

Full Length Article

Co-production of bioethanol and furfural from poplar wood via low temperature ($\leq 90^\circ\text{C}$) acid hydrotropic fractionation (AHF)

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GRAPHICAL ABSTRACT



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ABSTRACT

Poplar wood was fractionated into a water-insoluble cellulosic solid (WIS) fraction and a spent liquor that contained mainly dissolved lignin and xylan using an acid hydrotrope, *p*-Toluenesulfonic acid (*p*-TsOH), at low temperatures ($\leq 90^\circ\text{C}$). Reaction-kinetics-based severities were used to scale-up fractionation using 100 g wood at *p*-TsOH concentration 50 wt% and 90°C for 112 min. The WIS and spent liquor from a scale-up run were used to produce bioethanol and furfural, respectively. At 15% WIS loading (w/v), maximal ethanol concentration was 52.47 g/L with a fermentation efficiency of 68.3%. Direct dehydration of the virgin spent liquor resulted in a maximum furfural concentration of 5.44 g/L at 68.4% yield. Precipitating lignin in the spent liquor increased furfural concentration to 6.18 g/L and yield to 77.7%. These results demonstrate the potential of acid hydrotrope fractionation for forest biorefinery.

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1. Introduction

Using fossil-based fuels and chemicals is an environmental concern because of climate change resulting from greenhouse gas emissions. Recently, lignocellulose-based biofuels (e.g., bioethanol) and bio-based chemicals (e.g., lactic acid, furfural) have attracted increasing attention because lignocelluloses are renewable, capable of carbon sequestration, and abundant and available in many regions of the world [1]. Woody biomass has several advantages over herbaceous biomass and agriculture residues, such as high density, which facilitates logistics and transportation, and year round harvesting capability, which reduces storage [2]. However, woody biomass is more recalcitrant than herbaceous biomass for bioconversion, partially due to its high lignin content and strong physical integrity [3,4]. Conventional dilute acid or alkaline pretreatments are not effective on woody biomass [5], whereas effective pretreatment methods, such as sulfite [6,7], solvent [8–11], or steam explosion [12,13] are expensive, partly due to the requirement of high temperatures that increased capital cost. On the other hand, developing economic forest biorefinery requires valorization of all major components of wood [14]. A few approaches have been explored with some level of success [10,15,16], however, more work is needed. Lignin valorization remains especially difficult, partly due to lignin condensation [17,18] by most pretreatment processes include those mentioned above [7–13] that use harsh chemicals under high temperature.

Recently, we developed an acid hydrotrope fractionation (AHF) process that used an aromatic acid, i.e., *p*-Toluenesulfonic acid (*p*-TsOH), to solubilize approximately 90% of poplar wood lignin below 90 °C for a very short period of time, < 30 min, which allowed for producing lignin with a low degree of condensation [19–21] to facilitate subsequent valorization. By definition hydrotropes can solubilize hydrophobic materials, lignin in the present study, in aqueous systems through aggregation [22,23], though the exact mechanism of hydrotropic actions is still unclear [23]. However, it is understood that *p*-TsOH as a strong acid can cleave ether bonds [19,21] to facilitate lignin dissolution in aqueous *p*-TsOH solution through aggregation. There is a minimal hydrotropic concentration (MHC) below which solute (lignin) precipitate, which facilitates lignin separation through precipitation by simply diluting hydrotrope concentration in the spent liquor below its MHC.

An aromatic salt-based hydrotrope fraction was extensively studied for wood pulping a half century ago [24] and for pretreatment of

biomass for biorefinery in recent years [25,26]. However, aromatic salt-based hydrotropes are effective only at high temperatures, > 150 °C, for a long period of several hours. As a result, aromatic salt-based hydrotropic process suffers the same problems of existing pretreatment/fractionation technologies, such as lignin condensation to result in difficulties for lignin valorization and high capital cost due to high process temperature and pressure. AHF differentiates itself from conventional aromatic salt-based hydrotropic fractionation for its effectiveness at low temperatures, i.e., below the boiling point of water, and for highly selective and rapid dissolution of lignin (< 30 min) and hemicelluloses simultaneously and substantially. As a result, AHF substantially improves cellulose accessibility to enzymes [27], which leads to effective enzymatic sugar production [19]. Low-temperature AHF also resulted in substantially reduced lignin condensation to facilitate lignin valorization. As a hydrotrope, separation of *p*-TsOH from the dissolved lignin can be achieved by diluting the spent liquor to the minimal hydrotrope concentration (MHC) [19,28]. The amount of water to be evaporated for reconcentration is extensive; however, it is no more than that for weak black liquor evaporation in commercial kraft pulp mills. The dissolved xylan can then be dehydrated into furfural using the *p*-TsOH in the lignin precipitated spent liquor [29] without additional catalysts as will be evaluated in this study, which can improve product portfolio for biorefinery. *p*-TsOH can then be re-used. Initial evaluation showed over 98% of *p*-TsOH can be recovered in the washing filtrates [20], suggesting *p*-TsOH consumption through fractionation reactions is minimal and high recovery of over 95% is achievable through multiple cycles reuse. The spent liquor can also be directly reused without dehydration and lignin separation without losing its efficacy for a couple of cycles [19], which can save thermal energy for evaporation.

In view of the potential of using one chemical at low temperatures to valorize three major wood components with the advantages of reducing capital cost and easing chemical recovery, the objective of the present study was to conduct a careful evaluation of AHF for poplar wood biorefinery using *p*-TsOH. The study focused on optimizing (1) AHF for poplar wood bioconversion using reaction-kinetics-based severity factor for subsequent bioethanol production from the cellulosic solid fraction and (2) valorizing dissolved hemicelluloses in the spent liquor by producing furfural through batch dehydration (Fig. 1). The results obtained from the study can be used for future economic analysis studies for comparison with competing technologies.

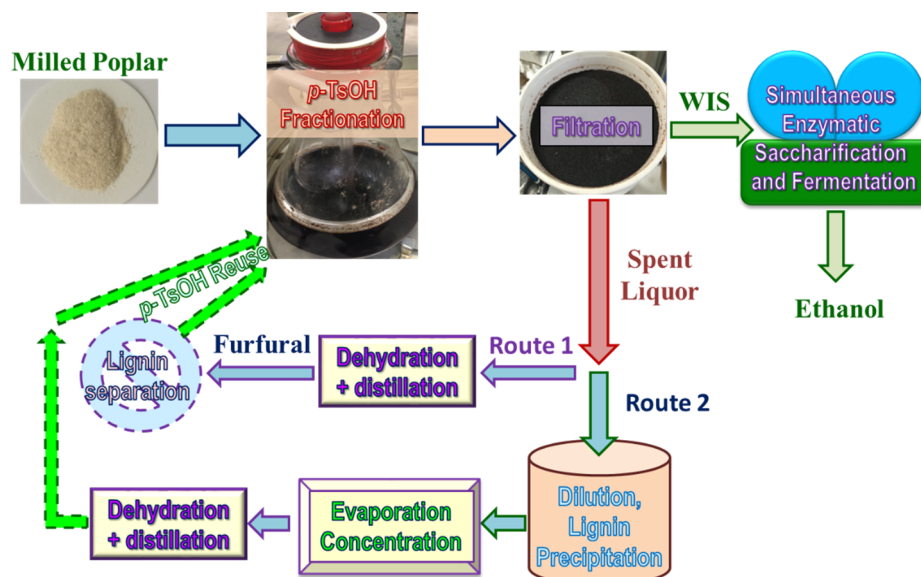


Fig. 1. A schematic flow diagram shows poplar fractionation using *p*-TsOH for the production of ethanol from the cellulosic solid residue with SSF/q-SSF, and furfural from the spent liquor. Process with dashed lines were not conducted in the present study.

2. Materials and methods

2.1. Materials

Poplar NE222 from *Populus deltoides* Bartr. ex Marsh $\times$ *P. nigra* L. was harvested from Hugo Sauer Nursery in Rhinelander, WI, USA, by Dr. Ronald Zalesny, Jr., USDA Forest Service, Northern Research Station. The logs were debarked and chipped at the USDA Forest Service, Forest Products Laboratory in Madison, WI, USA. The chips were screened using 32-mm-square holes. Wood chips were then ground to a 20 mesh using a Wiley mill (model No. 2, Arthur Thomas Co, Philadelphia, PA, USA).

The *p*-TsOH of ACS reagent grade was purchased from Sigma-Aldrich (St. Louis, MO, USA). A commercial complex cellulase, Cellic® CTec3 (abbreviated CTec3) was complementarily provided by Novozymes North America (Franklinton, NC, USA), with cellulase activity of 217 FPU/mL, as determined using filter paper assay according to the International Union of Pure and Applied Chemists [30].

2.2. Poplar fractionation using *p*-TsOH

AHF fractionations of poplar using *p*-TsOH were conducted under a wide range of conditions of *p*-TsOH concentration, 30–85 wt%, temperature, 30–80 °C, and reaction time, 5–60 min, to systematically study AHF selectivity in dissolving lignin and hemicelluloses, and retaining cellulose (Table S1). Each fractionation run was designated as PxxTyytzz, with xx, yy, and zz represent *p*-TsOH concentration in wt%, fractionation temperature in °C, and time in min. Aqueous *p*-TsOH solutions were prepared by dissolving desired amounts of *p*-TsOH in deionized (DI) water to make 100 g *p*-TsOH solution in conical flasks. The flasks were placed in a heated shaker (Model 4450, Thermo Scientific, Waltham, MA, USA) to facilitate dissolution. To prepare *p*-TsOH solutions at high concentrations (> 65 wt%), temperature was raised to approximately 100 °C using a heating plate to solubilize *p*-TsOH. After dissolution, the *p*-TsOH solutions were cooled to desired fractionation temperatures and placed on the same shaker, and 10 g (in oven dry weight) Wiley-milled poplar was then added into 100 mL solution, for a liquor to solid ratio of 10 (v/w).

The fractionation condition P50T90t112 was selected for bioethanol and furfural production study using 100 g of Wiley-milled poplar wood. The reaction was conducted in a water bath with agitation at 250 rpm for 112 min.

For all runs, undissolved solids and spent liquor without dilution were separated using vacuum filtration immediately at the end of each fractionation. The filtrate (*p*-TsOH spent liquor) collected from P50T90t112 was used to produce furfural through dehydration, and the solids were used to produce bioethanol through fermentation. All solids samples were thoroughly washed using DI water until pH of the filtrate reached 5–6. The washed water-insoluble cellulosic solids (WISs) were analyzed for chemical compositions. The *p*-TsOH spent liquors were analyzed for *p*-TsOH, sugars (glucose and xylose), formic acid, acetic acid, levulinic acid, 5-hydromethylfurfural (HMF), and furfural.

2.3. Enzymatic hydrolysis of WIS

Enzymatic hydrolysis of WIS was carried out in 125-mL Erlenmeyer flasks on a shaking bed incubator at 200 rpm and 50 °C (Model 4450, Thermo Scientific, Waltham, MA, USA) at solids loading of 1% (w/v) in 50 mM citrate buffer of pH 5.5. An elevated pH of 5.5 can reduce nonproductive cellulase binding to lignin, as we discovered previously [31,32]. CTec3 cellulase loading was 20 FPU/g cellulose. Aliquots of hydrolysate were taken periodically during hydrolysis (1, 3, 5, 6, 24, 48, 72 h). Each sample was centrifuged at 10,000 rpm for 5 min. The supernatant was filtered through a 0.22-μm membrane before sugar (glucose and xylose) analysis using high-performance liquid chromatography (HPLC).

2.4. Yeast strain and media

The yeast *Saccharomyces cerevisiae* YRH400, an engineered fungal strain for glucose and xylose fermentation [33], was generously provided by Drs. Ronald Hector and Bruce Dien at the USDA Agricultural Research Service. The strain was maintained at 4 °C on YPD agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar.

A colony from the YPD agar plate was transferred by a loop to 50 mL liquid YPD medium in a 125-mL flask and cultured on a rotary shaking bed incubator at 30 °C and 200 rpm for 24 h. The yeast concentration was monitored using optical density at 600 nm (OD₆₀₀) by a UV-vis spectrophotometer (Model 8453, Agilent Technologies, Palo Alto, CA, USA).

2.5. Enzymatic saccharification and fermentation of WIS

Enzymatic saccharification and fermentation experiments of the WIS from the pretreated poplar NE222 were carried out in 125-mL Erlenmeyer flasks on the same rotary shaking bed incubator described in the previous section. The enzymatic hydrolysis was conducted at 15% solids loading (w/v) with CTec3 dosage of 20 FPU/g cellulose. Hydrolysis was carried out at 50 °C and 200 rpm for 48, 24, and 0 h, respectively, before inoculation using yeast. Experiments with 0 h hydrolysis time are true simultaneous saccharification and fermentation (SSF), those with 48 and 24 h hydrolysis time are quasi (q)-SSF. The hydrolyzed or liquefied slurries were cooled to 37 °C before SSF and q-SSF on the rotary shaking bed incubator at 150 rpm. *S. cerevisiae* YRH400 broth was added to inoculate the enzyme-loaded poplar WIS at an initial OD₆₀₀ of 4. Nutrients (yeast extract 5 g/L, (NH₄)₂SO₄ 2 g/L, NaH₂PO₄ 5 g/L) were supplemented to the hydrolysate.

All fermentation runs were carried out in duplicates. Samples were withdrawn at 8, 24, 48, 72, 96, 120, 168, and 193 h and centrifuged at 10,000 rpm for 5 min. The supernatants were analyzed for glucose, xylose, xylitol, glycerol, and ethanol.

Fermentation efficiency, η (%), was calculated as the theoretical percentage of the amount of ethanol produced from the amount of carbohydrate in the pretreated solids fed into fermentation, expressed as

$$\eta (\%) = \frac{C_{\text{ethanol}} \times V_{\text{broth}}}{(0.511 * \frac{f_{\text{cellulose}}}{0.9} + 0.46 * \frac{f_{\text{hemicelluloses}}}{0.88}) \times m_{\text{WIS}}} \quad (1)$$

where C_{ethanol} and V_{broth} are the measured ethanol concentration (g/L) and the volume (L) of the fermentation broth; $f_{\text{cellulose}}$ and $f_{\text{hemicelluloses}}$ are the cellulose and hemicellulose content (g/g) in the pretreated poplar WIS, respectively. m_{WIS} is the total solids mass (g) in oven dry weight of the sample used in the enzymatic hydrolysis and fermentation.

2.6. Furfural production from *p*-TsOH spent liquor

Pure xylose solution was first used to optimize furfural production in batch mode catalyzed by *p*-TsOH. This is because approximately 90% of the dissolved xylan in spent liquor obtained from the large-scale run at P50T90t112 were monomeric xylose, as discussed later. The collected *p*-TsOH spent liquor from fractionation of poplar at P50T90t112 was then used to evaluate furfural production without adding catalysts using the optimal condition from the pure xylose study. To investigate the effect of lignin dissolved in the spent liquor on furfural production, two spent liquor preparation routes were adopted as shown in Fig. 1: (1) the spent liquor was directly dehydrated; or (2) the spent liquor was diluted 10 times using DI water to *p*-TsOH concentration of approximately 5.5% below *p*-TsOH MHC of 11.5% [19,28] to precipitate lignin through centrifugation. The lignin precipitated and diluted spent liquor was reconcentrated 10 times using vacuum evaporation at 60 °C.

Dehydration reaction for furfural production was conducted in a 25-mL stainless steel reactor containing 20 mL liquid in a sand bath (Techne F932D, Techne Inc.) at 140–170 °C for 4–7 min. After heating the sand to the target temperature, the stainless steel reactor was immersed in the sand, and kept for a set time. At the end of the reaction, the reactor was quenched immediately in an ice-water bath. The composition (glucose, xylose, formic acid, acetic acid, levulinic acid, HMF, and furfural) of the reacted liquor was analyzed by HPLC, as described below.

2.7. Analytical methods

The chemical compositions of untreated poplar and *p*-TsOH fractionated poplar WIS were analyzed by the Analytical Chemistry and Microscopy Laboratory at the USDA Forest Products Laboratory (Madison, WI, USA), as described previously [34].

The chemical compositions of *p*-TsOH spent liquors, enzymatic hydrolysates, and fermentation broths were determined by a HPLC system (Ultimate 3000, ThermoFisher Scientific) using a refractive index detector (RI-101, Shodex), as described previously [29]. Chromatographic species separation was achieved using a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm i.d.) at 60 °C with dilute sulfuric acid at 0.005 mol/L as the mobile phase at a flow rate of 0.6 mL/min.

3. Results and discussion

3.1. Fractionation mass balance and severity

Component mass balance for *p*-TsOH fractionation of poplar is determined from the WIS yields and compositional analysis of the fractionated WIS (Table S1). Overall, AHF using *p*-TsOH fractionation is highly selective in preserving cellulose (mostly retained in WIS) and dissolution of lignin and hemicelluloses. To facilitate process scale-up, we used a combined delignification factor (*CDF*) and a combined hydrolysis factor (*CHF*) developed previously [29,35] based on reaction kinetics to analyze lignin and xylan dissolution data. Using a bi-phasic assumption [36,37], i.e., both xylan and lignin contain a fast and a slow fraction, then, the fraction of xylan, X_R , and lignin, L_R , that remained in WIS can be expressed as

$$X_R = (1 - \theta - \theta_R)e^{-CHF} + \theta \cdot e^{-f \cdot CHF} + \theta_R \quad (2a)$$

$$CHF = \exp\left(\alpha - \frac{E}{RT} + \beta C\right) \cdot C \cdot t \quad (2b)$$

$$L_R = (1 - \theta' - \theta'_R)e^{-CDF} + \theta' \cdot e^{-f' \cdot CDF} + \theta'_R \quad (3a)$$

$$CDF = \exp\left(\alpha' - \frac{E'}{RT} + \beta' C\right) \cdot C \cdot t \quad (3b)$$

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. Fitting of the data of xylan and lignin that remained in WIS (Table S1) resulted in parameters as listed in Table 1.

Eqs. (2) and (3) indicate that *CHF* and *CDF* can predict xylan and lignin dissolution, as validated by experiments shown in Fig. 2. Therefore, xylan dissolution and delignification can be controlled by *CHF* and *CDF*, respectively, independent of individual fractionation conditions. Furthermore, *CHF* and *CDF* are true fractionation severity and can be used for process scale-up to alleviate experimental constraints posed by individual process conditions. For example, low acid concentrations are often preferred to reduce chemical recovery costs, a

Table 1

List of fitting parameters for Eqs. (2) and (3) using the data of xylan and lignin that remained in WIS (Table S1).

Parameter	Unit	Xylan	Lignin
α , α'	none	19.00	26.70
β , β'	L/mol	1.33	1.50
E , E'	J/mol	75,200	96,100
θ , θ'	none	0.25	0.55
f , f'	none	0.0088	0.0066
θ_R , θ'_R	none	0.125	0.135

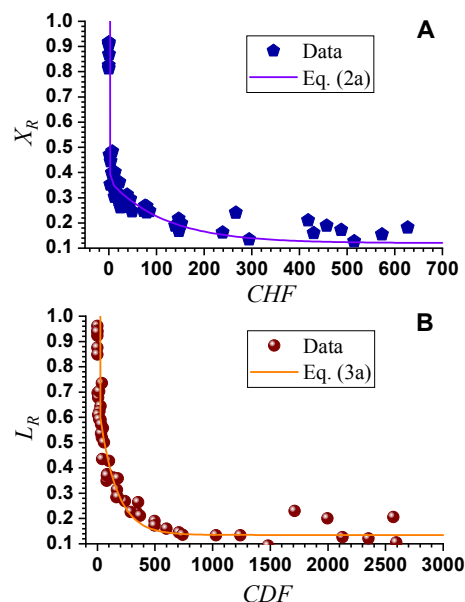


Fig. 2. Fittings of poplar dissolution data of xylan (A) and lignin (B) by *p*-TsOH using kinetic-based reaction severities, combined hydrolysis factor (*CHF*) and a combined delignification factor (*CDF*), respectively.

long reaction time can be used to compensate for a low acid loading.

Based on the results shown in Fig. 2, we chose to use a moderate *p*-TsOH concentration of 50 wt% but a higher temperature of 90 °C and a relatively long reaction time of 112 min, or P50T90t112, to achieve good delignification and dissolution of hemicelluloses for a scale-up run using 100 g of poplar. The corresponding severities for this condition were *CHF* = 78 and *CDF* = 296 according to Eqs. (2b) and (3b), respectively. The actual hemicelluloses and lignin dissolutions were approximately 79% and 84% (Table 2) based on WIS yield and chemical composition. The extensive lignin and hemicellulose removal ensured

Table 2

Chemical composition of poplar before and after *p*-TsOH scale-up fractionation. Numbers in parentheses are component retained in WIS.

	Untreated poplar	P50T90t112
WIS (%)		
Solid yield	100	47.7
Cellulose	45.7 ± 0.6	83.4 ± 0.1(87.0)
Hemicelluloses	16.4 ± 0.3	7.4 ± 0.1(21.4)
Lignin	23.4 ± 0.2	8.1 ± 0.1(16.3)
Spent liquor (g/L)		
<i>p</i> -TsOH		545.0 ± 9.1
Glucose		1.1 ± 0.1
Xylose		12.7 ± 0.3
Formic acid		0.2 ± 0
Acetic acid		4.5 ± 0.1
HMF		0
Furfural		0.7 ± 0.1

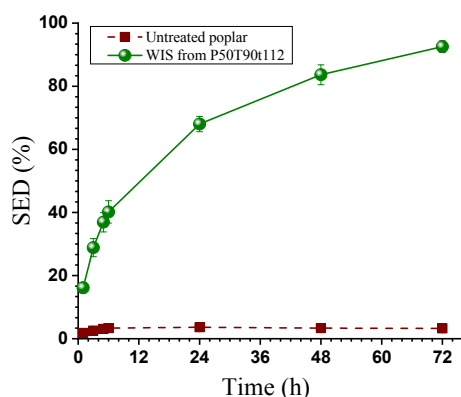


Fig. 3. Time-dependent substrate enzymatic digestibility of *p*-TsOH-fractionated poplar WIS at 1% (w/v) substrate and 50 °C with pH 5.5. CTec3 = 20 FPU/g cellulose.

excellent enzymatic digestibility of the fractionated WIS for sugar/biofuel production [27]. Results in Table 2 also indicate that cellulose dissolution was low, approximately 10% in the form of oligomers, as glucose concentration in the spent liquor was low. Most of the dissolved xylan was in the form of xylose. The amount of xylan dehydrated into furfural was low, approximately 10%.

3.2. Enzymatic hydrolysis of *p*-TsOH fractionated WIS and comparisons with literature data

The WIS from P50T90t112 along with the untreated poplar wood were enzymatically hydrolyzed. As shown in Fig. 3, *p*-TsOH fractionation substantially improved the solid substrate cellulose enzymatic digestibility (SED) as expected due to substantial delignification and dissolution of hemicelluloses (Table 2). Here SED is defined as the percentage of cellulose in WIS enzymatically saccharified into glucose. SED of WIS reached 93% after 72 h, compared with the untreated poplar, which was barely hydrolyzed, with SED less than 5%.

Table 3 compares the AHF with other pretreatment methods for enzymatic hydrolysis of poplar wood reported in literature. We also listed hemicellulose removal and lignin dissolution as both are important to remove the recalcitrance of lignocelluloses [27]. Due to variations in the poplar wood and enzymatic hydrolysis conditions, such comparisons can only provide qualitative information about the

performance of AHF. In general AHF is not as effective as SPORL [7] to enhance enzymatic saccharification of cellulose, partly because sulfite in SPORL substantially solubilized hemicelluloses and sulfonated lignin which reduced nonproductive cellulase binding. However, AHF performed better than high temperature pretreatments using hot-water [38] and dilute acid [39], mainly due to substantial lignin dissolution in AHF and slightly higher hemicellulose removal than hot-water treatment. A recent study by Prof. Saddler's group at the University of British Columbia showed that AHF using aqueous *p*-TsOH solution at 80 °C also performed better than a deep eutectic solvents system of lactic acid and betaine for fractionation of corn stover and willow wood conducted at a high temperature of 140 °C [40].

3.3. Ethanol production from WIS

Ethanol yield in a traditional separated hydrolysis and fermentation (SHF) is often negatively affected due to inhibition by substrate (glucose) and end-product (ethanol) [41]. SSF was developed to simplify process integration and eliminate end-product inhibition, thereby reducing production cost [42]. Prehydrolysis is often implemented to produce a certain amount of glucose to facilitate the growth of microorganisms for fermentation. SSF with prehydrolysis is called q-SSF. Time-dependent consumptions of glucose and xylose and production of ethanol and minor products (xylitol and glycerol) during fermentation using SSF and q-SSF are shown in Fig. 4A and B, respectively. Glucose concentration was below 6 g/L throughout the entire SSF process, suggesting SSF effectively eliminated glucose inhibition. Glucose concentration decreased and remained at a low level (~2.7 g/L) after 24 h. Ethanol concentration increased rapidly in the first 48 h. Fermentation was nearly completed in 72 h, with ethanol concentration over 51 g/L. Extending fermentation to 120 h resulted in a minor increase in ethanol concentration to approximately 52 g/L. Compared with glucose, the maximal xylose concentration only reached approximately 2 g/L, and decreased to approximately 0.5 g/L after 72 h. Fermentation by-product xylitol from xylose metabolism increased with time, with a maximum xylitol concentration of approximately 1.2 g/L attained at 120 h. Another fermentation by-product glycerol was also produced, with a maximum concentration of approximately 3.0 g/L at 168 h.

Ethanol fermentation efficiencies were compared among SFF and two q-SSF processes (Fig. 4C). Ethanol fermentation efficiencies of the two q-SSF runs were lower than that of the SSF run, indicating that the high initial sugar concentration did not result in more efficient

Table 3

Comparison of *p*-TsOH fractionation with other pretreatment methods for enzymatic saccharification of poplar wood.

Wood	Fractionation condition	Removal of xylan; lignin (%)	Enzyme loading (FPU/per g glucan)	Substrate enzymatic digestibility (SED) @ 72 h (%)	Method and Source
Poplar NE222 fibers	<i>p</i> -TsOH = 50 wt% T = 90 °C t = 112 min	80; 84	CTec 3 = 20	93 ± 2	Acid Hydrotrope This study
Poplar NE222 fibers	<i>p</i> -TsOH = 75 wt% T = 80 °C t = 20 min	81; 86	CTec 3 = 5 7.5 10 20	32 47 59 87	Acid Hydrotrope [19]
Poplar NE222 chips	T = 150 °C; t = 108 min NaHSO ₃ = 0.67%; @ H ₂ SO ₄ = 0.3% @ H ₂ SO ₄ = 0.5% H ₂ SO ₄ = 0.2%; @ NaHSO ₃ = 0% @ NaHSO ₃ = 1.3%	86; 8 96; 9 69; 2 79; 26	CTec 3 = 10	84 98 43 71	SPORL [7]
Poplar Pass ¼" screen	Hot-water @ 200 °C; 10 min	62; ~0	Spezyme = 15 + 40 IU Novo188	30	Hot-water [38]
Poplar saw dust	H ₂ SO ₄ = 4% T = 180 °C; t = 10 min	89; ~0	Celluclast 1.5 L = 30 + 30 IU Novo188	75	Dilute acid [39]

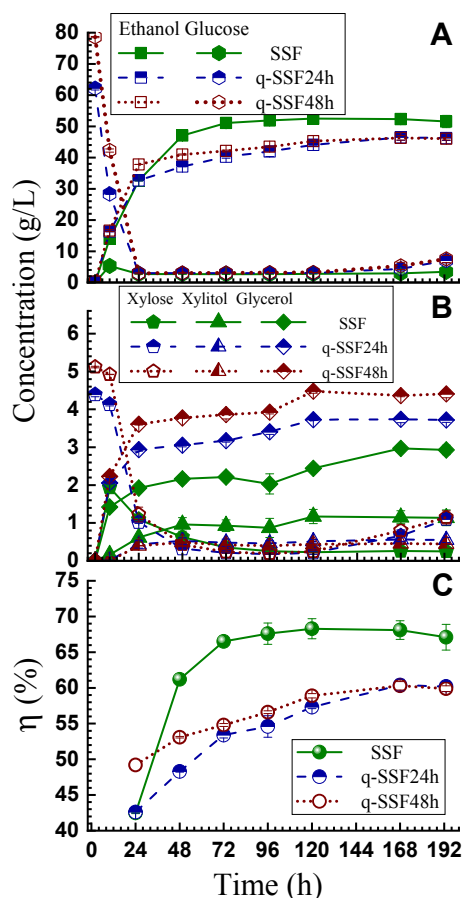


Fig. 4. Time-dependent data from simultaneous saccharification and fermentation (SSF) and quasi (q)-SSF of *p*-TsOH fractionated poplar WIS at 15% (w/v). CTec 3 = 20 FPU/g cellulose. (A) Ethanol and glucose concentrations; (B) xylose, xylitol, and glycerol concentrations; (C) fermentation efficiency (η). Yeast inoculation at time = 0.

fermentation. Glucose concentrations of 62.2 and 78.6 g/L, and xylose concentrations of 4.4 and 5.1 g/L, were achieved from the two q-SSF runs with prehydrolysis times of 48 and 24 h, respectively (Fig. 4A). Results showed that the q-SSF48h (prehydrolysis 48 h) produced a relatively higher ethanol concentration than the q-SSF24h (prehydrolysis 24 h) before 120 h, but both runs achieved the same terminal ethanol concentration of 46.4 g/L at 168 h, lower than that produced by SSF. Perhaps glucose inhibition became important as glucose concentrations in both q-SSF runs were greater than 50 g/L [43]. Glucose concentrations for both q-SSF runs decreased rapidly within the first 24 h of inoculation simply due to the availability of a high amount of glucose. The glucose concentration in the SSF run, however, had a slight increase from zero at the beginning of fermentation within the first 8 h of inoculation, indicating that the rate of glucose produced from enzymatic hydrolysis was higher than that of yeast utilized to produce ethanol. The glucose concentration for all fermentation runs was low after 24 h, at approximately 3 g/L.

Xylose consumption had characteristics similar to glucose for the corresponding fermentation runs (Fig. 4B). It appears that *S. cerevisiae* YRH400 consumes xylose almost immediately without delay. However, xylose consumption for the two q-SSF runs was low in the first 8 h due to the availability of high amounts of glucose. The fermentation by-product xylitol reached approximately 0.5 g/L in 8 h of SSF. Glycerol concentration increased with time. Maximum glycerol concentrations were 3.7 and 4.5 g/L for q-SSF24h and q-SSF48h, respectively. The SSF run produced a higher xylitol concentration and a lower glycerol concentration than the two q-SSF runs, indicating that the higher initial

glucose and xylose concentrations resulted in more glycerol production, whereas high initial xylose concentration did not lead to high xylitol production. This is perhaps because *S. cerevisiae* YRH 400 was engineered to ferment xylose by stable integration of the xylose reductase, xylitol dehydrogenase, and xylulokinase genes [33].

3.4. Furfural production from *p*-TsOH spent liquor

Furfural production from a pure xylose solution was conducted at xylose concentration of 12 g/L and *p*-TsOH concentration of 50 wt%, based on the concentrations of xylose and *p*-TsOH in the spent liquor from fractionating poplar at P50T90t112 (Table 2). Furfural yields were calculated based on the measured final furfural concentration (including furfural production in the fractionation process) as percentage of the theoretical achievable furfural from the initial xylose concentration in the pure xylose solution or in the *p*-TsOH liquor. High temperature and short reaction time resulted in high furfural yield (Table S2). Due to limitations in experimental apparatus, the shortest achievable reaction time was 4 min. As a result, reaction at 170 °C for 4 min produced the highest furfural yield of 66% theoretical, which is quite good for batch operations because of unavoidable side reactions, such as condensation with itself and sugars, which tend to reduce furfural yield [44]. Xylose was almost depleted with a negligible amount of formic acid formation.

Furfural production using *p*-TsOH spent liquor from P50T90t112 was conducted at 160 and 170 °C with various reaction duration times based on the results from pure xylose study discussed above. The hemicellulose removal data along with the xylose concentration in the spent liquor (Table 2) indicate that approximately 90% of the dissolved xylan in the spent liquor of P50T90t112 was in the form of xylose, with very low amount of xylooligomers. Therefore, using optimal conditions derived from the pure xylose solution study is valid. Comparisons were made between spent liquor with or without lignin precipitation. The spent liquor without lignin precipitation was directly dehydrated. Results indicated that xylose was not completely depleted after dehydration reactions. Similar to a study using pure xylose, a higher temperature of 170 °C and a short reaction time of 4 min resulted in the highest yield of 68.4% with residue xylose concentration of 1.9 g/L. Increasing reaction time reduced residue xylose concentration but did not increase furfural yield, most likely due to furfural condensation through side reactions [44]. Using batch distillation may reduce furfural condensation reactions. For example, furfural yields of 75% and 90% were obtained using a pure xylose solution of 5 g/L and hot-water-extracted lignocellulose hydrolysates with a significant amount of xylooligomers, respectively [45]. When using corn-cob directly, furfural yield of 75% theoretical was achieved through batch distillation [46]. These studies suggest that the furfural yield reported here can be further improved through batch distillation.

To study the effect of dissolved lignin on furfural production, lignin in the same *p*-TsOH spent liquor from P50T90t112 was precipitated as described in the experimental section (Fig. 1). The dehydration results using precipitated lignin and reconstituted spent liquor were compared with results from the virgin spent liquor. Xylose loss through evaporation for reconstitution was negligible (Table 4). Evaporation removed the volatile components with formic acid, acetic acid, and furfural removal of 100%, 68.2%, and 100%, respectively (Table 4). Similarly, reaction temperature at 170 °C for 4 min produced the highest furfural yield of 77.7%, higher than the 68.4% obtained using the virgin liquor without lignin precipitation. Residue xylose concentration was reduced from 1.9 g/L for the virgin spent liquor to 1.4 g/L in the lignin-precipitated liquor. This difference is equivalent to an increase of approximately 3 percentage points in furfural yield estimated from the xylose and furfural concentrations listed in Table 4. It is possible that the removal of volatile species through evaporation and precipitating lignin also contributed to improved furfural yield by reducing the side condensation reactions [45]. To demonstrate the effects

Table 4Compositional analysis of spent liquor from *p*-TsOH fractionated poplar with/without lignin precipitation after furfural production at different reaction conditions.

Component	Spent liquor without lignin precipitation									Spent liquor with lignin precipitation				
	virgin spent liquor	160 °C				170 °C				reconcentrated spent liquor	170 °C			
		5.5 min	6.0 min	6.5 min	7.0 min	4.0 min	4.5 min	5.0 min	5.5 min		4.0 min	4.5 min	5.0 min	5.5 min
Glucose (g/L)	1.23	0.60	0.60	0.54	0.32	0.59	0.49	0.49	0.25	1.18	0.52	0.34	0.34	0.08
Xylose (g/L)	12.43	1.56	1.56	1.52	1.06	1.87	1.75	1.49	0.87	11.99	1.38	1.04	0.98	0.70
Formic acid (g/L)	0.18	0.49	0.49	0.51	0.63	0.45	0.45	0.53	0.64	0	0.39	0.52	0.52	0.64
Acetic acid (g/L)	4.59	4.62	4.59	4.60	4.65	4.56	4.59	4.58	4.50	1.46	1.59	1.59	1.59	1.57
Levulinic acid (g/L)	0	0.49	0.53	0.53	0.66	0.45	0.46	0.54	0.70	0	0.60	0.75	0.74	0.90
HMF (g/L)	0	0.09	0.09	0.08	0.06	0.10	0.10	0.08	0.07	0	0.10	0.10	0.08	0.05
Furfural (g/L)	0.54	5.13	5.06	4.97	4.56	5.44	5.27	5.08	4.62	0	6.18 ^a	5.80 ^a	5.68 ^a	5.15 ^a
Furfural yield ^b (%)	0	64.5	63.6	62.5	57.3	68.4	66.3	63.8	58.1	0	77.7	72.9	71.4	64.8

^a Furfural concentration contains the furfural produced in the fractionation process and the furfural produced from the spent liquor by dehydration.^b It was calculated using the total furfural produced from the final liquor (including furfural production in the fractionation process) as the percentage of the theoretical achievable furfural from the amount of xylose in the *p*-TsOH spent liquor.

of the removal of acetic acid and dissolved lignin on furfural production, xylose dehydration experiments were conducted using pure xylose solution spiked with acetic acid and dissolved lignin. The results (Table S3) show the presence of acetic acid and dissolved lignin reduced furfural concentration.

A small amount of glucose in the spent liquor resulted in the formation of HMF (Table 4). Glucose was nearly-depleted with lignin precipitation.

4. Conclusions

This study demonstrated the potential of acid hydrotrope fractionation for forest biorefinery applications. Poplar wood was fractionated using *p*-TsOH into a water-insoluble solids (WIS) fraction and a xylose-rich spent liquor at 90 °C. Fermentation of WIS resulted in a maximum ethanol concentration of 52.47 g/L at 15% solids loading with a fermentation efficiency of 68.3%. Direct dehydration of the spent liquor in batch without any catalyst produced furfural yield of 68.4%. Precipitating lignin and removal acetic acid in the spent liquor increased furfural yield to 77.7%.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fuel.2019.05.155>.

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