

Article

Co-Producing Xylo-Oligosaccharides, 5-HMF, Furfural, Organic Acids, and Reducing Sugars from Waste Poplar Debris by Clean Hydrothermal Pretreatment

Yuheng Yang, Ruibing Cui, Wei Tang, Bo Fan and Yucai He * 

School of Pharmacy & Biological and Food Engineering, Changzhou University, Changzhou 213164, China; yangyuheng0725@163.com (Y.Y.); crb31829@163.com (R.C.); tw2022@cczu.edu.cn (W.T.); fanboxiaoyu@cczu.edu.cn (B.F.)

* Correspondence: heyucai2001@163.com

Abstract: The sustainable valorization of lignocellulosic biomass into value-added biobased chemicals has gained more and more attention on a large industrial scale. To efficiently utilize the abundant, inexpensive, and renewable biomass, it is necessary to employ an effective biomass pretreatment technology for breaking down hemicellulose and lignin. Hydrothermal pretreatment is an effective way to change the structure of lignocellulose and improve its enzymatic hydrolysis efficiency. The hydrothermal cleaning of waste poplar debris (PD) was conducted when the severity factor (LogR_0) score was 5.49. At 220 °C and a solid–liquid ratio of 1:10 for 90 min, the pretreatment liquor contained 4.90 g/L of xylo-oligosaccharides, 1.23 g/L of furfural, 0.41 g/L of formic acid, 2.42 g/L of acetic acid, and 0.57 g/L of 5-HMF. Additionally, 74.9% xylan and 82.4% lignin were removed. After 72 h of enzymatic saccharification, a high enzymolysis efficiency of PD was obtained. A series of characterizations (such as chemical composition analysis, hydrophobicity, lignin surface area, and cellulase accessibility) indicated that hydrothermal pretreatment destroyed the surface structure of PD, improved cellulose accessibility, decreased lignin surface area and weakened lignin hydrophobicity. In general, hydrothermal pretreatment is a simple, green, and environmentally friendly approach for sustainable pretreatment of PD using water as a solvent. It can efficiently break the surface structure of PD and remove lignin and xylan, acquiring high enzymolysis efficiency and realizing the co-production of 5-HMF, furfural, xylo-oligosaccharides, and organic acids. It provides an innovative idea for the value-added utilization of wood-based and straw-based biomass in a sustainable and cost-effective way, showing high potential in industrial application.



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1. Introduction

Recently, the efficient use of renewable biological resources has gained more and more attention because the world's storage capacity for fossil fuels has been limited [1]. The fast and limitless use of fossil fuels has led to the gradual depletion of resources and serious environmental pollution [2]. Bioenergy sources like ethanol and methanol can benefit from the utilization of lignocellulosic biomass (LCB), which is the most abundant, renewable, and biodegradable bioresource [3]. Efficient utilization of LCB is in line with the current trend of sustainable development [4]. However, due to their inherent stability, LCBs are either wasted or directly burned, resulting in inefficient utilization of biomass resources. One prevalent hardwood is poplar. A substantial quantity of sawdust is produced during

the processing of poplar into various products. This byproduct has the potential to be an abundant and inexpensive source of feedstock for biofuel production [5,6]. Hemicellulose, cellulose, and lignin are the three main components of LCB [7]. The network structure in LCB is stabilized by the combination of cellulose's surface hydrogen bond adhesion and the van der Waals force [8], as well as by lignin's ether and glycosidic bond connections. The presence of hemicellulose and lignin around cellulose makes it difficult for cellulase to adhere to the material [9]. In order to increase the yield of reduced sugar, it is essential to pretreat lignocellulosic biomass by eliminating hemicellulose and lignin [10]. Currently, various different types of pretreatment techniques have been reported, including alkali pretreatment [11], hydrothermal treatment [12], steam explosion pretreatment [13], dilute acid pretreatment [14], fungal degradation pretreatment [15], irradiation pretreatment [16], wet explosion pretreatment [17], organic solvent pretreatment [18], and ionic liquid (ILs) pretreatment [19], have been used to improve enzymatic hydrolysis. While there are a number of potential benefits to pretreatment, there are also a number of drawbacks. These drawbacks, such as the potential for environmental pollution, the high expense of chemical addition, poor efficiency, stringent operating parameters, etc., contribute to the high cost of pretreatment and further diminish its commercial viability. From the perspective of sustainable development, the recycling of waste through hydrothermal pretreatment and other technologies can greatly reduce environmental pollution, promote resource recycling, and have a positive impact on the economy, society, and the environment. In recent years, hydrothermal pretreatment of biomass has aroused wide interest for many reasons, including its convenient operation, short running time, low cost, less corrosion of equipment, low risk, green environmental protection, etc.

Hydrothermal pretreatment is an effective process for the valorization of biomass, which can be conducted by using water as solvent at high temperatures ($>160\text{ }^{\circ}\text{C}$) [20]. By eliminating the need for additional chemical reagents and noble metals, hydrothermal pretreatment has gained more and more attention in biorefinery. In addition, it eliminates the need for time-consuming and costly drying processes by utilizing liquid water, which is in contrast to other treatment methods. As mentioned in the report, the disadvantages of concentrated acid hydrolysis are the higher toxicity and corrosivity that require corrosive-resistant reactors or specialized non-metallic constructions and the indispensable need for an acid recovery process to make the process economically feasible [21]. Hydrothermal processes make use of water molecules, which are not only as a solvent but also as a reactant and chemical catalyst. Hydrolysis, which results from the ionization of water, can depolymerize polysaccharides by hydrolyzing heterocyclic ether linkages and selectively eliminating acetyl groups. Water changes its polarity, diffusivity, and viscosity when heated. These alterations to the water's physical characteristics can facilitate various molecular transfers or hydrolysis events. Furthermore, hemicellulose's acetyl groups are broken down into organic acids like formic acid (FA), levulinic acid (LA), and acetic acid (AA). These acids not only catalyze the process automatically, but they may also help depolymerize polysaccharides into the monosaccharides that are necessary. The hydrothermal treatment can destroy the lignocellulosic structure of biomass, thereby enhancing its enzymolysis efficiency and increasing the sugar yield. Hydrothermal pretreatment is an effective biomass conversion technology. Treating biomass under high temperature and pressure can remove hemicellulose, destroy the connection between lignin and cellulose, and make the biomass structure looser, thus improving its biodegradation performance. This treatment process facilitates the contact between the enzyme and cellulose in the subsequent enzymolysis process, thus improving the enzymolysis efficiency. Specifically, an increase in hydrothermal temperature can significantly affect the composition and structure of biomass, making it more conducive to the role of enzymes in the enzymolysis process. In addition, hy-

hydrothermal pretreatment time is also an important factor. In a certain range, prolonging the pretreatment time is helpful to increase the sugar production of biomass. However, too long a pretreatment time may lead to excessive degradation of biomass, which in turn reduces sugar production. Therefore, it is necessary to find the appropriate hydrothermal pretreatment time and temperature conditions to achieve the best enzymolysis of sugar production. Tripathi et al. investigated various aspects of hydrothermal treatment of ligno-cellulosic waste to achieve efficient and economical PHA production [22]. This treatment technology provides strong support for the resource utilization of biomass [23]. Oladzad et al. used hydrothermal pretreatment for biomass treatment and obtained an enzymolysis efficiency of 53.8% at 140 °C and 90 min. It improved the accessibility and surface area of the biomass (increasing the structural porosity) and made it more vulnerable to enzyme attack. However, due to the high residual lignin content, a large amount of cellulose (28.6% glucose under the most demanding pretreatment conditions) remained unhydrolyzed and existed in solid residues [24]. Enzymatic hydrolysis allows for the utilization of vast quantities of monosaccharides (such as glucose and xylose) to generate bioenergy or biochemicals.

Wang et al. pretreated PD with Fenton reagents to obtain a maximum ethanol yield of 406 mg/g [25]. Zhang et al. used white-rot fungi and alkaline to treat PD and obtained the highest reducing sugar yield of 89.3% [26]. Ziaei-Rad and Pazouki pretreated PD with low-cost ionic liquid [TEA][HSO₄]. After pretreatment, lignin removal and xylan separation were 70.1% and 90.0%, respectively [27]. Nevertheless, there is limited information about the efficient valorization of PD into value-added chemicals (e.g., xylo-oligosaccharides, 5-HMF, furfural, organic acids, and reducing sugars) through hydrothermal pretreatment. Less equipment corrosion, reduced operational costs, and better green environmental protection are achieved by hydrothermal pretreatment compared to the traditional pretreatment methods [28]. Hydrothermal treatment has a wide range of applications in industrial biorefineries, environmental sustainability, and economic viability. In industrial biorefineries, hydrothermal treatment disrupts biomass's complex structure, enhancing enzymolysis efficiency. Economically, hydrothermal treatment shows great potential due to its low-performance cost, which is feasible for industrial biorefineries. Optimizing conditions and improving conversion efficiency can further reduce production costs and enhance market competitiveness, realizing the resource utilization and sustainable development of biomass. Consequently, this work was conducted to examine how hydrothermal pretreatment affected the structure, enzymatic hydrolysis, and saccharification capabilities of PD. Moreover, the treatment conditions (time and temperature) of hydrothermal pretreatment were optimized. The changes and enzymatic hydrolysis of cellulose, hemicellulose, and lignin in PD under various pretreatment conditions were investigated. The changes in PD properties before and after pretreatment (such as cellulase accessibility, lignin surface area, hydrophobicity, etc.) explained the reason for the improvement of PD enzymatic hydrolysis efficiency. The aim of this work is to establish an efficient hydrothermal pretreatment system for PD. In addition, biomass conversion products are usually cleaner than fossil fuels, which helps to reduce the pressure on the environment and fills the gap in this aspect of biomass conversion.

2. Materials and Methods

2.1. Reagents and Materials

A hamlet in Qinghai Province, China, was surveyed for poplar debris (PD). Sigma-Aldrich Trading Co., Ltd. (Shanghai, China) supplied the cellulase (Cellic® Ctec.2). Other commercial sources supplied the reagents utilized in the experiment, including the Congo red.

2.2. Hydrothermal Treatment

The 100 mL stainless steel reactor was supplemented with 60 mL of deionized water and 6 g of PD. The reactor was pretreated by swirling it at 500 rpm at 160–220 °C for 30–120 min in an oil bath that included dimethylsilicone oil. The reactor was cooled quickly in an ice water bath after pretreatment. The pretreatment liquid was separated from the solid by a vacuum filter pump. The pretreatment liquid was collected in a 50 mL centrifuge tube and placed in a refrigerator at 4 °C for use. After pretreatment, the solids were transferred to 500 mesh nylon bags, and then rinsed repeatedly with tap water until neutral, and placed in a refrigerator at 4 °C for use. After being rinsed with water until it becomes neutral, the product was put in a refrigerator set at 4 °C until needed. Researchers use the recovered biomass samples for things like saccharification and other experiments. The severity factor [29] (LogR_0) can be used to compare hydrothermal pretreatment experiments conducted under different operating conditions. It is derived using the performance temperature (T , °C) and the pretreatment time (t , min).

$$\text{LogR}_0 = \text{Log}(t \times \exp^{\frac{T - 100}{14.75}}) \quad (1)$$

2.3. Chemical Composition Analysis

The chemical components of PD were tested as follows: In total, 0.30 g solid was slowly dripped with 3 mL sulfuric acid (72%) in a 100 mL hydrolytic bottle. This bottle was placed in a water bath shaker (30 °C) for 1 h. Subsequently, 84 mL of deionized water was added to the bottle. By autoclaving at 121 °C for 1 h, the hydrolysates were collected and further analyzed. Agilent HPLC (Agilent 1260, Agilent Technologies, Santa Clara, CA, USA) equipped with an Aminex HPX-87H column was used to analyze the BR-hydrolysate for glucose, xylose, cellobiose, and arabinose. The mobile phase contained 5 mM H_2SO_4 , and its flow rate was 0.6 mL/min. Solid recovery (SLD), glucan recovery (GCR), xylan removal (XLR), glucose yield (YG), and reducing sugar yield (YRG) were calculated as below:

$$\text{SLD (\%DM)} = \frac{\text{Treated PD (g)}}{\text{Untreated PD (g)}} \times 100 \quad (2)$$

$$\text{GCR (\%DM)} = \frac{\text{Glucan in treated PD (g)}}{\text{Glucan in raw PD (g)}} \times 100 \quad (3)$$

$$\text{XLR (\%DM)} = (1 - \frac{\text{Xylan in pretreated PD (g)}}{\text{Xylan in raw PD (g)}}) \times 100 \quad (4)$$

$$\text{LGR (\%DM)} = (1 - \frac{\text{Lignin in pretreated PD (g)}}{\text{Lignin in raw PD (g)}}) \times 100 \quad (5)$$

$$\text{YG (\%DM)} = \frac{\text{Glucose released} \times 0.9}{\text{Glucan in PD}} \times 100 \quad (6)$$

$$\text{YRG (\%DM)} = \frac{\text{Reducing sugar released} \times 0.9}{\text{Glucan and xylan in PD}} \times 100 \quad (7)$$

2.4. Enzymatic Hydrolysis

For the enzymatic hydrolysis process, a 200 mL flask containing the treated PD was supplemented with 20 FPU/(g glucan) of commercial cellulase (CTec.2). A 50 mM citrate buffer solution was used to maintain a system pH of 4.8. The PD was enzymatically digested with cellulase for 72 h at 50 °C and 150 rpm. In total, 2 mL of the hydrolysate was then removed and centrifuged for 3 min at 8000 rpm to eliminate any solid residue. The HPLC determination was performed to quantify the concentration of cellobiose, glucose,

xylose, and arabinose. The relative saccharification activity (RSA) and enzymatic hydrolysis efficiency (EHE) were defined as follows:

$$\text{EHE (\%)} = \frac{\text{Glucose in enzymatic hydrolysate (g)} \times 0.9}{\text{Glucan in treated raw BR (g)}} \times 100 \quad (8)$$

$$\text{RSA (\%)} = \frac{\text{EHE of treated PD (\%)}}{\text{EHE of raw PD (\%)}} \times 100 \quad (9)$$

2.5. Accessibility, Hydrophobicity and Surface Lignin Area

2.5.1. Accessibility of Cellulose

Accessibility can be measured through a direct red dye adsorption test [30]. A Congo red dye absorption experiment was used to investigate the accessibility of both raw and processed PD. An appropriate dye concentration range is used to ensure that the results are accurate and reliable. Raw and pretreated PD samples (0.1 g) were mixed with various concentrations of Congo red solutions (0–4.0 g/L) for 1 day at 60 °C with agitation set at 150 rpm. Afterwards, the liquid in suspension was spun at 3000 rpm for 5 min to separate the supernatant. A UV/Vis spectrophotometer was used to examine the Congo red solutions and supernatant at 498 nm. The dye adsorption capability of treated sunflower straw was determined by calculating the accessibility using the Langmuir adsorption isotherm. Accessibility was calculated as previously reported [30].

2.5.2. Hydrophobicity

Lignin hydrophobicity can be assessed through the adsorption of Rose Bengal reagent [31]. Rose Bengal reagent solution (40 mg/L) was mixed with 10 mL of PD solutions ranging from 0.04 to 0.4 g/L. The mixture's pH was kept at 4.8. After 2 h, the sample was submerged in Rose Bengal solution while operating at 50 °C and 150 rpm. By centrifugation at 5000 rpm for 5 min, the recovered liquid was subsequently analyzed using an ultraviolet/visible spectrophotometer at 543 nm. Hydrophobicity (L/g) was calculated as previously described [31].

2.5.3. Surface Lignin Area

The surface lignin area of PD was evaluated through the Azure B dye adsorption. Azure B dye solution (ranging from 0 to 0.8 g/L) was added to the 50 mL flask with 0.2% (*w/v*) of the raw or treated PD. The pH level of the system was kept at 7.0. Shaking these mixtures was conducted at 150 rpm and 25 °C for 1 day. In order to evaluate the amount of adsorbed dye, the standard Azure B was measured at 647 nm using a UV-3200 PCS ultraviolet/visible spectrophotometer. The PD surface lignin area was determined using the Langmuir adsorption isotherm [32].

2.6. Statistical Analysis

The xylo-oligosaccharide concentration, relative saccharification activity, and hydrothermal pretreatment time and temperature were analyzed using analysis of variance (ANOVA). Data analysis was conducted using SPSS (Statistics 26) program 200.

3. Results and Discussion

3.1. Chemical Composition of PD Between and After Hydrothermal Pretreatment

Previous studies have shown that pretreatment's reaction duration and temperature might significantly impact its effectiveness [33]. Hydrothermal pretreatment altered the PD composition under various conditions, as shown in Table 1. The PD used in this study was composed of lignin (30.5%), glucan (37.6%), and xylan (20.5%). The chemical composition

of PD altered to varying degrees after the pretreatment (temperatures: 160–220 °C; reaction time: 30–120 min; liquid–solid mass ratio: 1:10). Raising the temperature of hydrothermal treatment increased its pretreatment strength, which in turn increased the glucan concentration and facilitated the removal of lignin. Morales et al. conducted hydrothermal pretreatment of walnuts. By raising the pretreatment temperature from 180 to 220 °C, the damage strength increased. The glucan content increased from 13.0% to 27.33%, and the lignin content decreased from 17.90% to 7.92% [34]. Table 1 shows that the recovery rates of glucan were in the range of 52.7–97.2%. Upon increasing pretreatment temperature from 160 to 220 °C, the removal of xylan rose from 15.5% to 78.5%, and the delignification elevated from 37.7% to 85.3%. These results demonstrated that hydrothermal pretreatment successfully eliminated lignin and hemicellulose from biomass, while preserving a significant amount of glucan and providing sufficient substrate for future research.

Table 1. Component changes in raw and after hydrothermal treatment of PD.

No.	Temperature, °C	Time, min	Glucan, % DM	Xylan, % DM	Lignin, % DM	Recovery, %		Removal, %	
						Solid	Glucan	Xylan	Lignin
Raw	/	/	37.6 ± 0.2	14.7 ± 0.3	30.5 ± 0.1	/	/	/	/
1	160	30	39.4 ± 0.1	13.4 ± 0.2	20.5 ± 0.1	92.7 ± 0.1	97.2 ± 0.3	15.5 ± 0.1	37.7 ± 0.1
2	160	60	40.2 ± 0.1	12.5 ± 0.1	20.3 ± 0.1	90.6 ± 0.2	96.8 ± 0.1	22.9 ± 0.1	39.8 ± 0.1
3	160	90	41.9 ± 0.2	11.7 ± 0.2	17.5 ± 0.1	83.7 ± 0.1	93.2 ± 0.3	33.4 ± 0.2	52.1 ± 0.3
4	160	120	46.5 ± 0.1	10.2 ± 0.1	15.5 ± 0.1	74.2 ± 0.3	91.8 ± 0.2	48.4 ± 0.1	62.2 ± 0.2
5	180	30	44.8 ± 0.1	10.9 ± 0.1	17.0 ± 0.2	80.9 ± 0.1	96.4 ± 0.1	39.9 ± 0.1	54.9 ± 0.1
6	180	60	50.5 ± 0.2	11.3 ± 0.1	16.6 ± 0.1	69.6 ± 0.3	93.4 ± 0.1	46.4 ± 0.12	62.1 ± 0.3
7	180	90	51.8 ± 0.1	10.8 ± 0.2	15.1 ± 0.1	63.0 ± 0.1	86.8 ± 0.2	53.8 ± 0.1	68.9 ± 0.2
8	180	120	52.4 ± 0.1	10.1 ± 0.1	14.5 ± 0.1	60.5 ± 0.2	84.3 ± 0.3	58.5 ± 0.2	71.2 ± 0.1
9	200	30	52.1 ± 0.2	10.6 ± 0.3	14.8 ± 0.1	61.8 ± 0.2	85.6 ± 0.1	55.3 ± 0.1	70.1 ± 0.2
10	200	60	52.7 ± 0.1	10.5 ± 0.1	14.7 ± 0.1	57.6 ± 0.3	80.9 ± 0.2	58.8 ± 0.2	72.2 ± 0.3
11	200	90	53.6 ± 0.3	10.2 ± 0.1	15.6 ± 0.2	51.4 ± 0.1	73.3 ± 0.1	64.2 ± 0.1	73.8 ± 0.1
12	200	120	54.1 ± 0.2	10.0 ± 0.3	14.3 ± 0.1	47.3 ± 0.1	68.0 ± 0.2	67.8 ± 0.1	77.8 ± 0.1
13	220	30	53.9 ± 0.1	10.0 ± 0.1	15.2 ± 0.1	49.7 ± 0.2	71.2 ± 0.3	66.1 ± 0.2	75.3 ± 0.2
14	220	60	54.2 ± 0.1	9.8 ± 0.2	14.9 ± 0.1	43.4 ± 0.1	62.5 ± 0.1	71.1 ± 0.1	78.8 ± 0.3
15	220	90	55.8 ± 0.2	9.5 ± 0.1	13.8 ± 0.1	38.8 ± 0.2	57.6 ± 0.1	74.9 ± 0.1	82.4 ± 0.1
16	220	120	57.2 ± 0.3	9.1 ± 0.1	12.9 ± 0.1	34.7 ± 0.1	52.7 ± 0.2	78.5 ± 0.1	85.3 ± 0.2

Dimethylsilicone oil was used in the oil bath as the reaction instrument for hydrothermal pretreatment in this work. Although the lignin removal rate at a higher temperature of 220 °C was higher than that at a lower temperature gradient (Figure 1a), because dimethylsilicone oil releases toxic organic compounds like formaldehyde and benzene when heated, it can irritate the skin and eyes, affect the respiratory system, and worsen long-term or heavy contact exposure. It also has a strong odor and tastes unpleasant. Additionally, these dangerous compounds will have negative effects on human health, research work is more prone to hazards at higher temperatures, and heating and cooling use more energy. In contrast to other research [35], the lignin polymerization and cellulose surface deposition caused by high operating temperature had the opposite effect on the lignin removal effect in this work. Accordingly, 220 °C was selected as the appropriate temperature for pretreatment.

Meanwhile, further research on the effect of different reaction duration on PD pretreatment was studied. As shown in Figure 1b, with the extension of hydrothermal time, the recovery of glucan decreased gradually. The removal of xylan and delignification increased slowly. It was clear that the reaction duration had a certain effect on the chemical composition of PD. Figure 1c displays the effects of the treatment temperature on the chemical components. As the reaction temperature elevated from 160 °C to 220 °C (solid–liquid mass ratio 1:10, 120 min), the content of xylan dropped from 10.2% to 9.1%, while the content of

glucan significantly increased from 46.5% to 57.2% (Figure 1c). Upon elevating the reaction time from 30 to 120 min (solid–liquid mass ratio 1:10, 220 °C), the glucan content in the pretreated PD increased from 53.9% to 57.2%, and the lignin content decreased from 15.2% to 12.9%. While the xylan content in pretreated PD was close (Figure 1d), Batista et al. conducted hydrothermal pretreatment of sugarcane straw at 170 °C, and the recovery of glucan decreased from 16.35% to 9.8% by increasing treatment time from 5 min to 15 min. The removal of xylan increased from 47.25% to 85.45%, and the delignification increased from 5.69% to 19.42% [36]. Nevertheless, a longer reaction time may use more energy, and a higher glucan recovery could provide a more plentiful substrate for future enzymatic hydrolysis operations. Therefore, the ideal condition for this study was determined to be 90 min of reaction at a temperature of 220 °C. Simultaneously, the data significance analysis and single-factor ANOVA testing on several pretreatment conditions were conducted in this study. It was found that the data of “F” are high, which may be caused by the selection of complicated model for the data analysis. While all of them revealed the significance ($p < 0.001$) (Table 2). In the future work, more reasonable models will be sought to analyze experiment results.

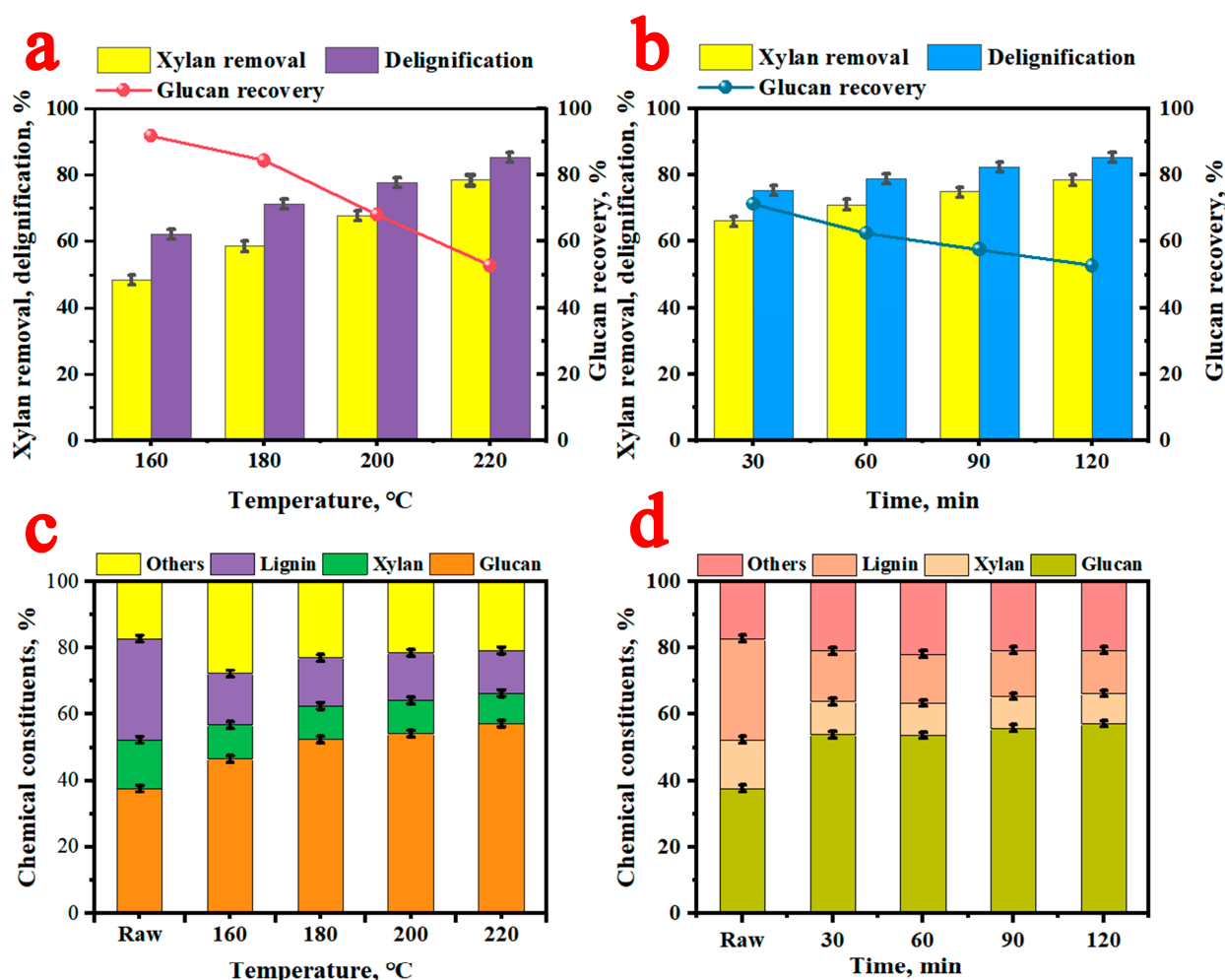


Figure 1. Effect of different hydrothermal temperature (160–220 °C) on delignification, xylan removal, and glucan recovery [120 min, solid–liquid mass ratio of 1:10] (a). Effect of different hydrothermal times (30–120 min) on delignification, xylan removal, and glucan recovery [220 °C, solid–liquid mass ratio of 1:10] (b). Chemical constituents after different hydrothermal temperatures (160–220 °C) on delignification, xylan removal, and glucan recovery [120 min, solid–liquid mass ratio of 1:10] (c). Chemical constituents after different hydrothermal time (30–120 min) on delignification, xylan removal, and glucan recovery [220 °C, solid–liquid mass ratio of 1:10] (d).

Table 2. Statistical analysis of xylo-oligosaccharide content, relative saccharification activity, and hydrothermal pretreatment time and temperature.

Test of Intersubjective Effects						
Variance Source	Dependent Variable	Class III Sum of Squares	Degree of Freedom	Mean Square	F	p
Modified model	Xylo-oligosaccharide	86.605a	16	5.413	6.22113×10^{32}	<0.001
	Relative saccharification	816.331a	16	51.021	1.8325×10^{32}	<0.001
Intercept	Xylo-oligosaccharide	15.8	1	15.8	1.81592×10^{33}	<0.001
	Relative saccharification	814.454	1	814.454	2.92525×10^{33}	<0.001
Temperature	Xylo-oligosaccharide	40.17	3	13.39	1.53896×10^{33}	<0.001
	Relative saccharification	496.993	3	165.664	5.95013×10^{32}	<0.001
Time	Xylo-oligosaccharide	15.873	3	5.291	6.08097×10^{32}	<0.001
	Relative saccharification	146.321	3	48.774	1.7518×10^{32}	<0.001
Temperature $\times$ Time	Xylo-oligosaccharide	28.263	9	3.14	3.60926×10^{32}	<0.001
	Relative saccharification	54.466	9	6.052	2.17359×10^{31}	<0.001
Errors	Xylo-oligosaccharide	2.96×10^{-31}	34	8.70×10^{-33}		
	Relative saccharification	9.47×10^{-30}	34	2.78×10^{-31}		
Total	Xylo-oligosaccharide	123.401	51			
	Relative saccharification	2713.138	51			
Corrected Total	Xylo-oligosaccharide	86.605	50			
	Relative saccharification	816.331	50			

Note: Confidence interval is 95%. “a” means significant at 95% confidence level.

3.2. Chemical Composition of Hydrothermal Treated PD's Prehydrolysate

Pretreatment solutions undergo changes in pH and composition as a result of ionization of water to form hydrated hydrogen ions during high-temperature hydrothermal pretreatment, which is sensitive to variations in reaction time and temperature. The hydrothermal treated PD's prehydrolysate's pH dropped from 5.35 to 2.93 as the hydrothermal temperature rose, as indicated in Tables 3 and 4, due to the increased production of water and hydrogen ions by water ionization. The content of glucose and xylose in the pretreatment liquor increased gradually (Table 3). It was observed that the glucose concentration elevated from 0.73 to 2.52 g/L, and the xylose concentration increased from 2.81 to 4.43 g/L (Table 3). The liquor's pH slightly decreased from 3.22 to 2.93 as the reaction duration increased from 30 to 120 min (220 °C). In the reaction liquors, formic acid, acetic acid, 5-HMF, and furfural produced significantly with increasing LogR₀ (Table 4 and Figure 2a). Different LogR₀ values could affect xylan and lignin removal (Figure 2b). Acid catalysis might facilitate the conversion of xylans in lignocellulose to oligosaccharides or monosaccharides [37]. Therefore, the presence of xylose and XOSs in the filtrate obtained from hydrothermal pretreatment could be used as an indicator to determine the level of xylan dissolution. Because of its unique structure and function, XOSs have a broad application prospect in industrial production. Apart from being an antioxidant additive and low-calorie sweetener, XOSs have demonstrated a broad range of uses in the food, healthcare, and pharmaceutical industries. XOSs with varying molecular weights can form through hydrothermal pretreatment under a weak acidic environment. Results from the determination of the XOS content in the hydrothermal treatment solution are presented in Table 3 and Figure 2a, with a maximum concentration of 4.9 g/L at 220 °C. The XOS content dropped to 3.81 g/L after 120 min at 220 °C, while the xylose concentration in the treatment solution was raised from 3.98 g/L to 4.43 g/L. The treatment period was increased from 30 to 120 min at 220 °C (Table 3), with LogR₀ values ranging from 5.01 to 5.61. An investigation was conducted to determine the correlation between XOS concentration and LogR₀. When LogR₀ was 5.49, the XOS concentration reached the highest (Figure 2a). The XOS concentration decreased to a certain extent when LogR₀ was greater or less than 5.49. The XOS concentration fell slightly with increasing xylose content when the reaction time was increased from 90 to

120 min at 220 °C, although the change was not substantial. This might be ascribed to that XOSs can be degraded into xylose at high temperatures [38]. The hydrolysis process breaks down xylan into xylose. Under the optimum reaction conditions (solid–liquid ratio of 1:10, 220 °C, 90 min), 4.9 g/L of XOSs was acquired.

Table 3. Main sugar constituents of PD's prehydrolysate.

No.	Temperature, °C	Time, min	Constituents, g/L			pH
			Glucose	Xylose	Xylo-Oligosaccharides	
1	160	30	0.04 ± 0.01	0.03 ± 0.01	1.00 ± 0.03	5.35
2	160	60	0.05 ± 0.01	0.04 ± 0.01	1.02 ± 0.05	4.37
3	160	90	0.10 ± 0.01	0.15 ± 0.02	1.03 ± 0.05	3.99
4	160	120	0.14 ± 0.02	0.37 ± 0.01	1.34 ± 0.06	3.69
5	180	30	0.06 ± 0.01	0.06 ± 0.01	1.51 ± 0.05	4.20
6	180	60	0.14 ± 0.02	0.09 ± 0.02	1.64 ± 0.08	3.38
7	180	90	0.16 ± 0.02	0.18 ± 0.01	1.76 ± 0.06	3.32
8	180	120	0.20 ± 0.01	1.24 ± 0.06	1.85 ± 0.08	3.39
9	200	30	0.19 ± 0.02	1.84 ± 0.07	2.13 ± 0.10	3.20
10	200	60	0.48 ± 0.02	2.05 ± 0.09	2.43 ± 0.12	3.03
11	200	90	0.73 ± 0.04	3.14 ± 0.10	2.49 ± 0.11	2.98
12	200	120	1.14 ± 0.05	3.29 ± 0.12	2.81 ± 0.12	2.94
13	220	30	0.73 ± 0.03	2.81 ± 0.11	3.59 ± 0.18	3.22
14	220	60	1.01 ± 0.05	3.01 ± 0.15	3.73 ± 0.19	3.11
15	220	90	1.67 ± 0.08	3.98 ± 0.19	4.90 ± 0.24	2.99
16	220	120	2.52 ± 0.12	4.43 ± 0.22	3.81 ± 0.19	2.93

Table 4. Main byproducts in PD's prehydrolysate.

No.	Temperature, °C	Time, min	log R_0	Formic Acid, g/L	Acetic Acid, g/L	5-HMF, g/L	Furfural, g/L
1	160	30	3.24	N.D.	N.D.	N.D.	N.D.
2	160	60	3.54	0.03 ± 0.01	0.04 ± 0.01	N.D.	N.D.
3	160	90	3.72	0.06 ± 0.01	0.09 ± 0.01	N.D.	N.D.
4	160	120	3.85	0.08 ± 0.01	0.22 ± 0.01	N.D.	N.D.
5	180	30	3.83	0.01 ± 0.01	0.05 ± 0.01	N.D.	N.D.
6	180	60	4.13	0.06 ± 0.01	0.38 ± 0.02	0.12 ± 0.01	N.D.
7	180	90	4.31	0.15 ± 0.01	0.85 ± 0.01	0.22 ± 0.03	0.04 ± 0.01
8	180	120	4.43	0.18 ± 0.02	1.21 ± 0.05	0.32 ± 0.01	0.06 ± 0.01
9	200	30	4.42	0.15 ± 0.01	0.53 ± 0.02	0.03 ± 0.01	0.36 ± 0.01
10	200	60	4.72	0.19 ± 0.01	1.18 ± 0.05	0.19 ± 0.02	0.51 ± 0.02
11	200	90	4.90	0.27 ± 0.01	2.10 ± 0.11	0.42 ± 0.10	0.71 ± 0.03
12	200	120	5.02	0.34 ± 0.02	2.29 ± 0.10	0.60 ± 0.02	0.82 ± 0.03
13	220	30	5.01	0.16 ± 0.02	2.10 ± 0.09	0.18 ± 0.03	0.90 ± 0.03
14	220	60	5.31	0.28 ± 0.03	2.13 ± 0.13	0.27 ± 0.01	1.11 ± 0.01
15	220	90	5.49	0.41 ± 0.01	2.42 ± 0.12	0.57 ± 0.02	1.23 ± 0.05
16	220	120	5.61	0.50 ± 0.02	2.63 ± 0.13	0.64 ± 0.01	1.27 ± 0.05

3.3. Enzymatic Hydrolysis of Raw and Hydrothermal Treated PD

An essential indication to measure the efficacy of hydrothermal pretreatment is enzymatic hydrolysis activity, which is directly related to biomass use [39]. Improved enzymatic saccharification of xylan is accomplished by hydrothermal pretreatment of sunflower straw, leading to a 44.6% yield of XOSs. Generating glucose and decreasing sugars are the primary outcomes of enzymatic hydrolysis in biomass [40]. The enzymatic hydrolysis, xylan removal, and delignification are all measured in this study. Increasing the pretreatment temperature and prolonging the pretreatment duration can enhance xylan removal, and delignification and reduce sugar yield (Figure 3a,b). The hydrothermal temperature was raised from 160 °C to 220 °C during the temperature optimization procedure, and the

reducing sugar yields of untreated PD increased from 6.3% to 41.4% (Figure 3a). Enzymolysis efficiency was substantially enhanced as the reaction temperature was raised. By eliminating more lignin and hemicellulose at higher temperatures, cellulase may be able to bind with cellulose more effectively, leading to increased enzymatic saccharification [41]. Figure 3b shows that the reaction for 120 min at 220 °C resulted in a greater enzymatic hydrolysis effect than the 90 min pretreatment. However, the energy consumption was lower and the concentration of XOS produced was higher when the reaction was carried out at 220 °C in 90 min compared to those in 120 min. The oil bath's dimethyl silicone oil could also become viscous and produce toxic byproducts like formaldehyde, carbon monoxide, methane, and methanol when heated over 220 °C, which might shorten the oil's useful life and pose safety risks [42]. Thus, a pretreatment temperature of 220 °C was chosen after careful consideration. The results showed that reducing sugar yielded 48.6% with PD following hydrothermal pretreatment (220 °C, 90 min). In hydrothermal pretreatment, the treatment duration and temperature have significant influences on biomass composition and enzymolysis efficiency. With the increase in temperature and the extension of treatment time, the content of main components in biomass such as xylan, lignin, and glucan will change. In particular, the removal rates of lignin and xylan will increase, and the loss of glucan may also occur. These alterations are due to the high temperature and prolonged treatment duration that destroys the structure of the biomass cell wall, allowing easily degradable components such as hemicellulose to be removed. The change in treatment duration and temperature also directly affects the efficiency of enzymolysis. Notably, suitable temperature and treatment time can improve the enzymolysis efficacy of cellulose. For example, at a specific temperature and time (such as 220 °C and 90 min), a higher enzymatic saccharification can be obtained. However, if the treatment temperature and treatment time continue to increase, the xylan removal can be further improved, while the enzymolysis efficiency may decrease. Excessive treatment intensity may lead to the loss and structural damage of cellulose, and thus weaken its enzymatic hydrolysis. Consequently, time and temperature in hydrothermal treatment are the key factors affecting biomass composition and enzymolysis efficiency, which need to be reasonably regulated according to biomass types and specific needs in practical applications. In addition, different combinations of time and temperature can also lead to the formation of pseudolignin. Pseudolignin is a structural analog of lignin, which is formed by a complex reaction of carbohydrate degradation, oxidation, and polymerization. It can inhibit cellulase activity and reduce the conversion rate of lignocellulose. It is speculated that the substance was produced at 220 °C for 120 min. Enzymolysis was inhibited, so the enzymolysis efficiency was not significantly improved compared with 90 min. The subsequent enzymatic digestibility is affected by major parameters such as the degree of delignification and the removal of xylan.

It is clear that the process of lignocellulose enzymatic hydrolysis can be impeded when biomass fibers are loaded with lignin and hemicellulose through covalent connections, forming a thick three-dimensional network structure that is both extremely resistant and biodegradable [43]. For this reason, the xylan and lignin removal rates serve as important metrics for gauging the efficacy of the pretreatment. Increasing the delignification and xylan removal rates led to higher yields of reducing sugars, as displayed in Figure 3a and b. The findings demonstrated that enzymatic hydrolysis efficiency might be substantially enhanced with effective delignification and xylan removal [44]. If the temperature is appropriate, a high sugar yield could be acquired only by water pretreatment without adding other solvents or acid-base substances. This proved that hydrothermal pretreatment is an efficient and hygienic way to prepare PD for treatment.

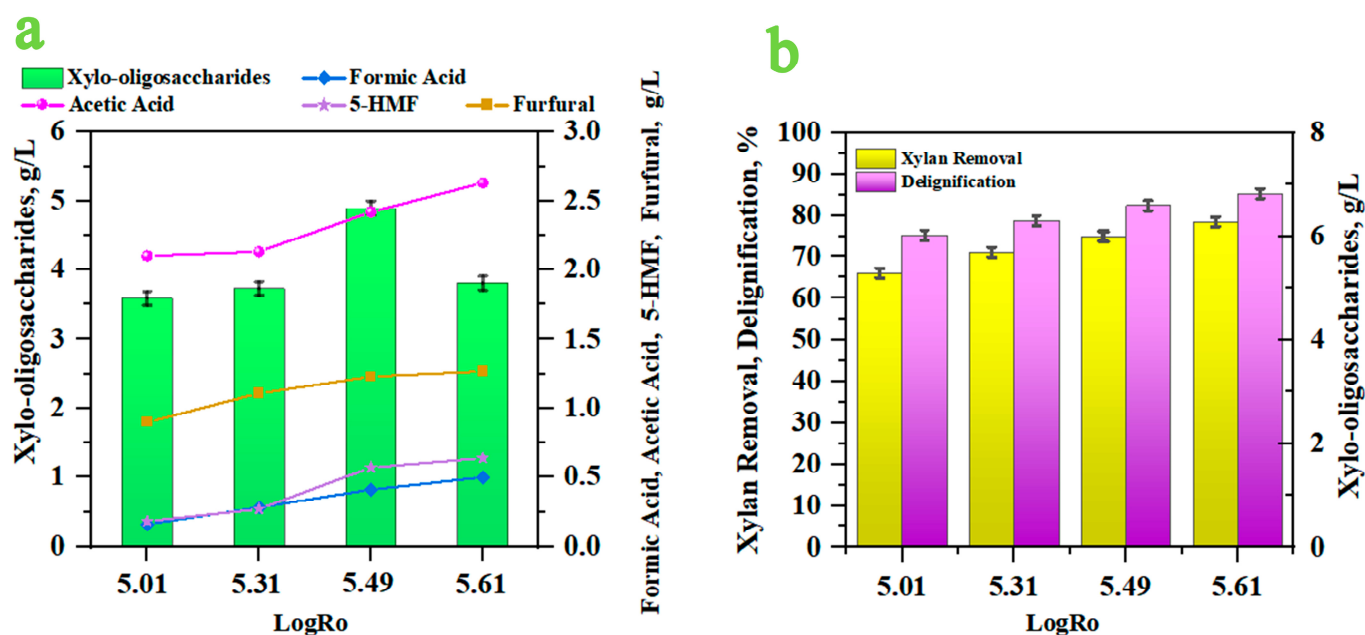


Figure 2. The effects of LogRo on the formation of XOSs, formic acid, acetic acid, 5-HMF, and furfural at 220 °C [30–120 min, solid–liquid mass ratio 1:10] (a). The relationship between LogRo, xylan removal ratio, delignification ratio 220 °C [30–120 min, solid–liquid mass ratio of 1:10] (b).

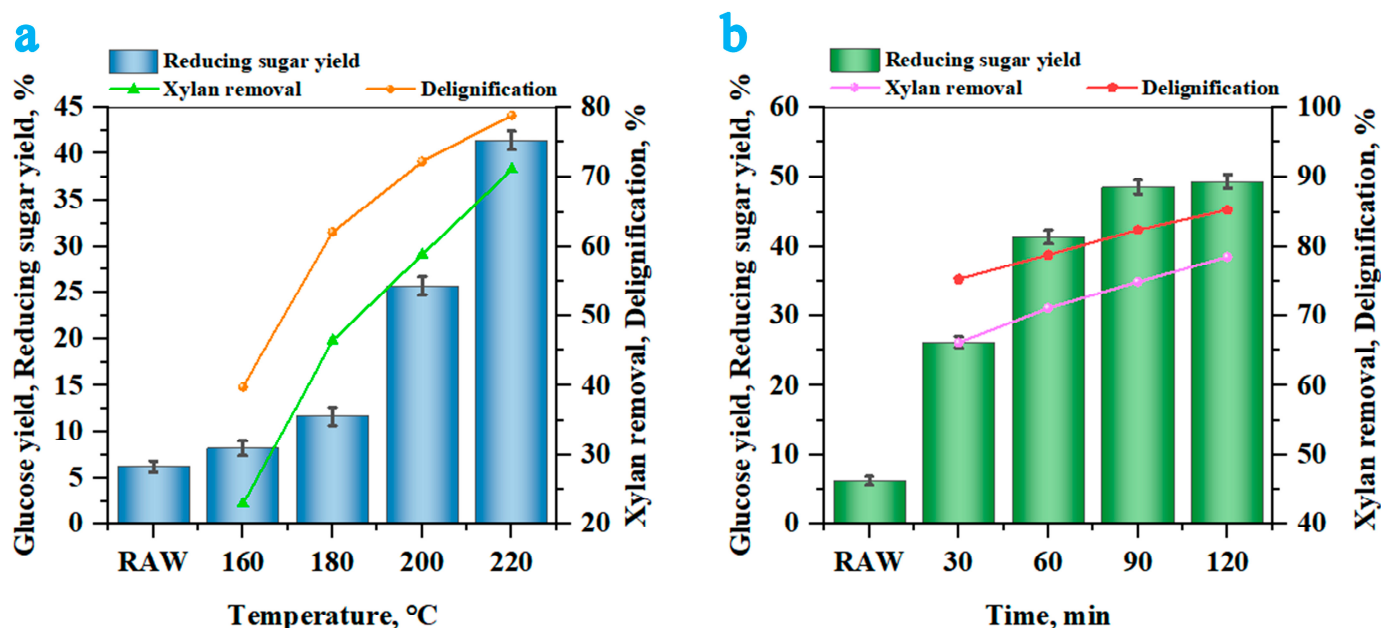


Figure 3. Xylan removal, delignification, and reducing sugar yield after hydrothermal treatment of PD at different temperatures (160–220 °C) for 60 min [Pretreatment: solid–liquid mass ratio of 1:10; enzymatic hydrolysis: 72 h] (a). Xylan removal, delignification, and reducing sugar yield after hydrothermal treatment of PD for 30–120 min at 220 °C [Pretreatment: solid–liquid mass ratio of 1:10; enzymatic hydrolysis: 72 h] (b).

3.4. Characterization of Raw and Treated PD

3.4.1. Cellulose Accessibility

The enzymolysis ability is related to the cellulose accessibility [30,32]. Dye adsorption can be used to assess cellulose accessibility. An anionic dye belonging to the benzidine class, Congo Red is insoluble in acids and bases but readily soluble in water, and it also produces a red colloidal solution, making it an attractive substitute for other macromolecules like enzymes and proteins utilized in biotransformation [45]. In this study, Congo red dye

was used to adsorb PD before and after hydrothermal pretreatment, and the adsorption value was used to judge the accessibility. The relationship between pretreatment time and accessibility is illustrated in Figure 3a. As the pretreatment time elevated from 30 to 120 min (220 °C), the accessibility increased from 177.3 to 365.2 mg/g. A calculated R^2 value of 0.87 (Figure 4a) indicated a clear linear correlation between accessibility and pretreatment time. Figure 4b displays that the reducing sugar yield was positively correlated with accessibility ($R^2 = 0.84$). A linear fitting was also found between the glucose yield and accessibility ($R^2 = 0.91$). Increasing cellulose accessibility by hydrothermal pretreatment could enhance the glucose and reduce sugar yields. Accessibility might increase as a result of longer reaction duration and higher performance temperature. The physical barrier formed by lignin was broken. These indicated that hydrothermal pretreatment could effectively remove lignin and xylan in PD, so that cellulase might better contact with the cellulose surface for enhancing the subsequent enzymolysis process, effectively improving the hydrolysis efficiency.

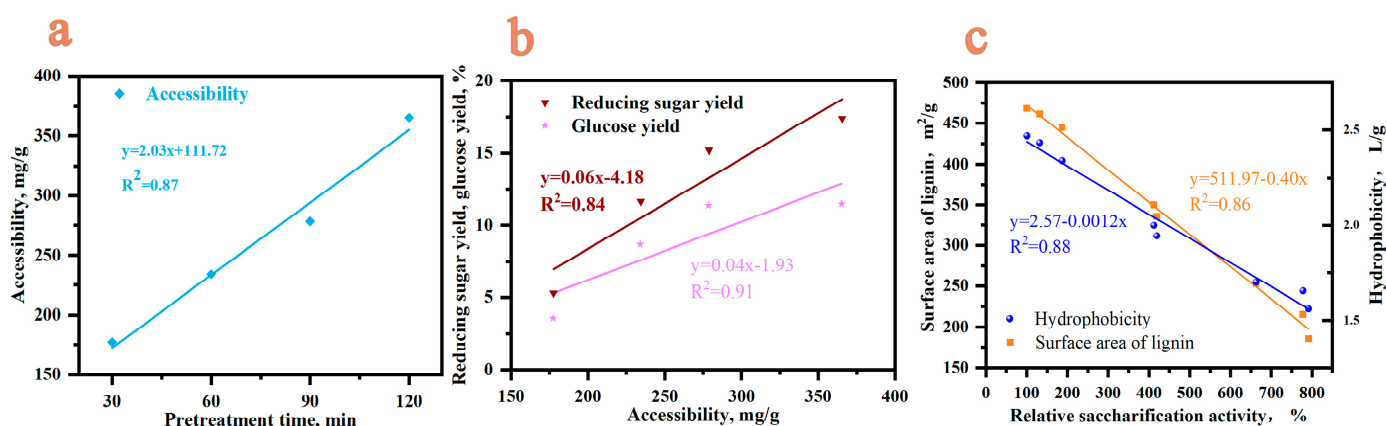


Figure 4. The linear relationship between pretreatment time and accessibility (a); the relationship between the accessibility and reducing sugar yield and glucose yield (b); the linear correlations between hydrophobicity, surface area of lignin, and relative saccharification activity (c).

3.4.2. Surface Properties of Lignin

According to the report by Uma Maheswari et al. [46], a pretreatment with humic acid showed a negative linear association between lignin surface area, hydrophobicity, and relative saccharification activity. So, it is necessary to study how enzymatic hydrolysis efficiency relates to lignin surface features. The impact of hydrothermal pretreatment on lignin was examined by measuring the lignin surface area and hydrophobicity through the azure B and rose red dye adsorption processes. Following PD treatment, the specific surface area decreased, and the surface lignin area was negatively correlated with the relative saccharification activity ($R^2 = 0.86$) (Figure 4c). Lignin could be significantly removed after hydrothermal pretreatment, resulting in a decrease in the lignin surface area and hydrophobicity. In the bioprocess of enzymatic saccharification, lignin in lignocellulose might not only hinder the enzymatic hydrolysis efficiency of cellulose but also cause the non-productive adsorption of cellulase due to the hydrophobic and electrostatic interactions. Non-specific binding of cellulases to lignin can hinder the hydrolysis of cellulose [47]. So, it is necessary to explore the hydrophobicity of untreated and treated PD. The adsorption experiments of Rose Red dye were used to examine the alterations in lignin hydrophobicity (Figure 4c). Similarly, hydrophobicity and enzymolysis efficiency were correlated ($R^2 = 0.88$). As shown in Figure 4c, after pretreatment, the hydrophobicity of PD dropped from 2.47 L/g to 1.56 L/g, displaying the relationship between the relative saccharification activity and hydrophobicity ($R^2 = 0.86$). A negative correlation was observed between lignin-specific surface area, hydrophobicity, and relative saccharification

activity. Hydrothermal pretreatment led to an improvement in enzymatic hydrolysis efficiency compared to raw PD. The surface area and hydrophobicity of lignin decreased correspondingly as a consequence of notable delignification effects. The process of eroding and removing the lignin surface weakened the surface lignin area and lignin hydrophobicity. The delignification, surface lignin area, and hydrophilic significantly greatly improved after hydrothermal pretreatment, while relative saccharification activity and accessibility were observed to be higher than those of the raw PD (Figure 5).

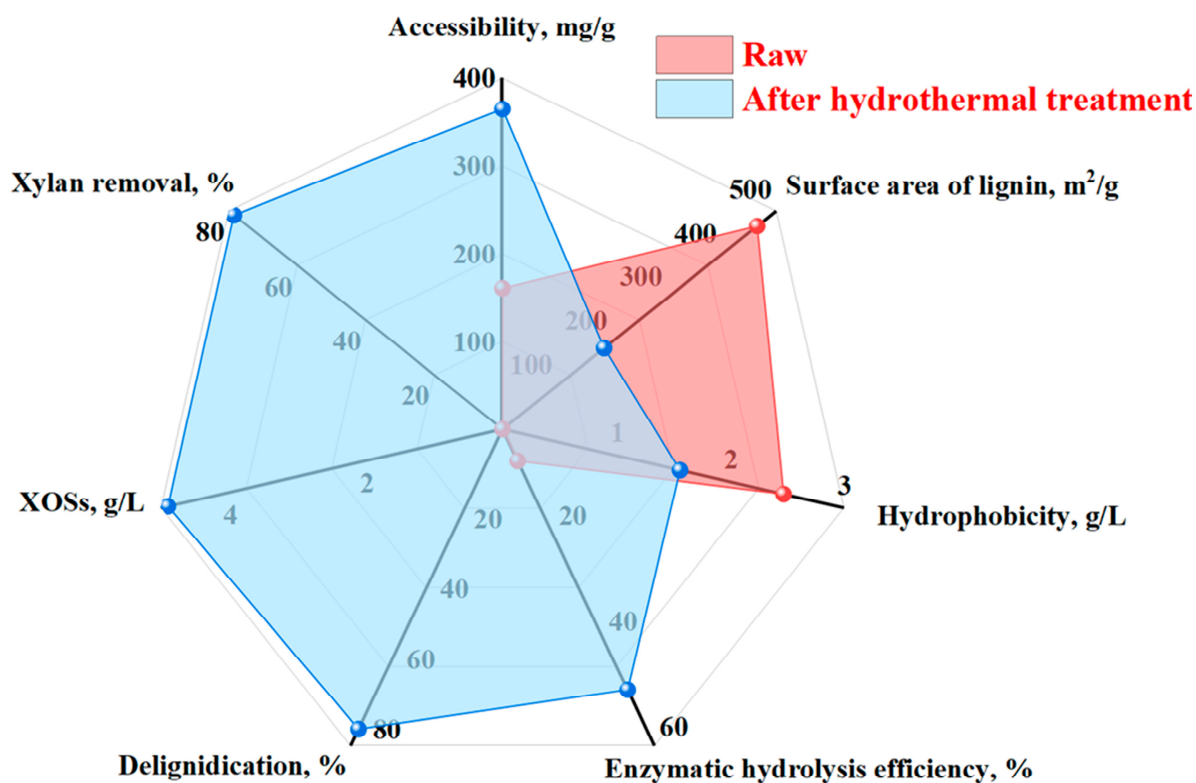


Figure 5. Radar images of accessibility, surface area of lignin, hydrophobicity, enzymatic hydrolysis efficiency, delignification, XOSs, and xylan removal through the hydrothermal treatment of PD.

3.5. Mass Balance of Hydrothermal Pretreatment

The mass balance map provides a detailed description of the enzyme saccharification of both the raw material and treated PD (Figure 6a). One hundred grams of PD comprising 37.6 g of glucan, 14.7 g of xylan, and 30.5 g of lignin were processed in this study. With a solid–liquid ratio of 1:10, the pretreatment of PD was carried out at 220 °C for 90 min. In total, 38.8 g of residue was obtained, which was composed of 21.6 g of glucan, 3.7 g of xylan, and 5.4 g of lignin. In pretreatment liquor, some valuable byproducts formed, including 4.9 g of xylo-oligosaccharide, 0.41 g of formic acid, 2.42 g of acetic acid, 0.57 g of 5-HMF, and 1.23 g of furfural. The majority of the glucan was preserved, and a considerable amount of lignin and xylan was removed. After the enzymatic hydrolysis of pretreated PD with cellulases, 10.5 g of glucose and 1.8 g of xylose were produced. In this study, hydrothermal pretreatment was used to treat PD, and the removal rates of lignin and xylan were 82.4% and 74.9%, respectively. Hydrothermal pretreatment could improve the accessibility of cellulose, reduce the surface area and hydrophobicity of lignin, improve the non-productive adsorption between cellulose and lignin, and elevate the efficiency of enzymatic hydrolysis (Figure 6b).

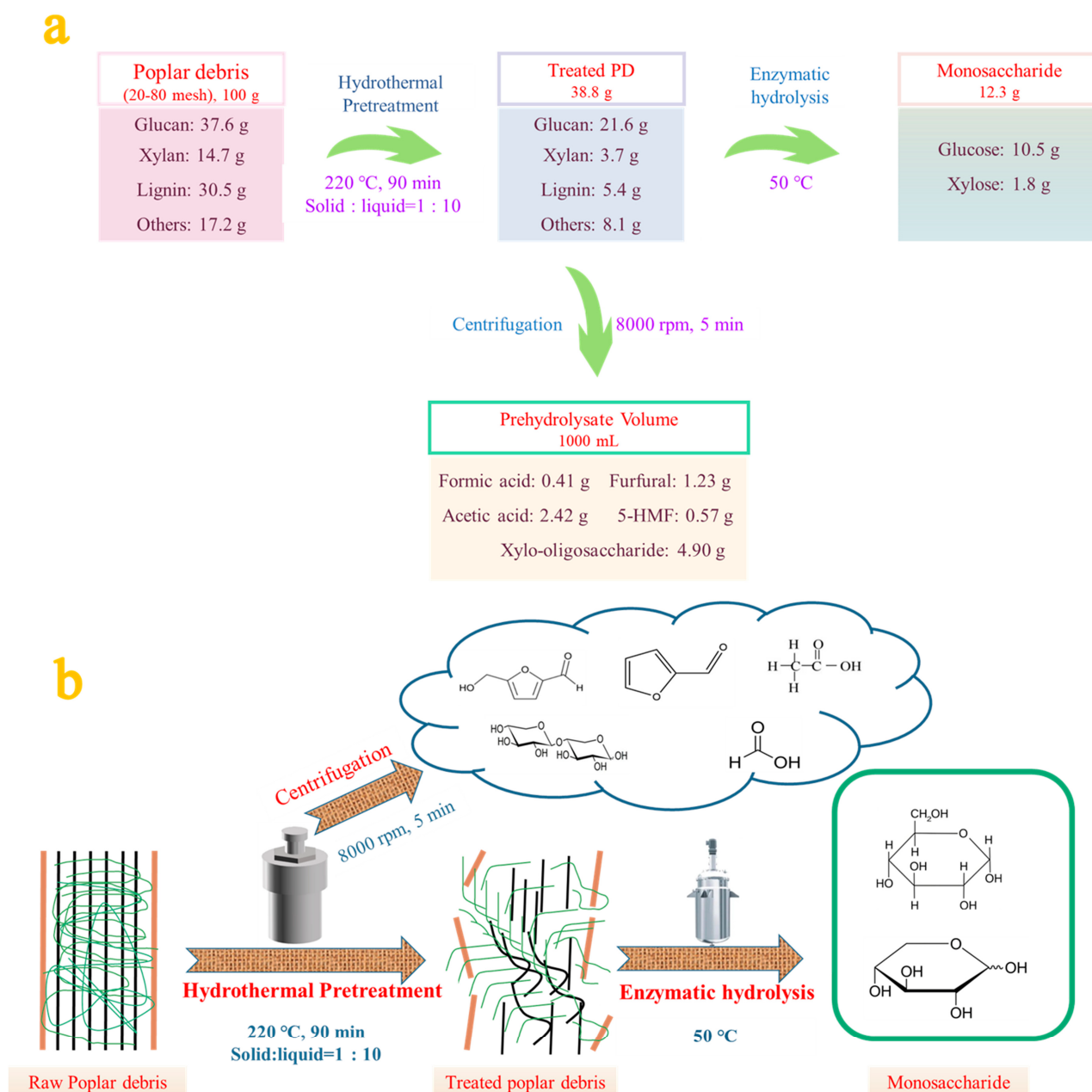


Figure 6. Mass balance of hydrothermal treatment PD and co-production (a); schematic representation of reaction (b).

It is of great interest to explore an effectual valorization way to utilize lignocellulosic biomass since it is a potential replacement for fossil fuels and other non-renewable resources [48]. All around the world, poplar trees can be found in forests. Poplar is widely distributed in the forest all over the world. It can be used as feedstock for producing fermentable sugars and biobased chemicals after the effective treatment [49]. In addition, water is very common and cheap, and hydrothermal pretreatment is easy to perform [50]. The cost of large-scale performance of this pretreatment process is very low. It is clear that hydrothermal pretreatment can improve the removal rate of lignin, possibly by changing the structure of lignin, thus affecting its properties such as solubility, thermal stability, and biodegradability. The removal rate of lignin can be enhanced by hydrothermal pretreatment, which may alter the structure of lignin and impact its solubility, thermal stability, and biodegradability, among other qualities. Pretreatment with hydrothermal energy resulted in a more porous structure, less hydrophobicity and lignin surface area, and improved

accessibility. Hydrothermal pretreatment of several substrates, including lignocellulose, might be explored for use in future research as a means to increase efficiency, decrease costs, and encourage effective exploitation of different substrates. Altering the solvent system, incorporating a specific amount of DES [51], ionic liquid [19], dilute acid, [52] and dilute alkali [53] into the water may further enhance the hydrothermal pretreatment efficiency. The efficient conversion of biomass into bio-based chemicals can be achieved by further enhancing the pretreatment's ability to improve the enzymatic saccharification.

4. Conclusions

In this work, lignin and xylan were successfully removed from PD and its enzymatic digestion efficiency was significantly enhanced after hydrothermal treatment (220 °C; 90 min; $\text{Log}R_0 = 5.49$; liquid–solid mass ratio = 10:1). There were abundant co-products (formic acid, acetic acid, 5-HMF, furfural, and XOSs) in the prehydrolysate. The hydrothermal treatment process increased the accessibility of PD cellulose to cellulase, reduced the surface area of lignin, and weakened the hydrophobicity, resulting in PD becoming looser and more porous, and significantly improving the efficiency of enzymatic hydrolysis. These results implied that hydrothermal treatment, as a clean pretreatment technology, could effectively valorize PD into value-added chemicals, providing a new idea for the sustainable and efficient utilization of PD. Different pretreatment techniques can effectively change the composition and structure of biomass and improve its conversion efficiency, promoting the green transformation of the energy industry and realizing the high-value utilization of biomass resources. In the future, we will explore more suitable temperature and pressure ranges, as well as develop new reactors and catalysts to achieve more efficient and environmentally friendly hydrothermal pretreatment processes, which can be carried out on a large industrial scale. By precisely regulating the treatment conditions, the conversion efficiency of biomass can be further improved, providing more possibilities for the production of bioenergy and bio-based chemicals.

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