Renewable Energy 204 (2023) 176–184

Contents lists available at ScienceDirect

Renewable Energy

journal homepage: www.elsevier.com/locate/renene

Integrated process for the co-production of bioethanol, furfural, and lignin nanoparticles from birch wood via acid hydrotropic fractionation

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ARTICLE INFO

Keywords:
Birch
p-Toluenesulfonic acid
Fractionation
Ethanol
Furfural
Lignin nanoparticles

ABSTRACT

This study proposes an efficient biorefining strategy for co-producing of bioethanol, furfural, and lignin nanoparticles (LNPs) from birch wood based on acid hydrotropic fractionation. Birch was fractionated into glucan-rich washed water-insoluble solids (WIS) fraction and xylose-rich spent liquor (SL) fraction with a low *p*-toluenesulfonic acid (*p*-TsOH) concentration of 15% (w/v). The obtained WIS was utilized to produce 75.5 g/L ethanol (76.3% ethanol yield based on initial sugars in the enzymatic hydrolysate) through separate hydrolysis and fermentation. Meanwhile, the xylose-rich SL was concentrated and catalyzed by dehydration to produce furfural with tetrahydrofuran (THF) as a co-solvent. The furfural concentration of 27.3 g/L (78.2% theoretical yield) was achieved from the 3-fold concentrated SL (without lignin precipitation) with a 3:1 THF-SL ratio at 160 °C for 7 min. The enzymatic hydrolysis solid residue of WIS was further fractionated by *p*-TsOH to prepare LNPs with an average particle size of 37.4 nm. The mass balance showed that 6.5 t oven-dry birch wood produced 1.0 t ethanol, 0.61 t furfural and 0.63 t LNPs.

1. Introduction

The use of bio-degradable lignocellulosic biomass to produce biofuels and other high added-value bio-chemicals and materials through the biorefinery concept has attracted significant attention for several decades. This is attributed to the abundance and renewability of the raw materials used to produce biofuels, serving as a substitute for nonrenewable fossil-based fuels and chemicals [1]. Lignocellulosic biomass exhibits high recalcitrance because of the complex intertwined network of cellulose, hemicelluloses, and lignin [2]. Thus, fractionation or pretreatment is required to disrupt the three-dimensional structure and facilitate enzyme accessibility to biomass [3]. Many diverse fractionation or pretreatment technologies have been developed, including physical [4], chemical [5], biological [6], and combined methods [7]. However, these pretreatments have their pros and cons with regards to energy consumption and production cost. Besides, these methods have difficulties in the economic recovery of chemicals, a prerequisite for sustainability [8,9].

Acid hydrotropic fractionation (AHF) process was recently developed that uses an aromatic acid (*p*-toluenesulfonic acid, *p*-TsOH) to simultaneously solubilize approximately 90% of wood lignin and a substantial amount of hemicelluloses [9,10]. AHF is considered as a potential pretreatment method alternative to conventional acid/alkali pretreatment because of low operating temperature at atmospheric pressure and rapid fractionation along with the potential of acid hydrotrope recycle. The structure characteristics of hydrotropes are similar to those of surfactants containing both a hydrophilic and a hydrophobic functional group [10,11]. Hydrotropes can solubilize hydrophobic substances such as lignin in aqueous systems through aggregation [12] and the lignin can be recovered from the hydrotrope solutions by simple dilution with water. AHF has a good delignification ability of hardwood (poplar [13,14]), grass (reed [14], *Miscanthus* [15], and switchgrass [16]), agricultural resource (corncobs [15], wheat straw [17,18], walnut shell [19], bagasse [20], and corn stover [21]), while has a tough delignification ability of softwood (masson pine [13] and *Radiata* pine [14]) because the dissolution of the syringyl (S) and *p*-hydroxyphenyl (H) units were easier than that of guaiacyl (G) unit [14].

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https://doi.org/10.1016/j.renene.2022.12.111
Received 4 April 2022; Received in revised form 23 November 2022; Accepted 27 December 2022
Available online 31 December 2022

AHF substantially improved cellulose accessibility to enzymes, enabling effective enzymatic sugar production. Furthermore, *p*-TsOH in the fractionation spent liquor (SL) can catalyze the dehydration of dissolved xylan into furfural without additional catalysts [12]. Furfural is a promising renewable platform molecule for sustainable production of C_4 and C_5 chemicals [22]. Low-temperature AHF also decreased lignin condensation, facilitating lignin valorization [23,24]. Separating dissolved lignin is achieved simply by diluting the *p*-TsOH in SL to the minimal hydrotrope concentration (MHC) [9,12]. The *p*-TsOH is then reused after concentration. The amount of water evaporation is extensive; however, it is similar to that used for weak black liquor evaporation in commercial Kraft pulp mills. The SL can also be directly reused without lignin separation without losing its efficacy [12]; this can save energy for evaporation.

Ji et al. [25] demonstrated effective production of furfural, phenolic compounds, and ethanol using *p*-TsOH but using γ-valerolactone (GVL) for fractionation, an extra process step with additional chemical cost as GVL is a very expensive solvent. To improve enzymatic saccharification, dissolution of xylan has found to be most effective [26]. On the other hand, it is very desirable to reduce *p*-TsOH concentration to decrease load for chemical recovery. As demonstrated previously using poplar wood [9,12] and herbaceous biomass [16,17], the reduced fractionation severity due to low *p*-TsOH loading can be compensated by using elevated temperatures (< 100 °C) and extended reaction time to ensure substantial dissolution of xylan and therefore to improve enzymatic sugars and furfural production.

Zhu et al. [12] proposed that hardwood poplar was fractionated with *p*-TsOH at the concentration of 55%; after filtration, the washed water-insoluble solids (WIS) were used to produce bioethanol via simultaneous saccharification and fermentation, while the SL was diluted to remove the lignin (because high lignin content could affect the furfural production), and then dehydrated to prepare furfural. However, the precipitated lignin was not studied further and the furfural concentration is relatively low. Therefore, this study aimed to evaluate the effectiveness of AHF for birch wood biorefinery using *p*-TsOH at lower concentrations than those reported in previous studies and with a multi-product portfolio for valorization all three components of lignocellulosic biomass. The specific objectives are (1) to optimize AHF for birch wood bioconversion using a reaction kinetics based severity factor for bioethanol production from the fractionated WIS, (2) to produce furfural from the dissolved xylose in the SL or concentrated SL (for high furfural concentration) in batch dehydration with/without co-solvent (with co-solvent to inhibit furfural decomposition to byproducts such as levulinic acid, formic acid and humins [27]), and with/without lignin separation for defining better route of *p*-TsOH recovery, and (3) to produce lignin nanoparticles (LNPs) from the enzymatic hydrolysis solid residue (EHSR) of WIS using further *p*-TsOH fractionation. In General, this work proposes a *p*-TsOH pretreatment at a low *p*-TsOH concentration to fractionate all lignocellulosic components and especially utilizes the EHSR for co-producing bioethanol, furfural, and lignin nanoparticles. Compared with the previous study [12], a higher concentration of bioethanol with separated hydrolysis and fermentation, and a higher concentration of furfural with a co-solvent system were produced, and especially lignin nanoparticles were generated from the EHSR via *p*-TsOH fractionation. The findings of this study will guide the development of a potential technology for biochemical conversion of birch wood.

2. Materials and methods

2.1. Materials

Birch wood logs was debarked and chipped at the USDA Forest Products Laboratory. The wood chips were hammer milled and then Wiley milled (model No. 2, Arthur Thomas Co, Philadelphia, PA, USA) to 20-mesh. The *p*-TsOH (ACS reagent grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagent-grade tetrahydrofuran (THF) (>99% purity) was obtained from Fisher Scientific (Pittsburgh, PA, USA). Other chemicals, such as sulfuric acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

A commercial complex cellulase, Cellic® CTec 3 (abbreviated CTec 3), was provided by Novozymes North America (Franklinton, North Carolina, USA). Its cellulase activity was 217 FPU/mL determined using filter paper assay as per the International Union of Pure and Applied Chemists [28].

2.2. Birch fractionation using p-TsOH

Wiley milled birch wood of 5 g (in oven dry weight) were fractionated using 50 mL aqueous *p*-TsOH solutions of different concentrations (15%, 30%, 45%, 60%, and 75%, w/v) at 90 °C for various reaction times (60, 120, 180, and 240 min). A low *p*-TsOH concentration of 5% at 80 °C for 70 min was also studied. The selected large-scale fractionation run using 100 g birch wood was carried out based on the results of optimization study using 5 g wood.

For all runs, vacuum filtration was used to directly separate undissolved solids and SL at the end of each fractionation. The filtrate (SL) collected from 100 g birch fractionation was used to produce furfural through dehydration. Deionized (DI) water was used to thoroughly wash the separated solid samples until the fluid reached a pH of 5–6. The WIS was then used to produce bioethanol through separate hydrolysis and fermentation (SHF).

2.3. Enzymatic hydrolysis of WIS

Enzymatic hydrolysis of all WISs was performed in 125 mL Erlenmeyer flasks on an incubator shaker at 200 rpm and 50 °C with solid loading of 1% (w/v) at pH 5.5 [12]. CTec 3 cellulase loading of 20 FPU/g glucan was used. After 72 h of hydrolysis, enzymatic hydrolysates were centrifuged at 10,000 rpm for 5 min. The supernatant was analyzed for monosaccharides via HPLC.

Solid substrate loading of 10% (w/v) was used in enzymatic saccharification to produce glucose for SHF. The hydrolysate was centrifuged and the supernatant was used for subsequent SHF.

2.4. Separate hydrolysis and fermentation

The yeast *Saccharomyces cerevisiae* YRH400, an engineered fungal strain for glucose and xylose fermentation, was obtained from the USDA Agriculture Research Service [12]. The strain was maintained at 4 °C on YPD agar plates supplemented with 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar.

A single colony from the YPD agar plate was inoculated into 50 mL liquid YPD medium in a 125 mL flask and cultured at 30 °C and 200 rpm for 24 h. A UV–vis spectrophotometer (Model 8453, Agilent Technologies, Palo Alto, CA, USA) was used to monitor the yeast concentration based on optical density at 600 nm (OD_{600}).

Part of the supernatants of the enzymatic hydrolysates from the 10% solid loading run was concentrated using a BÜCHI vacuum rotary evaporator R-210 at 60 °C and 160 mbar. Both the un-concentrated and the concentrated hydrolysates were supplemented with yeast extract (5 g/L), $(NH_4)_2SO_4$ (2 g/L), and NaH_2PO_4 (5 g/L) for fermentation in flasks at 30 °C and 150 rpm. The yeast loading was measured at an initial optical density (OD_{600}) of 5. Samples were withdrawn periodically (0–96 h) and centrifuged at 10,000 rpm for 5 min and then analyzed for glucose, xylose, fermentation products including ethanol, xylitol, and glycerol.

2.5. Furfural production from the p-TsOH spent liquor

Pure xylose solution was first used to optimize furfural production in batch mode catalyzed by *p*-TsOH. The collected SL of birch was then

used to evaluate furfural production under the conditions from the optimization study. SL was concentrated three folds through evaporation in a BÜCHI vacuum rotary evaporator R-210 at 60 °C and 160 mbar before dehydration to furfural. Two strategies were evaluated to investigate the effect of the dissolved lignin in SL on furfural production: (1) SL was directly dehydrated without lignin separation; (2) SL was first diluted with DI water three folds to separate lignin, and then re-concentrated three and six folds for dehydration [9].

Furfural dehydration was conducted at 150–210 °C for 5.5–25 min in a 25 mL stainless reactor containing 20 mL liquid with/without adding THF as co-solvent. A sand heating bath (Techne F932D, Techne Inc.) was used to heat the reactor. After heating the sand to the target temperature, the stainless reactor was completely immersed in the sand bath and kept for a period of time. At the end of the reaction, the stainless reactor was immediately quenched in an ice-water bath. The compositions of the reacted liquor were analyzed by HPLC.

2.6. Enzymatic hydrolysis solid residue for LNPs production

The EHSR from enzymatic hydrolysis of *p*-TsOH fractionated WIS was further fractionated to dissolve lignin using 55% (w/v) *p*-TsOH at 90 °C for 120 min, as described by Zhu et al. [29]. After filtration, the SL fraction containing primarily dissolved lignin was magnetically stirred at 600 rpm, and then added with deionized (DI) water at 2 mL/min to dilute the *p*-TsOH concentration below 10%. The diluted SL was centrifuged at 8000 rpm for 5 min and washed several times until the pH of the liquor became neutral. The obtained liquor contained LNPs. Transmission electron microscopy (TEM) was used for LNPs analysis. All aforementioned experiments were performed in duplicate to ensure the reproducibility of the experimental results.

2.7. Analytical methods

The chemical compositions (glucan, xylan, and lignin) of untreated birch and *p*-TsOH fractionated birch WISs were analyzed at the Analytical Chemistry and Microscopy Laboratory of the USDA Forest Products Laboratory (Madison, WI, USA), as described previously [30].

The HPLC system (Ultimate 3000, ThermoFisher Scientific) and refractive index detector (RI-101, Shodex) were used to determine the chemical compositions (glucose, xylose, xylitol, glycerol, ethanol, formic acid, acetic acid, levulinic acid, 5-hydromethylfurfural (5-HMF), and furfural) of SLs, enzymatic hydrolysates, and fermentation broths as described previously [31]. Chromatographic species separation was achieved using a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm i.d.). The separation was performed at 60 °C and a flow rate of 0.6 mL/min with dilute sulfuric acid (0.005 mol/L) as the mobile phase. At least two parallel samples were used in all analyses; data were presented as the mean of the replicates. Relative errors were determined to be within 5% for sugar and ethanol analysis and 10% for inhibitor assay. The experimental data were sorted, plotted and analyzed with Excel 2016 and Origin 9.0.

Transmission electron microscopy (TEM, Model, JEM-1400, Japan Electronics Corporation) was used to analyze the obtained particle size of LNPs [29]. The obtained LNPs solution was centrifuged at 10,000 rpm for 5 min. Then, the obtained solid fractions were dispersed using ethanol to obtain a suspension of LNPs (~ 1 g/L), which was added to a copper mesh slide, dried at room temperature for 12 h, and then observed by TEM. The particle size was analyzed by the software of Image Pro Plus. The optical density (OD) of the yeast was measured spectrophotometrically at 600 nm with the vessel diameter of 0.5 cm [12].

3. Results and discussion

3.1. Fractionation mass balance and severity

The analysis results of chemical compositions and yields of WISs from *p*-TsOH fractionated birch wood under different conditions along with the concentrations of glucose, xylose, formic acid, acetic acid, 5-HMF and furfural in the SLs (Table 1) showed that AHF was highly selective in preserving glucan (mainly retained in WIS) and dissolution of xylan and lignin. To facilitate process scale-up, a combined hydrolysis factor (*CHF*) and a combined delignification factor (*CDF*) developed previously [17,32] are used based on reaction kinetics for analyzing xylan and lignin dissolution data. By using a biphasic reaction model for both xylan and lignin (i.e., both xylan and lignin contains a fast and a slow fraction), the fraction of xylan, X_R, and lignin, L_R, that remained in WIS can be expressed as:

$$X_R = (1 \quad \theta \quad \theta_R) e^{\ CHF} + \theta \bullet e^{\ f \bullet CHF} + \theta_R \tag{1a}$$

$$CHF = \exp\left(\alpha \quad \frac{E}{RT} + \beta C\right) \bullet C \bullet t \tag{1b}$$

$$L_R = \ 1 \quad \theta' \quad \theta'_R) e^{\ CDF} + \theta' \bullet e^{\ f' \bullet CDF} + \theta'_R \tag{2a}$$

$$CDF = exp\left(\alpha' \quad \frac{E'}{RT} + \beta' C\right) \bullet C \bullet t \tag{2b}$$

where C is the *p*-TsOH molar concentrations (mol/L), $R = 8.314$ (J/mol/K) is the universal gas content, t is reaction time in min, and T is reaction temperature in kelvins. α, α', and β, β' are adjustable parameters, E and E' are apparent activation energy (J/mol), θ and θ' are the initial fraction of slow-reacting xylan and lignin, respectively. f and f' are the ratios of reaction rates between slow and fast xylan and slow and fast lignin, respectively. θ_R and θ_R' are residual xylan and lignin, respectively [11,17].

Fitting the data in Table 1 using Eqs (1) and (2) resulted in adjustable parameters $\alpha = 25.29$, $\alpha' = 36.07$ and $\beta = 0$, $\beta' = 0.1$, apparent activation energy $E = 70{,}000$ and $E' = 96{,}000$ (J/mol), the fraction of slow reacting xylan and lignin of $\theta = 0.549$ and $\theta' = 0.911$, respectively, and the ratios of reaction rates between slow and fast xylan $f = 0.0014$ and between slow and fast lignin $f' = 0.000047$, and residual xylan and lignin $\theta_R = 0.15$ and $\theta_R' = 0.03$, respectively. Excellent fittings of the data were obtained as shown in Fig. 1. Therefore, xylan dissolution and delignification can be controlled by *CHF* and *CDF*, respectively, independent of individual fractionation conditions. This reaction kinetics was successfully used for wheat straw [17], poplar [23], switchgrass [16], and bleached eucalyptus kraft dry lap pulp [33]. This suggested that *CHF* and *CDF* were true fractionation severity and *CHF* could be a real indicator used for process scale-up. For example, low acid concentrations are often preferred to reduce chemical recovery costs, a long reaction time can be used to compensate for a low acid loading. Therefore, based on the results shown in Fig. 1, we chose to use a low *p*-TsOH concentration of 15% and a relatively long reaction time of 180 min to achieve good dissolution of xylan with low delignification for a scale-up run using 100 g of birch. The corresponding severities for this condition were $CHF = 1162.5$ and $CDF = 14563.9$ according to Eqs. (1b) and (2b), respectively. Although the $CHF = 1162.5$ seemed not very good as the curve showed in Fig. 1A, increasing the value of *CHF* increased the *p*-TsOH concentration (because the reaction temperature was fixed, and the order of influencing strength of fractionation conditions was as follows: treatment temperature > *p*-TsOH concentration > treatment time). The actual xylan and lignin dissolutions were approximately 71% and 43% based on WIS yield and chemical composition, and the glucan dissolution was very low (less than 3%). Most of the dissolved xylan was in the form of xylose (Table 1). The obtained WIS (P15T90t180) was used to produce ethanol by separate hydrolysis and fermentation (SHF), while

Table 1
Chemical compositions of *p*-TsOH fractionated birch wood samples under different treatment conditions. The numbers in parentheses are component yield based on the component in the untreated birch.

Sample label[a]	Solid yield (%)	WIS (%)			Spent liquor (g/L)					
		Glucan	Xylan	Lignin	Glucose	Xylose	Formic acid	Acetic acid	5-HMF	Furfural
Untreated birch	100	36.0 ± 0	23.1 ± 0.1	21.8 ± 0.1						
P5T80t70	89.3	39.9 ± 0.4(98.9)	17.8 ± 0.3(68.8)	22.3 ± 0.3(91.3)	0.13	7.98	0.08	4.62	0	0.02
P15T90t120	63.2	56.1 ± 0.2(98.3)	11.6 ± 0.1(31.8)	25.9 ± 0.1(75.2)	0.28	17.15	0.46	5.75	0.16	0.09
P30T90t120	51.6	68.9 ± 1.0(98.6)	11.5 ± 0.1(25.6)	18.4 ± 0.1(43.5)	0.48	19.03	0.46	5.94	0.24	0.24
P45T90t120	44.3	78.7 ± 1.1(96.7)	10.1 ± 0.2(19.4)	11.6 ± 0.4(23.6)	0.70	19.47	0.38	5.96	0.16	0.60
P60T90t120	42.0	82.0 ± 0.4(95.5)	8.5 ± 0.1(15.5)	8.9 ± 0.2(17.1)	0.86	19.36	0.12	6.14	0	0.98
P75T90t120	37.9	86.0 ± 0.3(90.5)	6.7 ± 0.1(11.0)	6.7 ± 0.1(11.7)	1.26	16.51	0.18	5.88	0	1.63
P15T90t60	64.8	54.9 ± 0.1(98.7)	12.8 ± 0.1(36.1)	25.2 ± 0.3(75.0)	0.17	14.62	0.37	5.40	0.16	0.06
P15T90t180	59.2	60.6 ± 1.5(99.5)	9.6 ± 0.1(24.7)	21.8 ± 0.2(59.2)	0.60	18.60	0.48	6.02	0.15	0.15
P15T90t240	57.8	61.0 ± 0.3(97.9)	9.1 ± 0.1(22.9)	11.6 ± 0.1(30.8)	0.68	19.40	0.45	5.96	0.12	0.29

[a] PxxTyytzz, xx stands for *p*-TsOH concentration in %, yy stands for reaction temperature in °C, zz stands for reaction time in min. Abbreviations: WIS, Washed water insoluble solids; 5-HMF, 5-Hydroxymethylfurfural; *p*-TsOH, *p*-Toluenesulfonic acid.

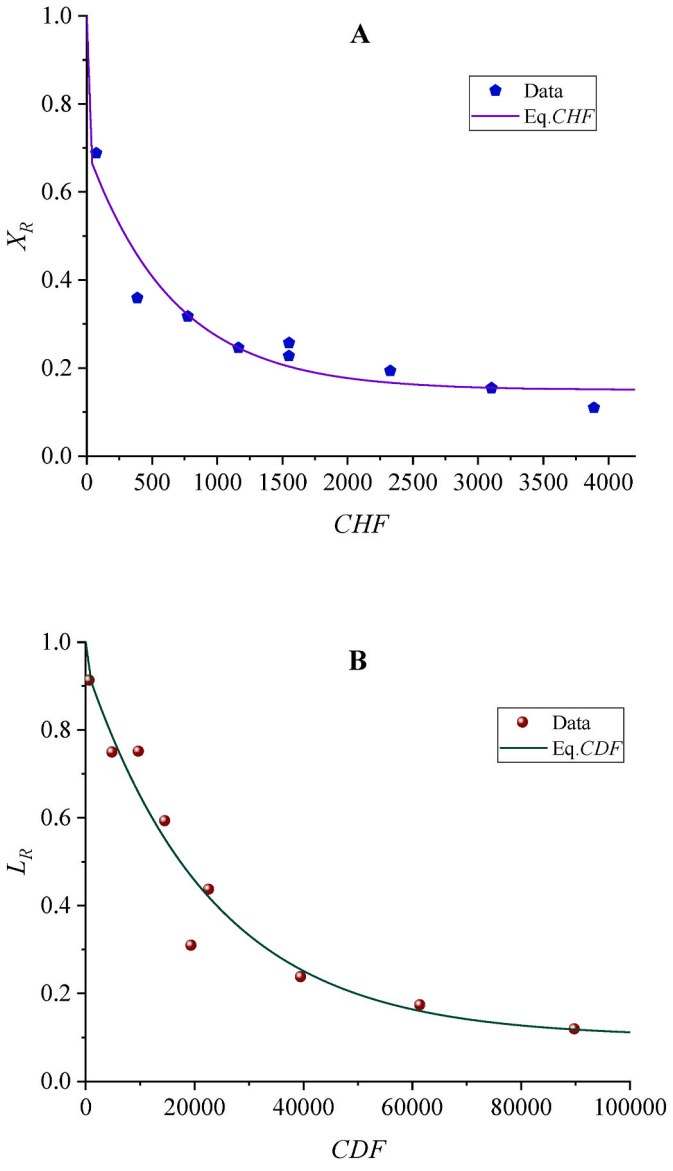

Fig. 1. Predictions of xylan and lignin dissolution by *p*-TsOH fractionation of birch using a combined hydrolysis factor (*CHF*) and a combined delignification factor (*CDF*), respectively, in comparison with experimental data.

the SL was used to prepare furfural. Zhu et al. [12] chose the condition of P50T90t112 to obtain a good delignification (83.7%) and dissolution of xylan (78.6%) for a scale-up run using 100 g of poplar. The corresponding severities *CHF* and *CDF* were 78 and 296, respectively. Ji et al. [34] used the *CHF* of approximately 3.90 as the optimal pretreatment severity for Hybrid poplar to balance enzymatic saccharification and the structural characteristics of lignin with approximately 90% of the xylan removal and a sugar yield of 76%. Therefore, proper reaction severities were chosen based on the xylan and lignin dissolutions.

The relationships between the glucose yield from WIS, and *CHF* and *CDF* are shown in Fig. 2, which demonstrated that glucose yield increased with the increase in *CHF* and/or *CDF* due to increasing xylan and/or lignin dissolution which improved substrate glucan accessibility to cellulase [35]. The highest glucose yield (99%) was observed at *CHF* of 3887.7 and *CDF* of 90026.1. In order to obtain a relatively high glucose yield (>85%) at a lower *p*-TsOH concentration, the conditions of *p*-TsOH of 15% and 180 min (P15T90t180) were selected from Fig. 2. The glucose yield was up to 88.4%. Under the same enzymatic hydrolysis conditions for the fractionated poplar (P50T90t112) as described by Zhu et al. [12], the glucose yield of WIS reached 93%. The results of Ji et al. [34] also showed that the sugar yield increased with the increase in *CHF*. Therefore, these results indicated that higher delignification and/or xylan removal could achieve a higher enzymatic hydrolysis

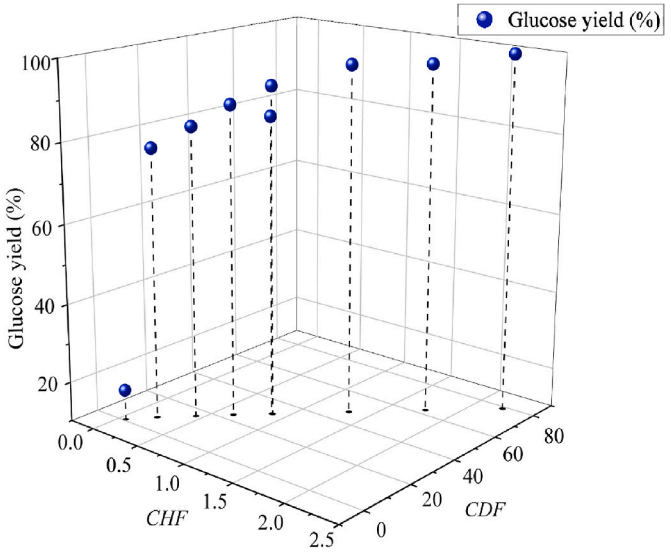

Fig. 2. Glucose yield of *p*-TsOH fractionated birch WIS with various combined hydrolysis factor (*CHF*) and combined delignification factor (*CDF*) at 1% (w/v) substrate and 50 °C with pH 5.5 for 72 h. Abbreviations: *p*-TsOH, *p*-Toluenesulfonic acid; WIS, Washed water insoluble solids.

performance.

The concentrations of glucose, xylose, formic acid, acetic acid, 5-HMF and furfural in the SLs (Table 1) showed that xylose (8.0–19.5 g/L) from the xylan hydrolysis was the main monosaccharide and a small amount of glucose (0.1–1.3 g/L) was also produced from glucan hydrolysis. A variety of carbohydrate-degradation products (formic acid, acetic acid, 5-HMF and furfural) were also produced, which inhibited the subsequential ethanol fermentation if they were not detoxified [36]. Acetic acid, released through the acetyl groups of hemicellulose, was the main degradation products from 4.6 to 6.1 g/L 5-HMF and furfural are formed by the degradation of sugars, while formic acid is produced from the degradation of 5-HMF and furfural [29,36]. The results of Ma et al. [17] demonstrated that glucose, xylose, formic acid, acetic acid, and furfural except 5-HMF were generated in the SL from the *p*-TsOH pretreated wheat straw.

3.2. Separate hydrolysis and fermentation of WIS

Separate hydrolysis and fermentation of *p*-TsOH fractionated birch WIS from run P15T90t180 was performed in a 250 mL Erlenmeyer flask at 10% (w/v) substrate loading. 57.8 g/L glucose and 11.0 g/L xylose were detected in the hydrolysate at 48 h, representing 88.2% of glucose yield. The hydrolysate was then concentrated by two and three folds via vacuum evaporation for subsequent ethanol fermentation. Glucose concentration was increased from 57.3 to 107.0 and 165.1 g/L, while xylose concentration was increased from 10.9 to 20.6 and 32.1 g/L, respectively.

Fig. 3 shows the performance of the fermentation runs using the un-concentrated and concentrated (by two and three folds) hydrolysates by *S. cerevisiase* YRH400. Ethanol yield was calculated as the percentage of ethanol determined to the ethanol theoretically produced from the initial glucose and xylose in the un-concentrated/concentrated hydrolysate [12]. As listed in Fig. 3A, after 30 h fermentation, glucose and xylose concentration decreased from 57.3 to 2.9 g/L, and 10.9 to 2.6 g/L, respectively, and ethanol concentration reached 27.8 g/L. The glucose consumption rate was 2.27 g/(L·h) before 24 h, and the xylose was not utilized before the glucose was almost completely consumed. Furthermore, the overall sugar utilization ratio and ethanol yield were 91.9% and 81.2%, respectively. The 2-fold concentrated enzymatic hydrolysate (glucose 107.0 g/L, xylose 20.6 g/L) also exhibited good fermentability. The ethanol concentration, sugar utilization ratio, and ethanol yield were 53.3 g/L, 94.6%, and 83.3%, respectively, fermentation time was prolonged to 72 h (Fig. 3B). The glucose consumption rate was 3.12 g/(L·h) before 24 h. Notably, the 3-fold concentrated enzymatic hydrolysate (glucose 165.1 g/L, xylose 32.1 g/L) was fermented slowly before 48 h, the glucose consumption rate was 1.81 g/(L·h) before 24 h. The ethanol concentration, sugar utilization ratio, and ethanol yield were 35.3 g/L, 47.6%, and 35.7%, respectively (Fig. 3C). This could be because the initial sugars concentration was very high (≈200 g/L, glucose 165.1 g/L and xylose 32.1 g/L), and the initial OD_{600} of the yeast inoculum was relatively low (OD_{600} = 5). Therefore, another yeast inoculum of OD_{600} = 15 was added at 50 h, and the fermentation process was allowed to continue for an additional 46 h. As a result, the ethanol concentration, sugar utilization ratio, and ethanol yield improved to 75.5 g/L, 91.5%, and 76.3%, respectively. The glucose consumption rate increased to 2.95 g/(L·h) between 50 and 72 h, indicating that high yeast inoculum increased the sugar consumption rate. Therefore, these results indicated that a higher concentration of yeast inoculum was needed when the sugar concentration was increased. Chu et al. [37] used the concentrated sugar solution (glucose 216.97 g/L, xylose 11.27 g/L) from the enzymatic hydrolysis of corn stover for ethanol fermentation with initial *S. cerevisiae* yeast inoculum of OD_{600} = 15. The results showed that the glucose consumption and ethanol yield was 99.48% and 94.98%, respectively. This indicated that the concentration of sugar solution was performed to increase glucose concentration, so as to increase subsequent ethanol concentration in the fermentation liquor [37].

S. cerevisiae YRH400 can also utilize xylose because it is engineered

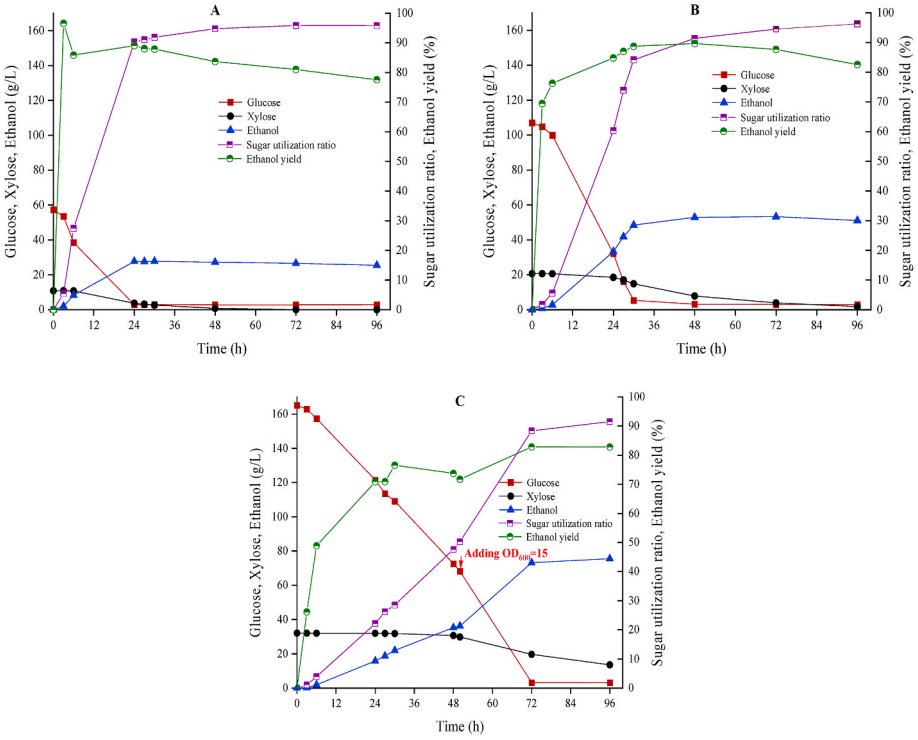

Fig. 3. Changes in the parameters during the ethanol fermentation of enzymatic hydrolysate of WIS (10% (w/v) substrate loading) (A), 2-fold concentrated enzymatic hydrolysate (B), and 3-fold concentrated enzymatic hydrolysate (C) by *S. cerevisiase* YRH400 with OD_{600} = 5. Abbreviations: WIS, Washed water insoluble solids; OD_{600}, Optical density at 600 nm.

with xylose fermentation genes that code for xylose reductase, xylitol dehydrogenase, and xylulokinase [12]. However, xylose accumulated more with increasing initial sugar concentration except for the un-concentrated hydrolysate, indicating that xylose utilization was more difficult than glucose even if the xylose fermentation genes were engineered. The xylose consumption rate was 0.24, 0.25, and 0.28 g/(L·h) of the un-concentrated hydrolysate (6–48 h), the 2-fold concentrated hydrolysate (6–72 h), and the 3-fold concentrated hydrolysate (30–96 h), respectively. The fermentation by-product xylitol was also produced, increased with the increasing of initial sugar concentration, but less than 0.9 g/L (data not shown in Fig. 3). Another fermentation by-product glycerol was generated, and its concentration increased with the increase in sugar concentration, reaching the highest value of 1.9, 5.3, 8.8 g/L at the un-concentrated hydrolysate, 2-fold concentrated hydrolysate and 3-fold concentrated enzymatic hydrolysate, respectively (data not shown in Fig. 3). The higher the initial sugar concentration, the higher the glycerol concentration. This is because the initial sugar is high, yeast secretes glycerol to balance in order to relieve the osmotic stress of sugar on itself [38].

3.3. Furfural production

Furfural production from a simulation xylose solution was conducted under at two sets of conditions: xylose and *p*-TsOH concentrations of 20 g/L and 15% (w/v), and 55 g/L and 45% (w/v), respectively. The experiment was performed based on the concentrations of xylose and *p*-TsOH in the SL of P15T90t180 before and after 3-fold concentration (Table 1). Furfural yield was calculated based on the measured terminal furfural concentration (including furfural production in the fractionation process) as a percentage of the theoretically achievable furfural from the initial xylose concentration in the simulation xylose solution or the SL. High temperature and short reaction time enhanced furfural yield at xylose concentration of 20 g/L and *p*-TsOH concentration of 15% (w/v) (Table S1). The reaction at 200 °C for 6 min produced the highest furfural yield of 62.6% (theoretical). The results of Danon et al. [39] showed that furfural yield of 30% was obtained in monophasic system at 170 °C. Therefore, the furfural yield (62.6%) of this simulation xylose solution in this study is excellent for batch operations at high temperature for a short time without halide salts or biphasic system.

Furfural yield was tended to reduce because of unavoidable side reactions, such as condensation and self-polymerization [40,41]. Xylose was almost depleted with a negligible amount of formic acid (0.20 g/L). Furfural yield did not increase at higher xylose and *p*-TsOH concentrations (Table S2). The maximum furfural concentration of 14.7 g/L (corresponding to 45.1% furfural yield) was achieved at 160 °C for 7 min with the remaining xylose concentration of only 0.63 g/L. This is attributed to severe unavoidable side reactions, which tends to reduce furfural yield. Therefore, alternative methods should be adopted to improve the furfural yield. Cai et al. [42] revealed that tetrahydrofuran (THF) as a novel single-phase co-solvent can promote higher yields of fuel precursors furfural, 5-HMF, and levulinic acid from biomass. THF is a promising organic solvent that is less toxic and miscible with water over a wide range of reaction conditions. Also, it can be synthesized directly from furfural at high selectivity through catalytic de-carbonylation to furan followed by ring hydrogenation. Besides, THF can be easily recovered by room temperature vacuum distillation due to its low boiling point [43]. Therefore, THF was used as a co-solvent in this study to increase the furfural yield. Furthermore, we compared furfural yield from THF co-solvent and non-solvent reactions of simulation xylose solution (Table S2). THF improved furfural yield from 45.1% to 75.7% at 160 °C for 7 min, compared to the non-solvent, which indicates that THF catalyzes xylose dehydration to improve the furfural yield. Varying the solvent-water ratio in the co-solvent reactions showed that furfural yield decreased with a decrease in THF level. The results of Cai et al. [42] showed that THF improved furfural yields from 62% to 87% compared to the non-solvent case from the maple wood at 170 °C for 40 min. Cai et al. [44] further improved the furfural yield (95%) directly from maple wood when combined $FeCl_3$ with THF co-solvent.

According to the results from the aforementioned simulation xylose and *p*-TsOH solution, furfural produced from the un-concentrated and 3-fold concentrated *p*-TsOH SLs of P15T90t180 is listed in Table 2. And also, comparisons were made between SLs with/without lignin precipitation. The SL without lignin precipitation was directly dehydrated. According to the results, xylose was not completely depleted after dehydration reactions at 180 °C. The highest furfural yield of 65.9% with residue xylose concentration of 3.39 g/L was obtained at 180 °C for 6 min. Increasing reaction time reduced residue xylose concentration but did not increase furfural yield. This can be attributed to furfural condensation through sided reactions [40].

Direct dehydration of the virgin SL from poplar after *p*-TsOH fractionation resulted in a high furfural concentration of 5.44 g/L at 68.4% yield as described by Zhu et al. [12]. While, precipitating lignin in the SL increased the furfural concentration to 6.18 g/L and yield to 77.7%. Therefore, to study the effect of dissolved lignin on furfural production, we precipitated lignin in the SL. The dehydration results from the SL with lignin precipitation were compared with the results from the virgin SL. Heating at 180 °C for 6 min produced the highest furfural yield of 64.5%, slightly lower than 65.9% obtained using the virgin SL without lignin precipitation. For the virgin SL, residue xylose concentration increased from 3.4 to 4.1 g/L in the lignin precipitated liquor. This indicates that the SL without lignin precipitation can obtain a relatively high furfural yield, possibly because there is about 38% of the dissolved lignin in the SL, which has a minimal effect on the furfural yield. A small amount of glucose in the SL resulted in 5-HMF formation (Table 2).

To obtain higher xylose and furfural concentration, we concentrated SL three folds with/without lignin precipitation. Furfural production using 3-fold concentrated SL was conducted at 160–170 °C for various reaction times, with/without THF co-solvent, based on the results of the aforementioned simulation xylose experiment (Table S2). The furfural yield was 33.0%/28.4% with/without lignin precipitation at 170 °C for 6 min, and the remaining xylose concentration was 2.33 and 2.08 g/L, respectively. This can be attributed to furfural condensation through sided reactions [40]. Therefore, THF was added as a co-solvent to reduce the sided reactions and we reduced the reaction conditions to 160 °C and 7 min. As a result, furfural yield increased to 79.4%/78.2% with/-without lignin precipitation at THF/water of 3/1 and 160 °C for 7 min. Meanwhile, residue xylose concentration was 4.73/5.31 g/L with/-without lignin precipitation. Although the furfural yield of SL with lignin precipitation was slightly higher, the process of dilution to lignin precipitation, involving water addition and re-concentration by vacuum evaporation, required more energy. It was obviously expensive. Therefore, SL without lignin precipitation was better to produce furfural. Moreover, 5-HMF was produced from glucose dehydration and levulinic acid was generated from 5-HMF further degradation during the furfural preparation. As the result of Cai et al. [42], besides furfural, 5-HMF and levulinic acid were also produced from maple wood with THF co-solvent at the same time. Decreasing the ratio of THF to water led to a reduction in the furfural yield. The results of Wang et al. [45] showed a highest furfural yield of 76.7% with 100% xylose conversion was achieved at 200 °C for 45 min in γ-valerolactone (GVL)-water (85:15 v/v%) mixture. The furfural yield was similar to that of this study with THF co-solvent.

3.4. Lignin nanoparticles prepared from enzymatic hydrolysis solid residue

The EHSR obtained after enzymatic hydrolysis of WIS (P15T90t180) was further fractionated using 55% *p*-TsOH at 90 °C for 120 min. After solid-liquid separation, the SL was diluted with water below the MHC of *p*-TsOH to obtain LNPs with a yield of 71.3% based on the lignin in the EHSR. The results of TEM images analysis showed that the untreated EHSR were large and agglomerated particles with cellulosic fibers (Fig. S1A). LNPs produced from the SL of *p*-TsOH fractionated EHSR

Table 2
Compositional analysis of spent liquor from *p*-TsOH fractionated birch with/without lignin precipitation after furfural production at different reaction conditions.

Name	Un-concentrated spent liquor						3-Fold concentrated spent liquor							
Lignin precipitation	No			Yes			No				Yes			
T (°C)/t (min)	180/6	180/7	180/8	180/6	180/7	180/8	170/6	160/7			170/6	160/7		
THF/Water, v/v	0	0	0	0	0	0	0	3/1	2/1	1/1	0	3/1	2/1	1/1
Glucose[a] (g/L)	0.34	0.31	0.20	0.39	0.23	0.21	0.09	0.42	0.49	0.60	0.25	0.40	0.44	0.44
Xylose[a] (g/L)	3.39	2.55	1.72	4.13	1.95	1.81	2.08	5.31	6.17	7.22	2.33	4.73	4.95	4.89
Formic acid[a] (g/L)	0.70	0.432	0.52	0.53	0.44	0.44	1.72	1.33	1.38	0.37	1.49	0.23	0.36	0.48
Acetic acid[a] (g/L)	6.33	6.345	6.25	2.39	2.34	2.31	9.44	9.14	8.69	10.10	6.20	6.61	7.11	6.77
Levulinic acid[a] (g/L)	0.44	0.505	0.61	0.33	0.54	0.56	1.56	0	0	0.82	1.40	0.48	0.76	0.86
5-HMF[a] (g/L)	0.08	0.06	0.05	0.08	0.05	0.05	0.05	0.71	0.61	0.48	0.08	0.72	0.67	0.48
Furfural[a] (g/L)	7.83	7.71	7.38	7.67[b]	7.66[b]	7.45[b]	9.94	27.33	24.49	22.70	11.53[b]	27.48[b]	26.57[b]	23.01[b]
Furfural yield[c] (%)	65.9	64.9	62.1	64.5	64.5	62.7	28.4	78.2	70.1	65.0	33.3	79.4	76.8	66.5

[a] The concentration is calculated as the concentration in the original *p*-TsOH spent liquor.
[b] Furfural concentration contains the furfural produced in the fractionation process and the furfural produced from the spent liquor by dehydration.
[c] It was calculated using the total furfural produced from the original spent liquor as the percentage of the theoretical achievable furfural from the amount of xylose in the *p*-TsOH spent liquor. The initial xylose concentration in the un-concentrated spent liquor (with/without lignin precipitation) was 18.57 g/L, while the initial xylose concentration in 3-fold concentrated spent liquor with or without lignin precipitation was 54.06, 54.60 g/L, respectively.

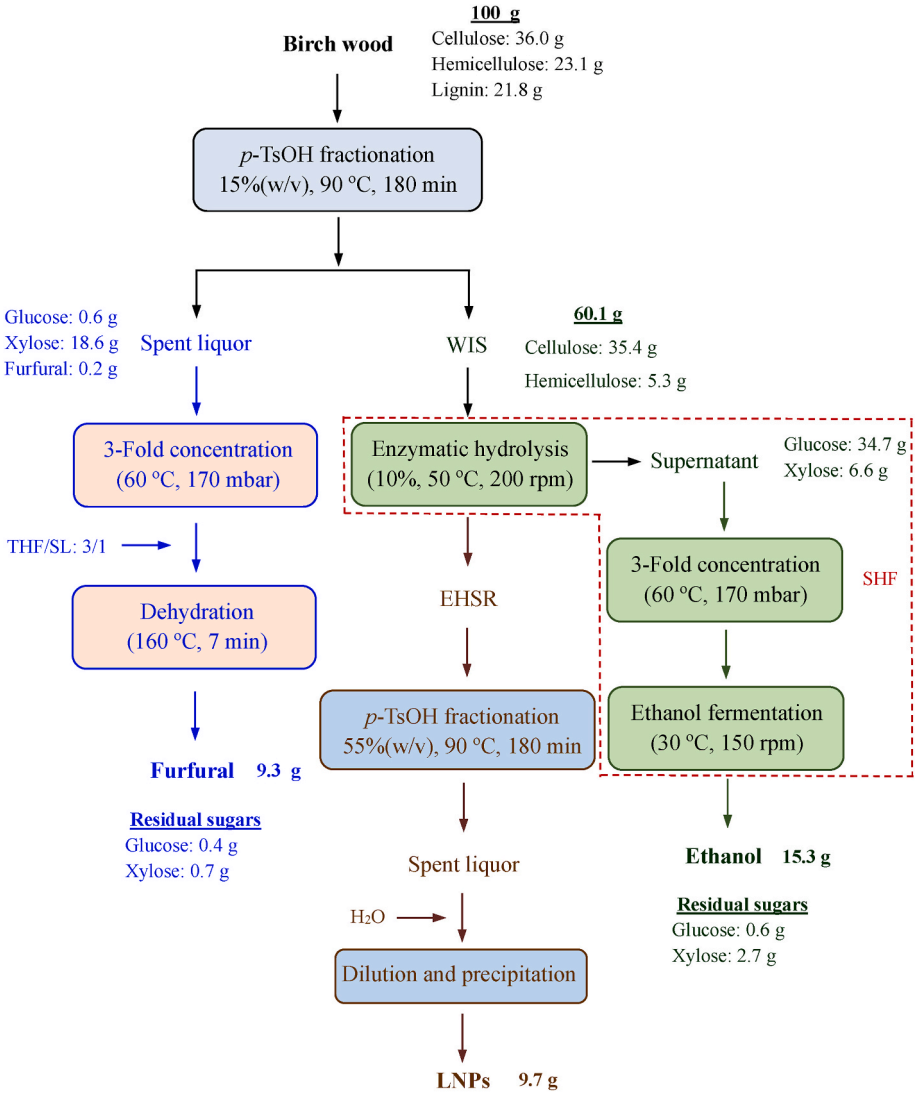

Fig. 4. Overall flow diagram of the integrated process.
Note: *p*-TsOH: *p*-Toluenesulfonic acid; WIS: Washed water-insoluble solids; THF: Tetrahydrofuran; SL: Spent liquor; LNPs: Lignin nanoparticles; EHSR: Enzymatic hydrolysis solid residue.

exhibited a very small average particle size of 37.4 nm (Fig. S1B). While the LNPs produced from the SL of *p*-TsOH fractionated birch wood displayed a big average particle size of 107.6 nm, indicating that a much smaller particle size of LNPs was obtained from EHSR. The LNPs produced from the SL of *p*-TsOH fractionated EHSR of the acid-catalyzed steam-exploded corn stover exhibited a very bigger average particle size of 98.0 nm [29] than that of this study. Tian et al. [46] reported that LNPs were directly prepared from the EHSRs of three steam pretreatment based lignocellulosic biomass (corn stover, poplar, and lodgepole pine) through solvent extraction and dialysis method, which gave a LNPs yield of 81.8%, 90.9% and 41.0% with a corresponding average particle size of 218, 131, and 104 nm, respectively. Therefore, the LNPs yield and average particle size depended on the type of biomass and the preparation method. The production of LNPs can be used as antioxidants, UV-protectants, antimicrobial agents, delivery agents, encapsulation agents, and adsorbents [47]. Zhu et al. [29] used the LNPs as adsorbents to partially remove the inhibitors in the prehydrolysate and improve the ethanol fermentability from 0.27 g/(L·h) (undetoxified prehydrolysate) to 0.61 g/(L·h).

3.5. Preliminary mass balance of the overall process

The mass balance of the entire process involved *p*-TsOH fractionation of birch, solid-liquid separation, enzymatic hydrolysis of the obtained WIS, ethanol fermentation by *S. cerevisiae* YRH400, SL conversion to furfural via dehydration, and enzymatic hydrolysis residue further *p*-TsOH fractionation to prepare LNPs (Fig. 4). Based on the results, 100 g oven-dry birch produced 15.3 g of ethanol, 9.3 g of furfural and 9.7 g of LNPs. When upscaled, 6.5 t oven-dry birch can produce 1.0 t ethanol, 0.61 t furfural and 0.63 t LNPs. Normally, 4.7–7 t oven-dry corn stover can produce 1.0 t ethanol [48]. Chu et al. [48] used sequential fermentation strategy (glucose fermentation by *S. cerevisiae*, followed by ethanol distillation, and finally xylose fermentation by *Pichia stipitis*) from green liquor-pretreated corn stover, one ton ethanol produced only need 4.75 t corn stover because this process ensured that each fermentation stage used the suitable microorganism, permitting high ethanol yields. However, this process only considered the utilization of glucan and xylan, the utilization of lignin was neglected. Zhu et al. [12] fractionated poplar with *p*-TsOH to co-produce ethanol and furfural. However, the precipitated lignin from the SL was not studied further and the obtained furfural concentration was low. After calculation, 1.0 t ethanol and 0.33 t furfural were produced from 6.0 t oven-dry poplar. Overall, the findings of this study show that the integrated process to co-produce ethanol, furfural, and lignin nanoparticles from all the three components in birch wood was more feasibility.

4. Conclusions

This study demonstrates the potential application of acid hydrotropic fractionation in forest biorefinery. Birch wood was fractionated using *p*-TsOH into water insoluble solids (WIS) fraction and a xylose-rich spent liquor (SL). Separate hydrolysis and fermentation of WIS resulted in a maximum ethanol concentration of 75.5 g/L; the enzymatic hydrolysis solid residue of WIS was further fractionated by *p*-TsOH to prepare lignin nanoparticles (LNPs); while the dehydration of the concentrated SL without lignin precipitation in batch using tetrahydrofuran as a co-solvent produced a furfural yield of 78.2%. Therefore, the integrated process was proposed to co-produce bioethanol, furfural and LNPs, thus offering a potential strategy for forest biorefinery.

CRediT authorship contribution statement

Junjun Zhu: Investigation, Methodology, Writing – original draft. **Ningxin Jiao:** Investigation, Methodology, Writing – original draft. **Jinlan Cheng:** Methodology, Formal analysis, Data curation, Data processing. **Han Zhang:** Methodology, Formal analysis, Data curation, Data processing. **Guangliu Xu:** Methodology, Formal analysis, Data curation, Data processing. **Yong Xu:** Resources, Validation. **J.Y. Zhu:** Conceptualization, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The research was supported by the National Key Research and Development Program of China (2017YFD0601001) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.renene.2022.12.111.

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