standard Procedure for Lignocellulose Fractionation

The substance of liquid phase was composed of 1,4-dioxane, HCl and deionized water. The 1,4-dioxane concentration in liquid flow was 90%, 70%, and 50% (*v*/*v*), corresponding to 900, 700, and 500 mL addition, according to a previous study [20], and the concentration of HCl was 4.2% (*v*/*v*). The deionized water was added to reach 1000 mL of the liquid volume. In solid phase, bamboo (100 g) and phenol (0, 1.0%, 1.5%, and 2.5%, *w*/*v*) were mixed with the solution for a subsequent fractionation process. The mixture was transferred to a stainless steel tubular homogeneous reactor (Shanghai Yanzheng Experimental Instrument Co., LTD., Shanghai, China), heated between 80 °C and 140 °C, and then kept for 1–5 h. Two parallel experiments were investigated, and detailed reaction conditions are shown in Table S1.

After the reaction, the mixture was filtered for solid-liquid separation. The solid portion was washed with 2000 mL deionized water, while the liquid portion was added into H_2O drop by drop to prepare two fractionated lignin samples (in order to ensure the pH value was 2, the volume of H_2O was 320 mL). The lignin in sample II is treated with phenol while that in sample I is not.

2.3. Enzymatic Hydrolysis

Commercial enzyme cellulase of Cellic Ctec2 from Novozymes (Beijing, China) was used. The filter paper activity of Cellic Ctec2 was 160 FPU/mL, and the contained cellobiase activity was 24 CBU/mL. The dosage of Cellic Ctec2 was 18 FPU/g glucan. The hydrolysis experiments were performed at 50 °C and 150 rpm for 72 h with three parallel experiments [21]. Acetate buffer solution (pH 4.8) and 5% (*w*/*v*) substrate concentration were proposed.

2.4. Analysis Methods

The NREL method was utilized to detect the material composition [9,22]. The amount of glucose, furfural and xylose were analyzed by HPLC-Waters 2695e (Waters, Milford, MA, USA) according to previous research [21].

The resultant solution of 1 mL was sampled for phenol analysis without any further treatment other than the addition of 100 μL as-prepared internal standard (100 mg octanol in 50 mL acetone) [23]. The solution (~1.1 mL) was analyzed with a gas chromatography (GC, Agilent 7890B series), according to the Reference [23]. The injection temperature was 250 °C with column temperature program of: 80 °C (1 min.), 20 °C/min. to 120 °C, 50 °C/min. to 280 °C, and 280 °C (2 min.). The detector temperature was of 300 °C. The production yields were calculated by following Equations (1)–(7).

$$\text{Furfural yield (\% of the theoretical value)} = (\text{Furfural detected} \times 132 \times 100)/(\text{Xylan in substrate} \times 96) \quad (1)$$

$$\text{Glucose yield (\% of the theoretical value)} = (\text{Glucose detected} \times 0.9 \times 100)/\text{The theoretical glucan value in substrate} \quad (2)$$

$$\text{Xylose yield (\% of the theoretical value)} = (\text{Xylose detected} \times 0.88 \times 100)/\text{The theoretical xylan value in substrate} \quad (3)$$

$$\text{Solid yield (\%)} = \text{Treated dry solid mass} \times 100/\text{Untreated dry solid mass} \quad (4)$$

$$\text{Retention ratio (\%)} = \text{The corresponding content of the treated material} \times \text{Solid yield} \times \quad (5)$$

$$\text{Removal ratio (\%)} = (\text{The corresponding content of the untreated material} - \text{The corresponding content of the treated material} \times \text{Solid yield}) \times 100/\text{The corresponding yield of the untreated material} \quad (6)$$

$$\text{Fractionated lignin ratio (\%)} = \text{Mass of acid extraction of lignin}/(\text{Mass of lignin in raw material} + \text{Mass of reacted phenol}) \quad (7)$$

2.5. Characterization of the Fractionated Lignin I and II

Mw and Mn, as well as polymer dispersity index (PDI) of the lignin-derived products, were testified by GPC-Waters 2695e (Waters, Milford, MA, USA) analysis, according Wen's method [24]. FT-IR was analyzed by Bruker-VERTEX 70 (Bruker, Germany) according to Xie's method [25]. The 2D HSQC analysis was performed according to Yang's method [26].

2.6. Statistical Analysis

The data analysis was evaluated by SPSS statistics 22.0 according to Zhou's method [27]. The *p*-values were examined for the significant difference that was influenced by phenol-acidic 1,4-dioxane fractionation process, and each value of p less than 0.05 indicated the difference was significant [27].

2.7. Severity Factor

The severity factor (expressed as $\log R_0$) was used to quantify the intensity of phenol-acidic 1,4-dioxane fractionation process of biomass by Equation (8) [27], in which t is the residence time (min.) and T is the temperature of the reaction condition. The reaction temperature and time duration can be used as a parameter to critically control the experiment conditions, as a stronger reaction intensity of Moso bamboo increased the mass of dissolved components. The severity of this experimental design was from 1.49 to 3.26, as shown in Table S1. These conditions were selected in order to ensure distributed severities within the equal range of reaction time and temperature.

$$\log R_0 = \log \{t \times exp\,[(T - 100)/14.75]\} \quad (8)$$

3. Results and Discussion

Figure 1 shows the overall scheme of the one-pot fractionation with acidic 1,4-dioxane. 1,4-dioxane of 900 mL, phenol (2.5%, *w*/*v*), and HCl (4.2%, *v*/*v*) catalyst were added to a 2000 mL stainless steel tubular reactor, together with 100 g of Moso bamboo. As was determined using the NREL method, Moso bamboo were composed of ash 1.80%, lignin 24.90%, glucan 46.00%, hemicellulose (a.k.a. xylan (24.00%), substitute for arabinan (0.30%), and mannan (0.20%)). After the reaction, a majority of the glucose in the solid fraction was recovered after the process of enzymatic hydrolysis, while most of the xylose was recovered as furfural in liquid hydrolysates. The solution was analyzed with GC in order to calculate the quantity of phenol in the process: 17.9 g of phenol was reacted with 25.0 g of lignin, indicating the numerical results of phenolation reaction. The total amount of fractionated lignin II containing phenolated lignin was 22.3 g, and the recovered yield of fractionated lignin II was 51.88% when the dosage of phenol was 2.5% (*w*/*v*) at 80 °C. Overall, the majority of the glucose was recovered from the enriched cellulosic pulp, a large fraction of furfural was obtained from xylan, and the phenolated lignin level was improved by increasing the phenol dosage, which resulted in a pulp fraction with a relatively higher purity.

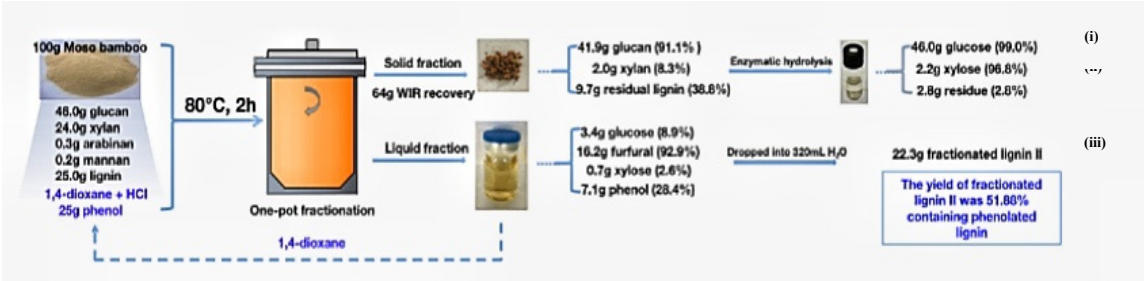


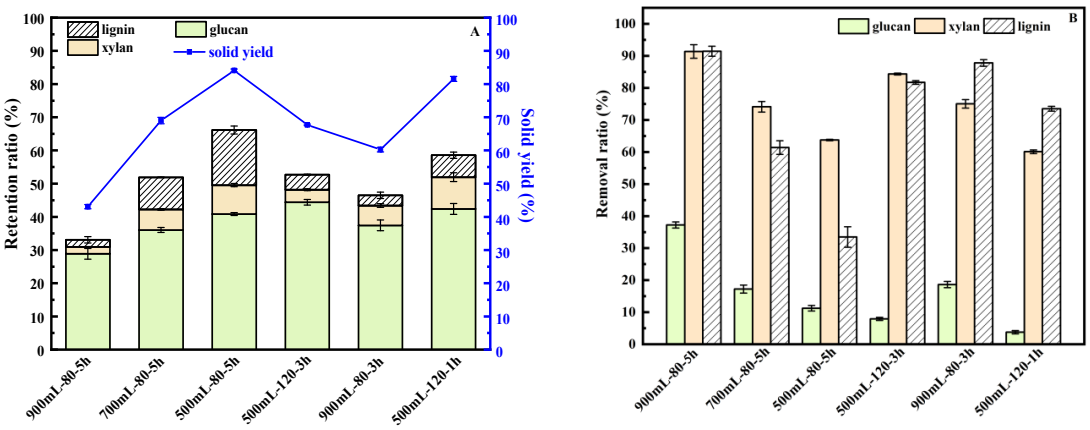
Figure 1. General scheme of the one-pot frationation process targeting (**i**) hemicellulose-derived furfural, (**ii**) lignin-derived phenolated lignin, and (**iii**) cellulosic pulp. WIR, water insoluble residues.

3.1. Influence of 1,4-Dioxane Dosage on Fractionation

Various solvents, including N,N-dimethylformamide, DMSO, methanol, acetone, 1,4-dioxane, and so on, would be appropriate options for lignin dissolution and separation [28]. As a common solvent for lignin, 1,4-dioxane still has some drawbacks, such as the irritation to eyes and upper respiratory tract, flammability, and higher cost [29]. Despite its weaknesses, 1,4-dioxane can be miscible with water and most organic solvents for dissolving more of lignin for value-added products.

Brown et al. reported that cyclic ethers, such as dioxane, could act as an efficient solvent for lignin extraction [30]. In the present case, 1,4-dioxane, boiling point at 100 °C, was selected as the extraction medium. This enables the separation of lignin from biomass into an acid solution and the subsequent recovery by distillation.

Figure 2 compared the distribution and composition of the biomass in the 1,4-dioxane/HCl system at various temperatures and reaction times. The retention ratio of glucan/xylan/lignin increased significantly by decreasing the 1,4-dioxane dosage from 900 to 500 mL/100 g bamboo ($p < 0.05$). The mass reduction of lignin with a 1,4-dioxane dosage of 500 mL/100 g bamboo during a 3 h reaction was 81.71%, while it was 87.80% under conditions of 900 mL/100 g bamboo with the same reaction time (Figure 2B). This phenomenon indicated that 1,4-dioxane played a key role in lignin dissolution and removal [20].



(**A**) (**B**)

Figure 2. Effects of 1,4-dioxane (500, 700, and 900 mL/100 g bamboo), reaction temperature (80 and 120 °C), and reaction duration (1, 3, and 5 h) on (**A**) the retention of various components, and (**B**) glucan/xylan/lignin removal ratio based on the original weight of components.

Glucan remained solid in the pulp for subsequent enzymatic hydrolysis for glucose. The retention ratio of glucan increased at a higher temperature of 120 °C within a shorter reaction time of 1 h with a lower 1,4-dioxane dosage of 500 mL/100 g bamboo. This indicated that glucan degradation mainly depends on the 1,4-dioxane dosage and reaction time. As a member of Gramineae plants, bamboo presents a typical structure with brittle skeleton that contributes to the enhanced degradation of cell wall polysaccharides during the fractionation process. This explains how the content of xylan was substantially reduced ($p < 0.05$) [31]. Stein et al., suggested that the separation of cellulose, hemicellulose, and lignin occurred due to the difference in their structure and solubility properties [32].

For any biorefinery scheme, the separation of various lignocellulose fractions is crucial. Table 1 showed the effects of 1,4-dioxane (500–900 mL/100 g bamboo) under the given temperatures and time conditions. Increasing the amount of 1,4-dioxane increased the efficiency of soluble product and increased the fractionated lignin I yield to 54.00%. However, a greater decomposition of glucan was observed when the substrate was treated with 1,4-dioxane of 900 mL/100 g bamboo. No apparent improvement in lignin extraction was observed under a higher 1,4-dioxane dosage. A 1,4-dioxane dosage of 900 mL/100 g bamboo was considered to be the optimal concentration for fractionation efficiency, although it showed a weaker selectivity regarding glucan fraction reservation (Table 1).

Table 1. Comparison of 1,4-dioxane addition during the fractionation of lignin.

Entry	1,4-Dioxane Addition (mL/100 g Bamboo)	T (°C)	Time (h)	Fractionated Lignin I Yield (%)
1	900	80	5	54.00 ± 0.88
2	700	80	5	42.24 ± 0.93
3	500	80	5	23.95 ± 0.91

3.2. Influence of Phenol Addition on Fractionation

When considering the HCl-catalyzed reaction, various phenol amounts (1.0–2.5%, *w*/*v*) and varying temperatures (from 80–140 °C) were applied in this study in order to observe their influence on biomass fractionation. The fractionation process was performed for a shorter reaction time of 2 h when compared to the process without phenol. The weight loss of glucan substantially increased from 3.32–16.74% as reaction temperature increased from 80–100 °C, as shown in Table 2.

Table 2. Comparison of phenol addition in the fractionation of lignin treated with 1,4-dioxane of 900 mL/100 g bamboo at various temperatures.

Entry	Phenol Addition (%, *w*/*v*)	T (°C)	Time (h)	Removal Ratio of Lignin (%)	Yield of Fractionated Lignin II (%)	Weight Loss of Glucan (%)	Enzymatic Hydrolysis (%)	Furfural Conversion (% Of Theoretical Yield)
1	1.0	80	2	78.62 ± 1.21	43.36 ± 1.39	3.32 ± 0.07	93.52 ± 1.13	89.78 ± 1.48
2	2.0	80	2	81.65 ± 0.76	46.54 ± 0.88	4.59 ± 0.11	95.14 ± 1.16	91.06 ± 1.26
3	2.5	80	2	84.83 ± 1.43	51.88 ± 0.75	8.92 ± 0.13	99.03 ± 0.97	92.92 ± 1.23
4	1.0	100	2	81.71 ± 1.28	37.92 ± 0.54	11.80 ± 0.42	96.25 ± 1.63	87.57 ± 0.79
5	1.5	100	2	85.22 ± 0.99	40.56 ± 0.92	15.50 ± 0.56	99.22 ± 0.86	88.94 ± 0.53
6	2.5	100	2	86.98 ± 0.23	42.79 ± 1.03	16.74 ± 1.01	99.26 ± 1.31	90.78 ± 1.09

The pulp yield sharply decreased as temperature increased up to 140 °C, as seen in Figure S1 in the supplementary data. The retention ratio of glucan was less than 10% under the reaction condition. This was because glucan was dramatically degraded into liquid in the presence of phenol at a temperature no less than 120 °C. Because the majority of the glucan degradation mostly occurred in the initial phase of delignification, a higher temperature would lead to more glucan degradation [33]. High temperatures were not conducive to a higher fractionated lignin II yield. The retention ratio of lignin in the solid phase was slightly greater than 80%, as shown in Figure S1. Lignin was condensed under high intensity reaction conditions, and the condensation reactions occurred on the aromatic ring of lignin (C2, C5, or C6), forming new structures and remaining in the solid phase [8]. In Table 2, the weight loss of glucan under the reaction condition of 80 °C, 2 h with the addition of phenol was less than 10% (Entry 3). However, the loss of glucan reached 37% under the treatment condition of 80 °C, 5 h without phenol addition (Figure 2).

Previous studies showed that the content of glucan was primarily affected by the reaction time. The internal crystalline structure of cellulose was damaged, and the degree of polymerization value of cellulose was decreased [6]. It can be observed that increasing the amount of phenol led to an increase in the soluble product yield, with 2.5% (*w*/*v*) of phenol being the optimal amount. More lignin was removed, as most was dissolved during the process, and the phenolated lignin was subsequently precipitated by placing it into water. Phenolated lignin is a more effective raw material for polymer production and has many potential uses in the fields of environment and energy [34]. The phenol reacted with the lignin and bound to its active site in order to form a higher activity product. A significantly higher extraction yield of fractionated lignin II (51.88%) was obtained ($p < 0.05$) with phenolated lignin being added.

3.3. Conversion of Hemicellulose to Furfural

Improved hydrolysis of the acid-catalyzed dioxane degraded a majority of the hemicellulose (a.k.a. xylan) into a liquid mixture for further furfural extraction. The previous results indicated that, under the acidic 1,4-dioxane experimental conditions, the removal ratio of Moso bamboo particles was, as follows: 33.48–91.45% of lignin, 60.10–91.37% of xylan, and 62.76–96.53% of glucan remained in the solid fraction (see Figure 2B).

Xylan depolymerization was observed via furfural formation in the aqueous phase. The highest furfural conversion yield of 93.81% of the theoretical value was consistent with highest furan yield in the presence of organic solvents and low water concentration (Table 3) [20]. Normally, xylose is produced during the depolymerization of xylan that is treated with a dilute acid. The dehydration of xylose treated with dilute Brønsted acid resulted in the formation of furfural, a platform molecule [35]. It can be observed that adding phenol to the one-pot fractionation system maintained the high yield of furfural conversion at approximately 90% of the theoretical as compared with the no-phenol-addition process (Tables 2 and 3). Increasing the amount of phenol led to an increase in the soluble product yield, with 2.5% (*w*/*v*) of phenol being the optimal amount. The formation of furfural from xylan in the hydrolysate had a high yield of 92.92%.

Table 3. Results of the theoretical furfural yield from Moso bamboo under various reaction conditions.

Entry	1,4-Dioxane Solvent Addition (mL/100 g Bamboo)	T (°C)	Time (h)	Furfural Conversion Yield (% of Theoretical Yield)
1	900	80	5	93.81 ± 1.05
2	700	80	5	69.03 ± 0.89
3	500	80	5	57.43 ± 1.01
4	500	120	3	80.42 ± 1.23
5	900	80	3	91.43 ± 0.77
6	500	120	1	72.46 ± 0.69

In previous studies, various organic solvents (e.g., γ-valerolactone (GVL), tetrahydrofuran (THF), and 2-methyltetrahydrofuran (2-MTHF)) were designed for furfural production from xylan and several catalysts were added in order to promote the production yield of furfural (Table 4) [36–41]. A bio-based system of 2-MTHF/H_2O was reported while using SnCl4 as catalyst with the highest furfural yield of 78.1%. With a silicoaluminophosphate (SAPO) catalyst by continuous stripping and minimizing of the amount of humin output, an improved furfural yield of 63% could be achieved; however, this manufacturing technique was expensive and complicated [37]. Ionic liquids are preferable solvents for the conversion of xylan-type hemicellulose that have gained wide attention. A selective conversion of hemicellulose fraction was performed in [bmim] [HSO_4] ionic liquids to produce 36.2% furfural [40].

In this study, we provided a simpler, more competitive, and highly efficient fuel precursor for a more cost-effective furfural separation process. Generally, reactions at higher temperature with longer time and using a higher dosage of 1,4-dioxane indicate a higher yield of furfural. HCl is a type of Brønsted acid, and it was found to be the most effective catalyst for xylan conversion efficiency [42]. As seen in the tables, furfural almost reached its maximum amount considering the chemical composition of bamboo, while arabinose had minimal influence on the furfural yield.

Table 4. Comparison of furfural production yield under various reaction conditions.

Starting Materials	Solvent	Catalyst	Conditions	Yield [a]	Reference
xylan	GVL-10% H_2O	$FeCl_3 \cdot 6H_2O$	170 °C, 100 min	68.6% [a]	Zhang et al. [36]
xylan	H_2O-THF	$AlCl_3 \cdot 6H_2O$-NaCl	140 °C, 45 min	64% [a]	Yang et al. [37]
xylan	2-MTHF-H_2O	NaCl-SO_4^{2-}/Sn-MMT	160 °C, 90 min	77.35% [a]	Lin et al. [38]
bagasse	H_2O/toluene	SAPO	170 °C, 8 h	63% [b]	Bhaumik et al. [39]
wheat straw	[bmim][HSO_4]	-	160 °C, 120 min	36.2% [b]	Dussan et al. [40]
corncob	H_2O	0.9 mmol H_2SO_4	160 °C, 5 h	71% [b]	Wang et al. [41]
Moso bamboo	H_2O/1,4-dioxane	HCl	80 °C, 3 h	91.47%	In this study
Moso bamboo	H_2O/phenol-acidic1,4-dioxane	HCl	80 °C, 2 h	92.92%	In this study

[a] furfural yield (accounted on C5 fraction in hemicellulose) [b] furfural yield (accounted on C5 fraction in biomass).

3.4. Conversion of Lignin to Phenolated Lignin

Multiple methods have been reported on the increase of lignin reactivity, including methylolation/hydroxymethylation, phenolation, demethylation, oxidation/reduction, and hydrolysis/hydrogenolysis. A high consumption of hazardous chemicals commonly was required in order to obtain a high conversion yield through oxidation/reduction and demethylation. It showed poor selectivity of compound formation during hydrolysis and hydrogenolysis, including the free ortho numbers and para positions that are associated with the aromatic ring. In addition, the hydroxymethylation of lignin showed a higher selectivity of maximum activation levels, but moderate yield. It has been reported that phenolated lignin has stronger mechanical behaviors than raw or hydroxymethylated lignin [43]. Furthermore, the β-O-4 bond could generate new reactive sites with aicidic catalysts [44]. Nonaka et al. reported that phenolation could be directly applied to wood pulp directly in order to obtain phenolated lignin [45].

GPC results showed that the added phenol had a positive result on the Mw of the lignin-derived products (see Table 5 and Figure S2). The Mw of lignin I and lignin II were lower than that of pretreated solid lignin [45]. As a typical organosolv, dioxane, with its appropriate solubility parameter, is an attractive isolation agent of lignin fraction [18]. Phenol, as a suppressor, was proven to prevent repolymerization of lignin [45]. This effect increased the solubility of the lignin with the reduced molecular weight. The products from the above strategies have higher monomer yields and selectivities than kraft or soda lignin. Phenolysis occurred through the attachment of phenol to lignin through the catalysis of acid, forming the substances with low molecular weight that corresponded to the results of Funaoka's research, as shown in Figure 3 [46].

Table 5. The Mw, the Mn, and polymer dispersity index (PDI) of the lignin-deriver products.

Lignin-Derived Products	Mn (g/mol)	Mw (g/mol)	PDI
lignin I	6.515×10^2 (±2.467%)	8.237×10^2 (±2.241%)	1.264 (±1.895%)
lignin II	5.611×10^2 (±3.048%)	6.936×10^2 (±2.924%)	1.236 (±4.224%)

Figure 3 presents the FT-IR spectra of the fractionated lignin I and II samples from the acid-catalyzed 1,4-dioxane-treated Moso bamboo. The bands at 1423 cm^{-1} corresponded to the aromatic skeletal vibrations, while the deformed C-H combining with the aromatic ring vibration was at 1461 cm^{-1} [25]. These two characteristic absorption peaks gradually receded with phenol addition, which was in accordance with the 2D-heteronuclear singular quantum correlation (HSQC) result. After phenolation, the peak intensity of lignin II at 1370 cm^{-1} was strengthened as compared to that of lignin I, indicating the increased content of phenolic hydroxyl. The impact of this increase was primarily due to the combination of phenol with lignin and not the hydrolysis of aryl ethers [47].

The phenolic hydroxyl group of lignin has important functions in further applications, specifically its positive influence on lignin molecule functionalization [48,49]. Higher substitution rates of lignin to phenol could be achieved through the enhanced lignin solubility and chemical reactivity related to the increased hydroxyl content [49,50]. Historically, the study of macromolecular applications has driven much of the research regarding developed lignin valorization by analyzing variations of hydroxyl content. This research contains a vast array of promising applications for carbon nanofibers [12,44]. It has been proven that the as-obtained product of phenolated lignin can be applied to macromolecular material production, in particular to the low-cost production of activated carbon fiber precursors [10,16,51], providing the opportunity to create the achievement of lignin-to-materials [9,52].

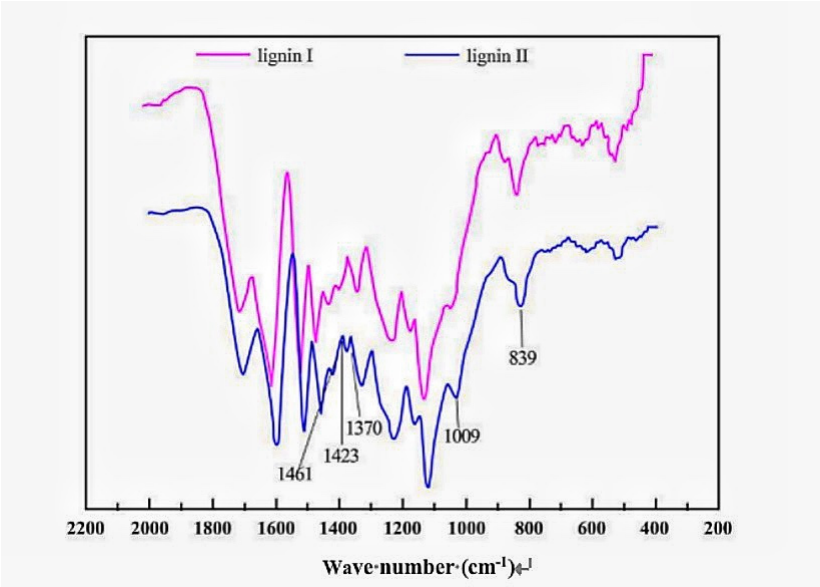
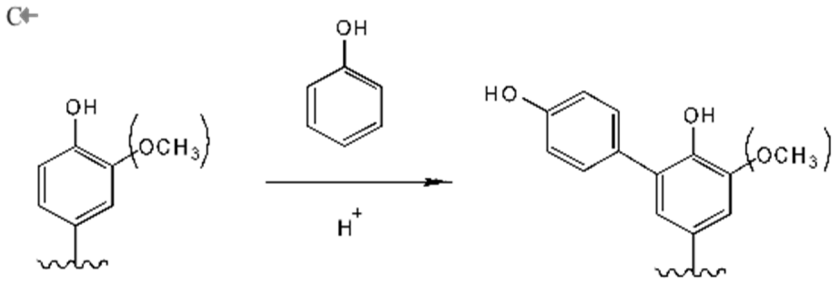


Figure 3. FT-IR spectra of Moso bamboo treated with acid-catalyzed 1,4-dioxane of 900 mL at 80 °C for 5 h and treated with 900 mL 1,4-dioxane at 80 °C with phenol (2.5%, *w*/*v*) for 2 h.

Figure 4 shows the 2D-HSQC spectra of the fractionated lignin I and II. The peak assignments/distribution were based on Ref. [26]. Figure 3 depicts the sub-units of the identified lignin. The cross signals of methoxyls (δC/δH 55.9/3.73), β-O-4, and the Cβ-Hβ correlations corresponding to the β-β linkages were in the side chain of lignin. It can be seen from the spectrum that the β-O-4 substructure intensities in the spectrum of lignin were obviously lower when compared to the original Moso bamboo lignin detected in previous work [24]. The β-O-4 linkage constitutes the most abundant link of lignin. During the process, bamboo was treated with acidic dioxane both with and without phenol; the β-O-4 linkages of the bamboo lignin were broken because of the lability of alkyl aryl ethers under acidic conditions. Thus, most of the cleavage occurred on the β-O-4 bonds. Weak signals of side-chain linkages (β-O-4, β-5, β-β) represent a low Mw and a relatively high content of phenolic hydroxyl in the lignin. Yang et al. observed similar results [26]. Hence, this lignin could be a valuable phenolic material.



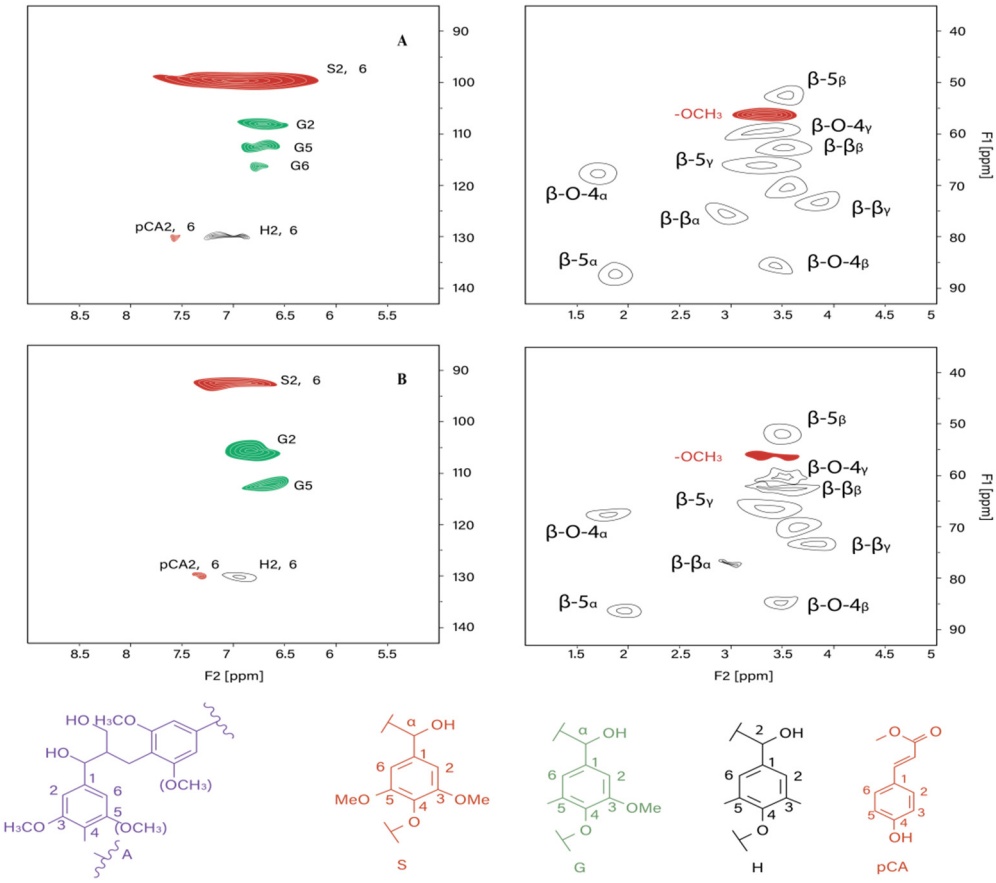


Figure 4. 2D-HSQC NMR spectra of lignin treated with 1,4-dioxane of 900 mL/100 g bamboo, acid-catalyzed at 80 °C for Moso bamboo. (**A**) Samples of the fractionated lignin I treated for 5 h without phenol. (**B**) Samples of the fractionated lignin II treated for 2 h with 2.5% phenol (*w*/*v*). (**C**) The reaction scheme of phenol with lignin units. The hollow area represents the reacted group.

The main 2D-HSQC spectra cross-signals of lignin in the aromatic region were appointed to the lignin units, including H, G, and S structures [26]. A significant difference was observed in the fractionated lignin II and I, treated with and without phenol respectively. The phenolation of lignin units was a crucial step toward achieving a higher phenolated lignin yield when compared to the reaction system without phenol addition [49]. The obvious disappearance of G_6 and $H_{2,6}$ linkages illustrated that phenolated lignin was produced by the reaction between phenol and lignin under acidic 1,4-dioxane with added phenol. Figure 4 shows the reaction scheme. Low intensity p-coumarate (PCA) signals were detected in the spectrum. The existence of phenol caused a frequency shift on the electron cloud of C_α.

3.5. Conversion of Carbohydrate Pulps to Fermentable Sugars

The effective saccharification of cellulose to fermentable sugars is the fundamental step required for carbohydrate pulp valorization [3,53,54]. Figure 5 shows both the glucose and xylose yields of raw Moso bamboo and acidic 1,4-dioxane treated residues. The lowest glucose yield of 36.38% was obtained after a 72-h enzymatic hydrolysis with raw material as the substrate. The efficiency of enzymatic hydrolysis was enhanced after reaction with acidic 1,4-dioxane. The glucose yield was improved with the increased dosage of 1,4-dioxane during the fractionation process. The glucose yield of 98.74% was obtained after 72 h from the substrate treated with 1,4-dioxane of 900 mL/100 g bamboo. Most of the xylose was recovered in the form of furfural in liquid hydrolysates and furfural yield was 93.81% of theoretical value (Table 3). There was still 2.10 g xylan (of 100 g Moso bamboo)

remained in the solid fraction after acid-catalyzed dioxane reaction. An extra 2.33 g of xylose was later recovered from xylan after enzymatic hydrolysis; thus, the overall yield ratio was 97.75%. Evidence suggested that the xylan removal improved the enzymatic hydrolysis of cellulose [55]. It can be seen from Figure 2 that the removal ratio of xylan and lignin was close to 90%, obtaining a solid pulp of high-purity glucan with a retention ratio over 90%. The glucose yield reached 92.46% of the theoretical value for 12 h after enzymatic hydrolysis (Figure 5). This was essentially ascribed to the effective removal of lignin and xylan from the treated substrates, so that cellulase and cellobiase had more space to get into glucan and hydrolyzed glucan to glucose [56].

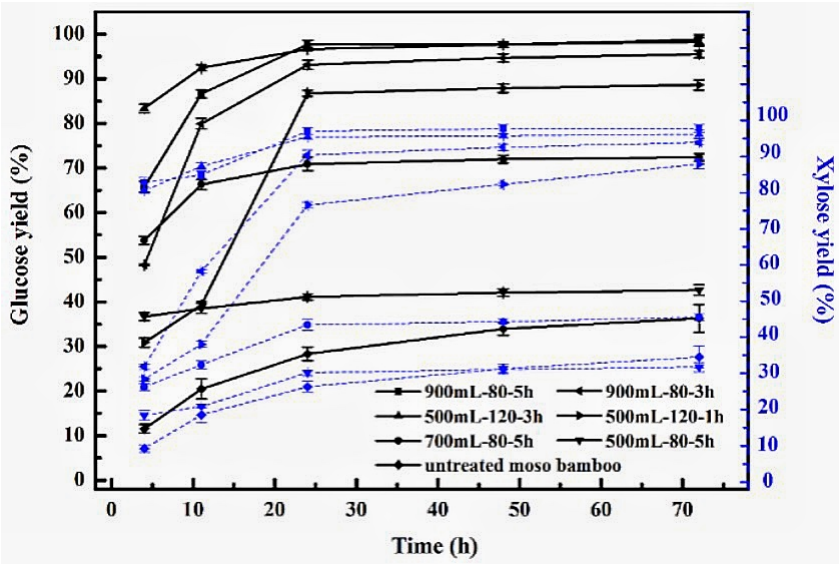


Figure 5. Effects of 1,4-dioxane (500, 700, and 900 mL/100 g bamboo), reaction temperature (80 and 120 °C), and reaction duration (1, 3, and 5 h) on the retention of various components, and glucan/xylan/lignin removal ratio based on the original weight of components.

After being treated in the presence of phenol, the remaining glucan rapidly depolymerized to glucose in the presence of enzymes (99.03% in 72 h), as seen from Table 2. Significant enhancement of enzymatic hydrolysis was achieved when most of the hemicellulose was removed and degraded to furfural under the conditions in this study. The high value was comparable to that obtained in the biodegradation of commercial Avicel [57]. The substrate that was treated in the absence of phenol showed similar enzymatic digestibility. This additional glucose production likely illustrated that phenol addition had no negative influence on enzyme binding and enzymatic digestibility.

Cellulase and xylanase are regular modular proteins with two modules—carbohydrate-binding module (CBM) and catalytic module [58]. Hence, it is necessary to know the adsorption process of cellulase and lignin in order to understand how glucan conversion is enhanced by the delignification of substrates. The influence of 1,4-dioxane dosage on alteration activities of β-glucosidase and exo-β-1,4-glucanase in the supernatants from the hydrolysis process could be observed in Figure S3 (see supplementary data). The activities of both β-glucosidase and exo-β-1,4-glucanase in the supernatant with the substrate that was treated with 1,4-dioxane of 500 mL/100 g bamboo at 120 °C for 3 h showed higher values of the overall trend compared with the enzyme activity of the sample treated with 1,4-dioxane of 700 mL/100 g bamboo at 80 °C for 5 h. This demonstrated that the decreased enzyme activity was due to a higher amount in the cellulase adsorption on lignin based on the difference in its content (81.71% vs. 61.40%) of the two samples. The increased degree of delignification reduced the effective adsorption sites for cellulase enzyme, and it made the binding force between lignin and cellulase weaker [58]. The substrate treated with 1,4-dioxane of 500 mL/100 g bamboo at 120 °C for 3 h was apparently preferably adsorbed onto the cellulase enzyme and it had a higher content of glucan with a larger surface area owing to the higher removal rate/amount of lignin and xylan. In addition, the higher content of lignin in the sample that was treated with 1,4-dioxane of 700 mL/100 g bamboo

at 80 °C for 5 h included the higher percentage of β-O-4 linkage. This indicated that the inhibitory actions of water-soluble components, such as the phenolics, could partially explain the observed inhibition of the enzymes. The inhibited activity of β-glucosidases caused by the simple phenolic-compounds was increased with their contact time [55].

4. Conclusions

An integrated process for selectively fractionating bamboo into furfural, phenolated lignin, and glucan in one pot has been investigated in an acid-catalyzed system that consists of phenol at mild temperature of 80 °C. Consequently, the disentanglement of the internal bamboo structure was achieved in a single step. In the aqueous solution, the conversion yield of furfural was of 92.92% and the lignin fractions could be converted to high performance fractionated lignin with the yield of 51.88% containing phenolic material. The high-purity glucan was exhibited with the enzymatic hydrolysis yield of 99.03%, which could be comparable to that of the commercial Avicel. This method presented a high efficiency and energy saving approach for lignocellulosic biomass fractionation and application.

Supplementary Materials: www.mdpi.com/1996-1073/13/20/5294/s1. Method of enzyme activity analyses, Table S1: The specific reaction condition during fractionation process, Figure S1: The effects of reaction temperature (120 °C and 140 °C) and phenol additions (1.0% and 1.5%, *w*/*v*) on lignin and glucan yield of Moso bamboo. lignin A: residual lignin; lignin B: dissolved lignin (phenolated lignin included), Figure S2: Molecular weight distributions for lignin fractions lignin I and lignin II, Figure S3: The effects of enzyme activities on Moso bamboo hydrolysis. A: The β-glucosidase activity during the time period 0 to 72 h of enzymatic hydrolysis. B: The exo-β-1,4-glucanase activity during the time period 0 to 72 h.

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