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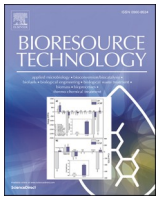

Upgrading dry acid pretreatment by post-hydrolysis for carbon efficient conversion of lignocellulose

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HIGHLIGHTS

- Upgrading DAP by post-hydrolysis for carbon efficient conversion of lignocellulose.
- A radar chart method was used for the comprehensive evaluation of DAP performance.
- One-pot post-hydrolysis and SSF without detoxification and sterilization.
- Preserved 45.2 % of original β-O-4 linkages in fractionated lignin.
- 66 % of lignocellulose carbon was recovered as ethanol, xylose and reactive lignin.

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ABSTRACT

Dry acid pretreatment (DAP) as a promising process for industrial biorefinery provide an efficient bioconversion of cellulose without free wastewater, although the partial xylan and lignin degrade to inhibitors or recondense. A biorefinery strategy for carbon efficient conversion of lignocellulose into bioethanol, xylose, and reactive lignin was developed by upgrading DAP with post-hydrolysis. The results showed that lignocellulose after mild DAP (175 °C, acid dosage of 15 mg/g dry material) obtained higher xylan recovery and lower inhibitors than that of general DAP. Subsequently, post-hydrolysis, simultaneous saccharification and ethanol fermentation were performed at solids loading of 20 wt% without detoxification and sterilization, resulting in xylose and ethanol yield of 71.8 % and 67.6 %. The fractionated lignin presented more reactive β-aryl ether linkages and less condensation than that from DAP. 66 % of lignocellulose carbon was recovered as ethanol, xylose and reactive lignin. This upgrading biorefinery strategy provided an easy-to-operate process for integrated utilization of lignocellulose.

1. Introduction

Dry acid pretreatment (DAP) was proposed to solve the dilute acid pretreatment problems and applied for various biofuels and biobased chemicals production due to its no wastewater generation, low energy costs, low erosion, good generality to various kinds of lignocellulosic feedstocks and high solids loading bioconversion (Han and Bao, 2018; He et al., 2022; He et al., 2014b; Zhang et al., 2011). However, this pretreatment performed at high severity conditions for the efficient enzymatic conversion of the cellulose, causes the over-degradation of xylan and lignin fractions and further results in the high content of pretreatment inhibitors, low xylose recovery and lignin recondensation. Although, various detoxification methods, such as overliming, washing and bio-detoxification, have been used after the DAP in the previous research to reduce the inhibitors, the waste water treatment, tedious operations and long processing time bring extra cost (Han et al., 2019; He et al., 2016; Zhou et al., 2017). In addition, majority of lignin residue from the DAP only meets with low value utilization, such as burning for power generation, because of its extensive condensation (Liu and Bao, 2017; Shuai et al., 2010). Meanwhile, physicochemical property of this lignin residue still has not been researched systematically. Therefore, an upgrading DAP should be developed for solving the above deficiencies by comprehensively considering xylose recovery, inhibitor generation, enzymatic hydrolysis of cellulose, and lignin reactivity.

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DAP at the low pretreatment severity can solve the above deficiencies, but large amount of xylooligosaccharides were generated (Han and Bao, 2018) and remained on surface of the pretreated material because of the very low liquid–solid ratio of this method, which caused strong inhibition on the enzymatic hydrolysis of cellulose (Qing et al., 2010; Zhang et al., 2015). A two-step pretreatment has been proposed for recovering more xylose by the relative low pretreatment severity at the first-step, and the post-hydrolysis of xylooligosaccharides in the pretreatment hydrolysate at the second step during the dilute acid or hot water pretreatment (Shevchenko et al., 2000; Yang et al., 2012; Zhang et al., 2015). However, a part of unhydrolyzed xylan remained in the pretreated material and the poor enzymatic hydrolysis yield of cellulose were caused by the low pretreatment severity of the first step, which strongly influenced on the maximization of carbon efficiency in lignocellulosic biomass. Furthermore, compared with dilute acid pretreatment, the biggest different is that no free liquid in the pretreated material after DAP method. The post-hydrolysis process of the pretreatment hydrolysate from dilute acid pretreatment is not suitable for the DAP. Even, no research about post-hydrolysis used during the DAP has been reported. Therefore, it is worth to further investigate the post-hydrolysis of the unhydrolyzed xylooligosaccharides and xylan remained on surface and inside of the solid part of the pretreated material for upgrading DAP as an integrated biorefinery process of lignocellulose.

In this study, an integrated biorefinery process of lignocellulose fractionation for co-production of xylose, cellulosic ethanol and active lignin by upgrading the dry acid pretreatment strategy with post-hydrolysis was proposed. Specifically, the DAP performance at the first-step was evaluated comprehensively by a radar chart of saccharide recovery, enzymatic hydrolysis of cellulose and inhibitors content. The post-hydrolysis of the residual xylan and xylooligosaccharides in the pretreated material was coupled with the sterilization simultaneously before fermentation. The simultaneous saccharification and ethanol fermentation at high solids loading of the above material without detoxification were investigated. The products of xylose, ethanol and lignin residue were recovered and carbon balance of the upgrading strategy was calculated. The property of lignin residue after fermentation were characterized. This strategy probably provided an easy-to-operate process for integrated utilization of lignocellulose.

2. Materials and methods

2.1. Raw materials and reagents

Wheat straw (WS, Dancheng, Henan, China) was washed, dried, and milled to pass through mesh diameter of 10 mm. The composition of WS included 38.7 % of glucan, 25.0 % of xylan, 14.9 % of lignin, and 5.2 % of ash, which was measured according to the NREL protocols (Sluiter et al., 2011).

The cellulase enzyme of Cellic CTec2 (Sigma-Aldrich, Shanghai, China) with the filter paper activity and the total protein content of 190.6 FPU/mL and 83.2 mg protein/mL was measured by the NREL protocol (Technical Report NREL/TP-510–42628) and the standard Bradford method, respectively (Adney and Baker, 2008; Bradford, 1976). All other chemicals and reagents were analytical pure and purchased from Aladdin Industrial Co. (Shanghai, China).

2.2. Dry acid pretreatment

The WS with moisture of 6.5 wt% was mixed with the diluted sulfuric acid solution at the solid to liquid ratio of 2:1 (w/w), and presoaked in a sealed plastic bag at the ambient temperature (18–25 °C) for 12 h. The sulfuric acid dosage was 10, 15, 20 and 25 mg/g the dry material (DM) of WS. The presoaked WS was fed into a 20 L pretreatment reactor with helical ribbon stirrer (He et al., 2014b) and reacted at the various temperature of 170, 175, 180 and 185 °C with agitation of 50 rpm for 3 min.

The structural carbohydrates (glucan and xylan), lignin, water soluble sugars (monosaccharides and oligosaccharides from the glucan and xylan in lignocellulose, respectively) and inhibitors in the pretreated WS were analyzed according to the protocols of NREL (Sluiter et al., 2008; Sluiter et al., 2011). The enzymatic hydrolysis of cellulose in pretreated WS were assessed by the modified protocols of NREL with the solids loading at 2.5 % (w/w), cellulase dosage of 20 FPU/g DM at 50 °C and 150 rpm for 72 h (Selig et al., 2008).

2.3. Post-hydrolysis of pretreated wheat straw

Pretreated WS was adjusted the solids loading to 20 % by deionized water (without washed and dried), then refined to the mean particle diameter of 100 ~ 200 μm by DSX-32 wet disk mill (Dongsheng Chemical Machinery Factory, Jiangsu, China). Then, post-hydrolysis of the pretreated and refined WS with initial pH 2.3–2.4 was performed at various sulfuric acid dosage (0.5, 1.0, 1.5 and 2.0 % (w/v)) and solids loading (10, 15 and 20 %), 121 °C for 2 h. After acid post-hydrolysis, water soluble substance was collected for measuring the free sugars (monomers and oligomers) and inhibitors concentration by HPLC according to the protocols of NREL (Sluiter et al., 2008).

2.4. Simultaneous saccharification and ethanol fermentation (SSF)

SSF operation was carried out in a 5 L bioreactor equipped with helical ribbon impeller according to previous study (Gu et al., 2015; Zhang et al., 2010). Briefly, the yeast used in present work was *Saccharomyces cerevisiae* PAT01 (CGMCC 18021) with high tolerance to phenolic inhibitors. Seed culture included 20 g glucose, 2 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 1 g $MgSO_4{\cdot}7H_2O$ and 10 g yeast extract per liter. SSF medium was similar with seed culture but excepted 20 g/L of glucose. The pretreated and post-hydrolyzed WS was pre-saccharification with cellulase dosage of 20 FPU/g DM at 50 °C and pH 4.8 for 24 h. The SSF was performed with inoculation of 10 % at 37 °C and pH 5.5 for 48 h.

After SSF, the fermentation slurry was performed solid–liquid separation. The solid lignin residue was washed and filtered for at least five times, then freeze-dried for subsequent analysis. The liquid was collected for measuring the glucose, xylose and ethanol concentration by HPLC.

2.5. HPLC analysis

Glucose, xylose, ethanol, and inhibitors, such as furfural, hydroxymethylfurfural (HMF) and acetic acid were analyzed using LC-20AD HPLC (Shimadzu, Japan), which equipped with a refractive index detector RID-20A and a Bio-rad Aminex HPX-87H column. The mobile phase was 5 mM H_2SO_4 at the rate of 0.6 mL/min and the column temperature was 65 °C. All samples were centrifuged to remove the cell mass and other water insoluble substances, and then filtered through a 0.22 μm filter before analysis.

2.6. Comprehensive evaluation of pretreatment performance

A radar chart method was used for the comprehensive evaluation of pretreatment performance. The radar chart has 3 axes represented three important results of the pretreated material including xylan recovery, enzymatic hydrolysis of cellulose and inhibitor content (acetic acid, furfural and HMF) from various pretreatment condition. The covered area constructed by the value on 3 axes corresponding to each pretreatment condition was calculated in the radar chart for comparison.

Data of the above results was independently standardized according to the following equation on the basis of their min and max value:

$$\bar{x} = \frac{x - x_{min}}{x_{max} - x_{min}} \quad (1)$$

Table 1
Composition of the pretreated wheat straw base on the dry matter after pretreatment.

Conditions	Dry matter recovery* (%)	CSP§	Insoluble faction				Soluble faction			
			Glucan (%)	Xylan (%)	Klason lignin (%)	Ash (%)	Glucose (%)	Oligo-glucose# (%)	Xylose (%)	Oligo-xylose# (%)
	Changing H_2SO_4 dosage at pretreatment temperature of 175 °C									
10 mg/g	91.47 ± 1.52	1.75	39.59 ± 0.57	13.34 ± 0.35	15.8 ± 0.83	5.6 ± 0.22	0.20 ± 0.11	1.06 ± 0.05	1.27 ± 0.35	11.83 ± 0.35
15 mg/g	86.93 ± 1.86	1.94	41.00 ± 1.11	9.26 ± 0.28	16.6 ± 1.03	5.9 ± 0.17	0.43 ± 0.10	1.29 ± 0.15	6.07 ± 0.51	12.49 ± 0.88
20 mg/g	87.77 ± 2.04	2.08	41.26 ± 0.43	6.14 ± 0.57	17.2 ± 1.21	5.9 ± 0.39	0.69 ± 0.08	1.47 ± 0.19	9.03 ± 2.58	12.74 ± 0.64
25 mg/g	81.45 ± 1.77	2.20	42.14 ± 0.28	3.64 ± 0.21	20.7 ± 1.85	6.3 ± 0.78	2.19 ± 0.34	1.32 ± 0.21	13.60 ± 0.72	10.82 ± 0.77
	Changing temperature at H_2SO_4 dosage of 15 mg/g dry wheat straw									
170 °C	88.70 ± 2.11	1.80	40.53 ± 0.45	10.37 ± 0.45	13.4 ± 1.11	5.8 ± 0.23	0.42 ± 0.13	3.01 ± 0.32	0.30 ± 0.13	16.45 ± 0.36
175 °C	86.93 ± 1.86	1.94	41.00 ± 1.11	9.26 ± 0.28	16.6 ± 1.03	5.9 ± 0.17	0.43 ± 0.10	1.29 ± 0.15	6.07 ± 0.51	12.49 ± 0.88
180 °C	87.99 ± 1.55	2.08	40.30 ± 1.51	8.41 ± 0.11	13.3 ± 1.31	5.9 ± 0.25	0.72 ± 0.09	1.59 ± 0.13	7.69 ± 0.44	10.14 ± 0.23
185 °C	78.05 ± 1.81	2.22	41.60 ± 0.84	4.17 ± 0.21	16.6 ± 1.51	6.6 ± 0.28	0.94 ± 0.23	2.23 ± 0.27	11.99 ± 0.83	10.14 ± 0.75

* Dry matter recovery means the percentage of dry matter after pretreatment based on the raw material.
§ CSP is the abbreviation of combined severity parameter.
# Oligo-glucose and Oligo-xylose are the abbreviation of oligomeric glucose and xylose, respectively.

where $\bar{x}$ is the standard value; x is the experimental value of the above results; x_{min} and x_{max} are the min and max value of total xylose recovery (0 and 100 %), glucose yield of enzymatic hydrolysis (0 and 100 %) and acetic acid (0 and 12.9 mg/g dry mater), furfural (0 and 6.57 mg/g dry mater) and HMF (0 and 4.1 mg/g dry mater) according to the previous studies (He et al., 2014a; He et al., 2014b; Zhang et al., 2011).

Then, $\bar{x}$ is the standard value of each above results was further normalized according to the following equation:

$$y = 1 - e^{-\bar{x}} \quad (2)$$

$$y = e^{-\bar{x}} \quad (3)$$

where y is the normalized value with the scale between 0 and 1. The normalized value of positive results (xylose recovery and enzymatic hydrolysis) and negative results (inhibitors content) contributed to the pretreatment performance was calculated by the Eqs. (2) and (3), respectively. Especially, the normalized value of inhibitors was represented by the weighted average of acetic acid (20 %), furfural (50 %) and HMF (30 %) on the base of their strength of inhibition. The normalized value of the 3 important results after various pretreatment condition was shown in the radar chart.

2.7. Calculation

The pretreatment severity of DAP was calculated according to the equation of the combined severity parameter (CSP) (Gu et al., 2018).

$$\mathrm{CSP} = \mathrm{Log}R_0 - \mathrm{pH} = \mathrm{Log}\left[t \cdot \exp\left(\frac{T\text{-}100}{14.75}\right)\right] - \mathrm{pH} \quad (4)$$

where T is pretreatment temperature (°C), t is pretreatment time (min), R_0 is the severity parameter of pretreatment. The dry dilute acid pretreatment was conducted at high solids content (70 % at the beginning and 50 % at the end) of lignocellulose biomass, and no free liquid was generated before and after the pretreatment, therefore the pH values was calculated according to the methods by Zhang et al (2015).

The glucan and xylan recovery from pretreatment and the total glucose and xylose yields from pretreatment, post-hydrolysis and enzymatic hydrolysis were calculated according to the previous method (Zhang et al., 2015). The ethanol yield is calculated according to the previous method (Zhang and Bao, 2012).

All the above results were expressed as mean ± standard deviation of independent experiments performed in two batches of fractionation processes at the same conditions. The standard deviations were employed as error bars in graphs with a confidence level of 0.95.

2.8. Characterization of pretreated wheat straw and lignin residue

Fourier transformed infrared spectroscopy (FT-IR) spectra of raw wheat straw and PWS samples were obtained using a Nicolet Magna-IR 550 spectrometer (Nicolet, Madison, WI, USA) at a wavelength setting ranging from 400 to 4000 cm^{-1}. The sample was mixed with KBr, then the mixture was ground and tableted (10 kpa). Measurements were done in duplicates and 256 scans were recorded for all samples at a 2 cm^{-1} resolution (Shen et al., 2016).

The X-Ray diffraction (XRD) patterns of the lignocellulose samples were analyzed by using a Rigaku D/MAX 2550 VB/PC XRD (Rigaku Corporation, Tokyo, Japan) with monochromatic CuKα radiation (1.54 Å) at 40 kV and 100 mA. Scans were obtained from 10 to 50 degrees 2θ (Bragg angle) at a 2°/minute scanning rate. Samples were milled prior to analysis and put through a 80–100 mesh sieve. The crystallinity index (*CrI*) was calculated following the previous method (Luterbacher et al., 2014).

Surface images of wheat straw before and after pretreatment were observed by scanning electron microscopy (SEM). The samples were lyophilized and coated with gold, and images were taken at 15 kV using a Hitachi S3400 N SEM (Hitachi, Kyoto, Japan).

The molecular weights of the lignin residue were determined by gel permeation chromatography (GPC) as previously reported (Li et al., 2018). For the collection of HSQC spectra, about 50 mg unacetylated lignin was fully dissolved in 0.5 mL DMSO-d_6. The samples were analyzed on a Bruker AV III 500 MHz spectrometer equipped with a DCH (^{13}C-optimized) cryoprobe (Billerica, MA). The test was conducted at 25 °C with Bruker's standard hsqcetgpsisp 2.2 pulse program (acquisition times 200 ms and 8 ms in 1H and 13C dimensions, inter-scan relaxation delay 1 s). Data were analyzed using Bruker's Topspin 4.0.8 software. The semi-quantitative of the 2D HSQC NMR experiments were

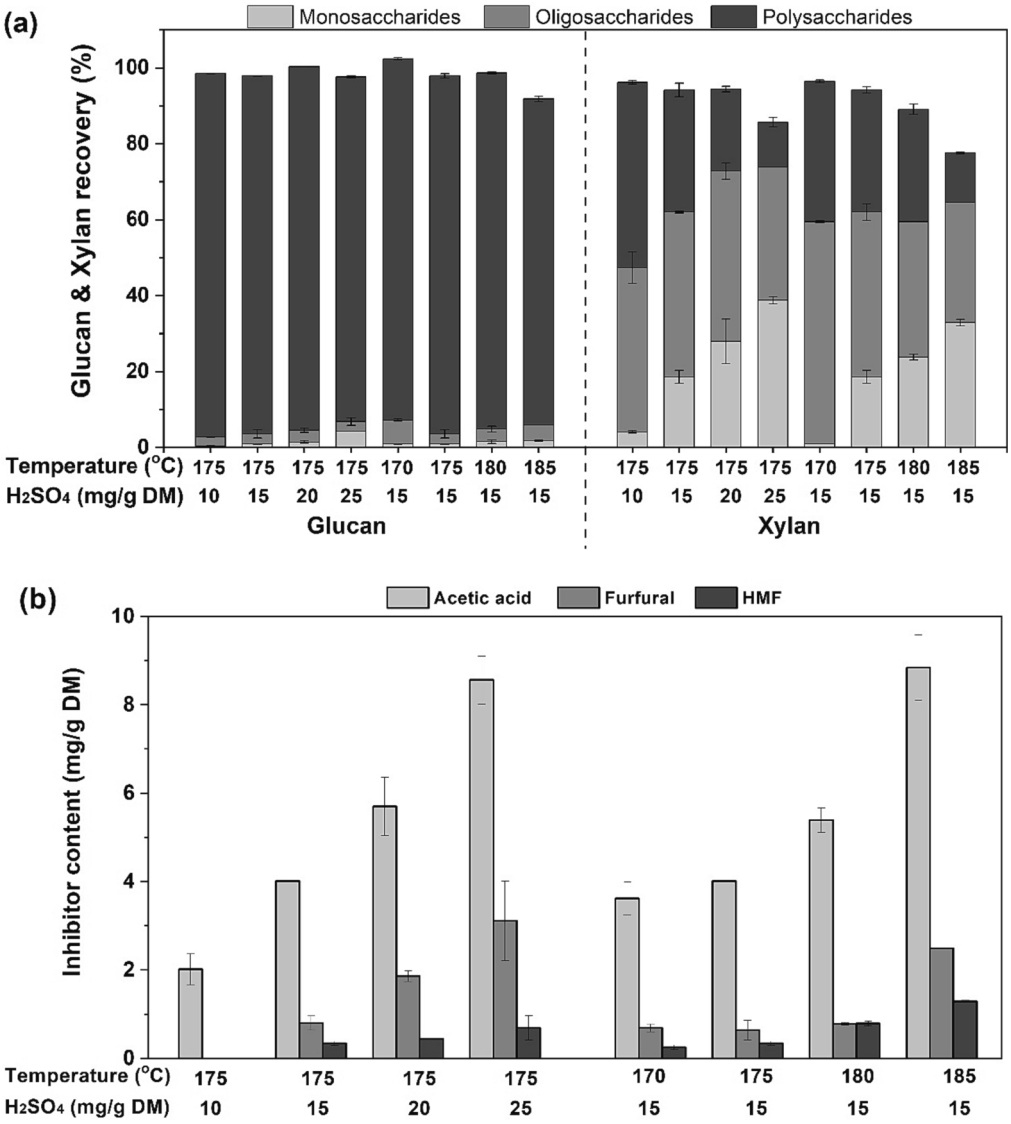


Fig. 1. Comprehensive evaluation of dry acid pretreatment performance. Pretreatment was performed at the ratio of solid to liquid 2:1 and residence time of 3 min unless mentioned elsewhere. Acid dosage of 10, 15, 20 and 25 mg/g dry wheat straw, temperature of 170, 175, 180 and 185 °C. (a) Recovery of glucan and xylan; (b) Inhibitors content in soluble fraction of the pretreated material; (c) Enzymatic hydrolysis of the pretreated material; (d) Radar chart for comprehensive evaluation of pretreatment.

conducted according to a previous method (Cai et al., 2020).

2.9. Carbon efficiency of the process

The carbon balance of agricultural waste biomass biorefinery determines the potential of mitigating greenhouse gas emissions of the process, which was evaluated based on carbon flow of this process. Molecular formula of the wheat straw components: glucan $C_6H_{10}O_5$, xylan $C_5H_8O_4$ and lignin $C_{10}H_{11.8}O_{4.48}$ (Wang et al., 2018). The carbon efficiency of the process is the amount of carbon in the products to the initial carbon in glucan, xylan and lignin.

3. Results and discussion

3.1. Comprehensive evaluation of dry acid pretreatment performance

The effect of acid dosage and temperature on the composition, enzymatic hydrolysis and inhibitors of the pretreated wheat straw was investigated. With the acid dosage and temperature increasing, the combined severity parameter (CSP) increased from 1.75 to 2.22, the glucan, lignin and ash content relative increased in the dry matter of pretreated wheat straw, while xylan in the water insoluble solids (WIS) fraction and the recovery of dry matter significantly decreased (Table 1). For the glucan and xylan recovery after pretreatment, the yield of glucose monomers and oligomers was almost below 10 %, while the yield of xylose monomers and oligomers increased from 47.1 to 73.9 % (Fig. 1a). For the pretreatment inhibitors, acetic acid and furfural significantly increased because of the xylan hydrolysis and further degradation, while HMF slightly increased (Fig. 1b). For enzymatic hydrolysis of WIS, the glucose yield obviously increased from 60 % to 97 %, while the xylose yield was almost below 20 % because of the low xylan in WIS (Fig. 1c). Based on the above results, the covered area in the radar chart was used for the comprehensive evaluation of pretreatment performance. The largest area in the radar chart was 0.79, which was obtained from the pretreatment condition at 175 °C and acid dosage of 15 mg/g DM (Fig. 1d). At this condition, the lower inhibitors concentration and the higher xylan recovery were obtained. Although, the glucose yield of enzymatic hydrolysis was 79.1 %, which would be

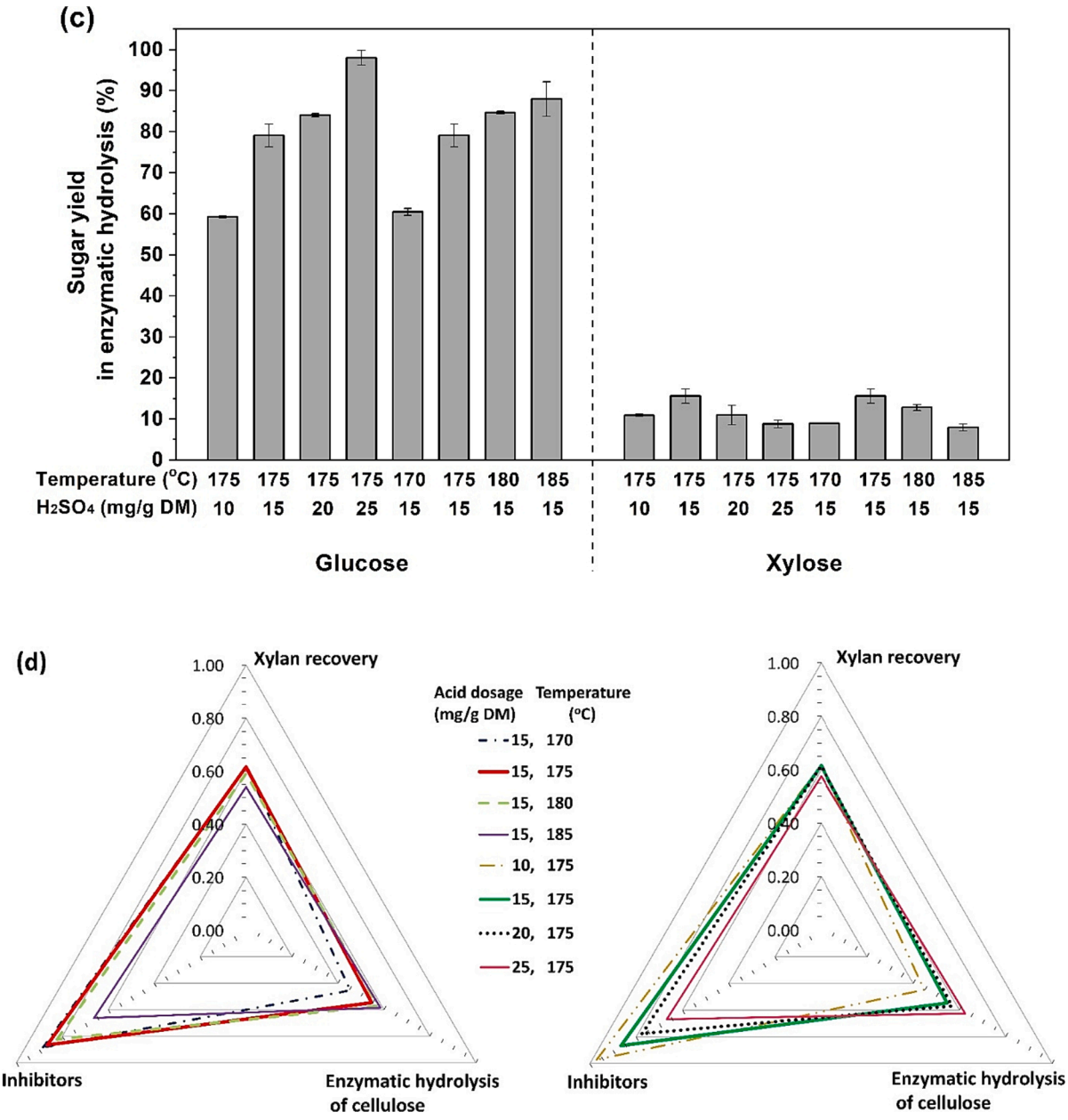


Fig. 1. (*continued*).

further improved during the subsequent post-hydrolysis step.

The result presented that increasing acid dosage could accelerate xylan hydrolysis and further conversed to xylose, while raising temperature had no significant effect on xylan hydrolysis to soluble sugars (xylose monomers and oligomers) and just improved hydrolysis of oligomers to monomers, which was consistent with the observation that large amount of xylooligosaccharides were generated by DAP at the low pretreatment severity in the previous research (Han and Bao, 2018). Compared with reaction temperature, acid dosage was more beneficial to destroy lignocellulose resistance, leading to improvement of enzymatic hydrolysis of cellulose in the pretreated material, which probably because of the increase in cellulose accessibility by removal of more xylan during DAP (Gu et al., 2018). Therefore, the above result indicated that DAP at a lower severity predominantly degraded hemicellulose, improved the accessibility of cellulose to enzymes and only produced minor inhibitors, which was suitable for the subsequent post-hydrolysis and SSF.

3.2. Characterization of pretreated wheat straw

The physicochemical properties of the pretreated WS were detected by using FT-IR, XRD and SEM. For FT-IR analysis, the bands at 1745 and 1240 cm^{-1} are mainly attributed to the stretching vibration of the C═O and C–O bonds of the acetyl ester in hemicellulose and the ether linkages between lignin and hemicellulose (Tjeerdsma and Militz, 2005). The decrease in the intensity of both bands at harsher pretreatment severities indicated that the dissolution/removal of hemicellulose from biomass. The bands at 1051, 1165, 1110 and 1515 cm^{-1} are mainly attributed to the stretching vibration of the primary and secondary OH groups, the C–O–C bond and the lignin aromatic rings (Lima et al., 2013). The increase in the intensity of these peaks was attributed to the relative increase in the lignin of PWS due to the removal of the hemicellulose fraction (see supplementary materials). For XRD patterns of WS samples, the peaks become narrower and more intense for crystalline cellulose, and the crystallinity indices (CrI) increased from 50.4 % to 68 % with the pretreatment severity increasing (see supplementary materials). The

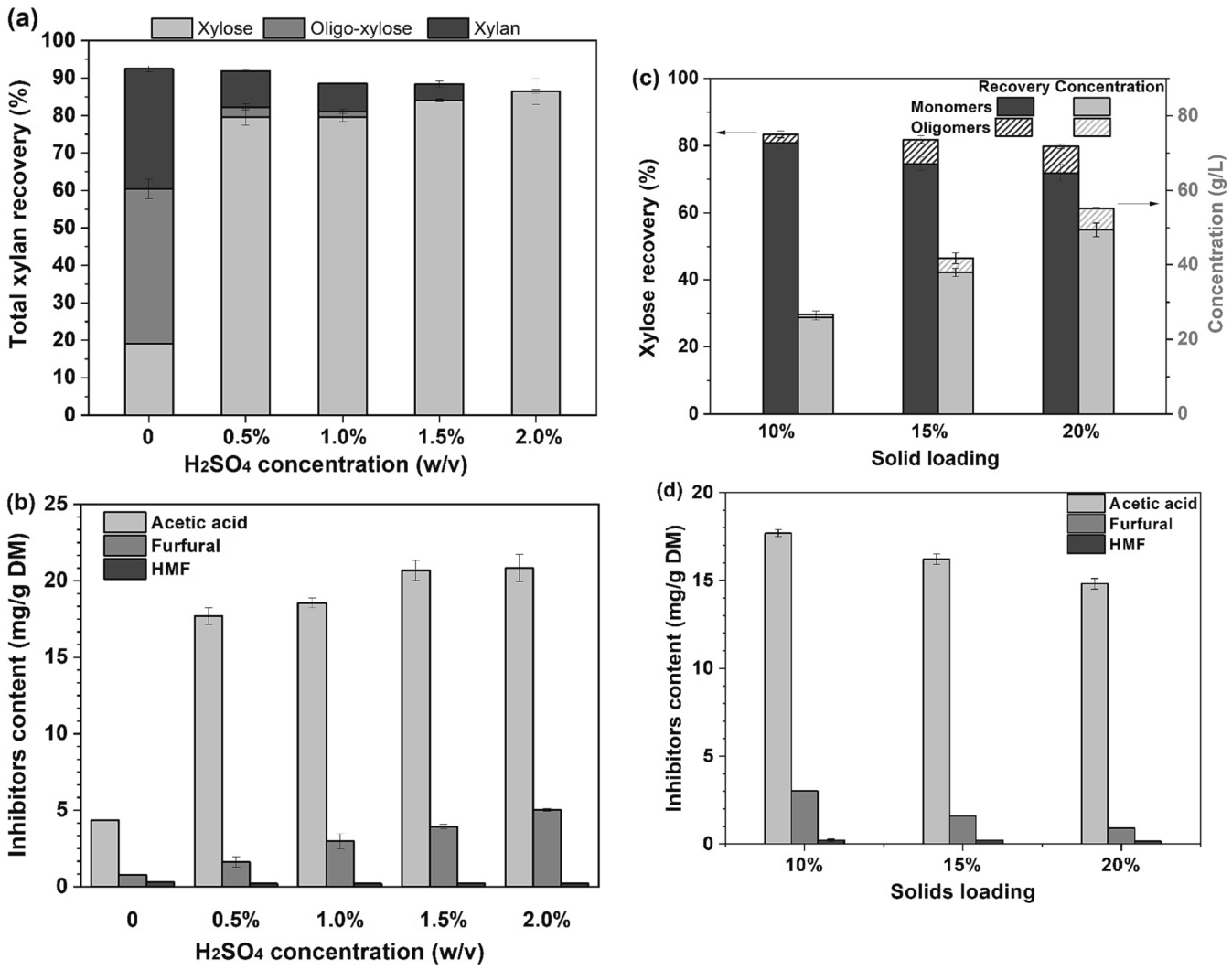


Fig. 2. Effect of post-hydrolysis on the xylose yield and inhibitors formation. Post-hydrolysis was performed at 120 °C for 2 h unless mentioned elsewhere. (a, b) Effect of acid dosage on the xylose yield and inhibitors formation: the acid dosage of 5, 10, 15 and 20 mg/g dry wheat straw, solids loading of 10 % ; (c, d) Effect of solids loading on the xylose yield and inhibitors formation: post-hydrolysis at the solids loading of 10, 15 and 20 % (w/w), acid dosage of 5 mg/g dry wheat straw.

increase could be attributed to the relative increase in the cellulose of the pretreated WS for the removal of amorphous hemicellulose, which consistent with the result of FT-IR. For the observation by SEM, the untreated WS presented the structure of whole macrofibers. The microstructure of pretreated WS was partially destroyed, and more fragments were produced with the pretreatment severity increasing (see supplementary materials). The slightly darker color of the pretreated WS could be attributed to the relocation of lignin at high pretreatment severity (Hansen et al., 2011).

3.3. Post-hydrolysis of pretreated wheat straw

The xylooligosaccharide and xylan remained on/in the pretreated material strongly inhibit cellulase activity and cannot be fermented by many organisms (Zhang et al., 2015). For recovering more monomeric xylose and further improving glucose yield of enzymatic hydrolysis, post-hydrolysis performed at various sulfuric acid dosage and solids loading was investigate. With the acid dosage increasing from 0 to 0.5 %, xylose recovery significantly increased from 18.6 % to 79.1 %, but no obvious improvement with acid dosage further increasing to 2.0 % (Fig. 2a). The acetic acid significantly increased from 4.1 to 20.5 mg/g DM and the furfural increased from 0.8 to 4.9 mg/g DM, while the HMF still kept at about 0.2 mg/g DM (Fig. 2b). With the solids loading increasing, the xylose recovery slightly reduced from 81.3 % to 71.8 %, while the corresponding concentration in the hydrolysate was increased from 26.9 to 50.6 g/L (Fig. 2c). Acetic acid and furfural slightly reduced from 18 to 15 mg/g DM and from 3.0 to 0.9 mg/g DM, respectively (Fig. 2d).

The results show that post-hydrolysis performed at sulfuric acid dosage of 0.5 % and solids loading of 20 % efficiently obtained high level of xylose, which was higher than that from the other post-hydrolysis method (Zhang et al., 2015), even closed to the desirable value (50–70 g/L) in the commercial production (Kim et al., 2013). In addition, the inhibitors such as acetic acid, furfural and HMF produced at this condition were lower than the minimum inhibitory concentration to *S. cerevisiae* and to cellulase activity (Ec et al., 2014; Kim et al., 2011).

3.4. SSF of pretreated and post-hydrolyzed wheat straw

The cellulose enzymatic hydrolysis and SSF of the pretreated and post-hydrolyzed WS were performed without any detoxification process (Fig. 3). For the cellulose enzymatic hydrolysis at solid loading of 20 %, the glucose yield of the pretreated and post-hydrolyzed WS (PPWS) was 85.4 % after 72 h' hydrolysis, which was higher than those of the pretreated WS at acid dosage of 15 and 25 mg/g DM, respectively (P15 and P25 in Fig. 3a). For SSF, the pre-saccharification process of 24 h was

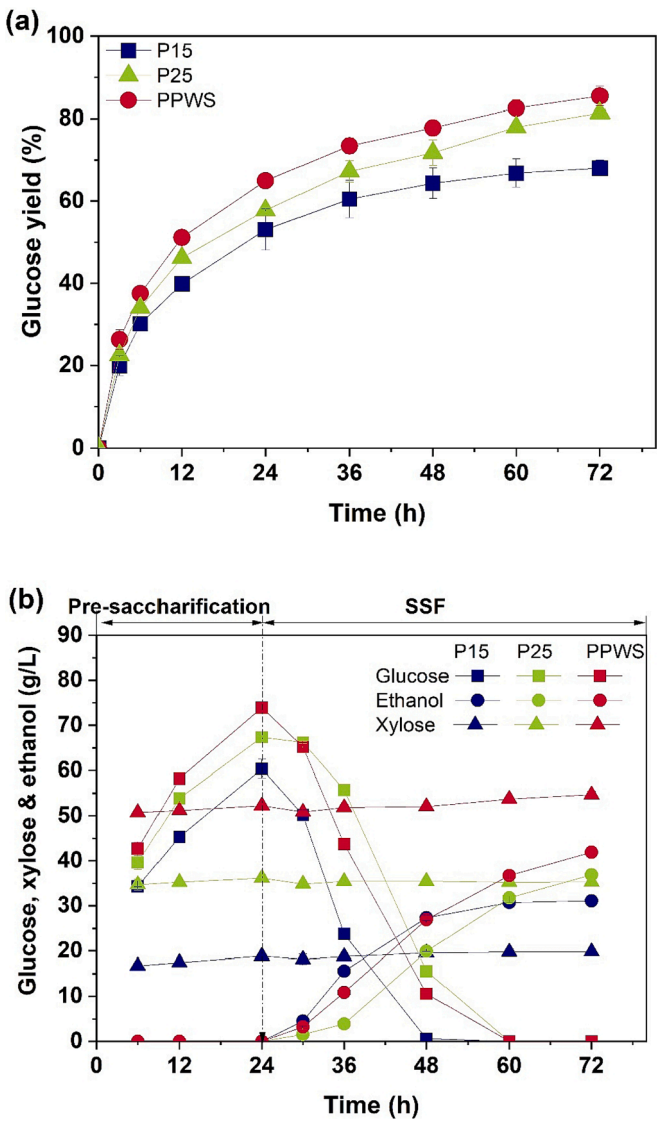


Fig. 3. Effect of fractionation strategies on enzymatic hydrolysis and ethanol fermentation. (a) Enzymatic hydrolysis was performed at solids loading of 20 % (w/w), enzyme dosage of 15 FPU/g DM, 50 °C, pH4.8 for 72 h; (b) Ethanol fermentation: pre-hydrolysis was performed at solids loading of 20 %, cellulase 15 FPU/g DM, 50 °C, pH4.8 for 24 h; Fermentation: inoculum of 10 % (v/v), 37 °C, pH5.5 for 48 h.

determined according to the result of the enzymatic hydrolysis. The PPWS provided the highest ethanol and xylose concentration of 41.8 g/L and 54.7 g/L (corresponding ethanol yield and xylose recovery of 73.9 % and 73.2 %) after SSF, respectively, which was higher than the P15 and P25. The xylose concentration remained almost constant during the whole SSF of all the three materials (Fig. 3b).

The results show that the PPWS could provide a better performance of the cellulose enzymatic hydrolysis and SSF under the condition without detoxification. The improvement of the cellulose enzymatic hydrolysis probably because of reducing the inhibition of xylooligosaccharide and xylan remained in the pretreated material on cellulase activity and the increase in cellulose accessibility by the post-hydrolysis process (Zhang et al., 2015). The higher ethanol from SSF of the PPWS might be caused by the lower inhibitors of furfural and HMF (about 0.1 g/L and 0.05 g/L) than the P25, while the synergistic effect of pretreatment and post-hydrolysis provided stronger pretreatment severity than the P15 (Fig. 1,2 and see supplementary materials).

3.5. Characterization of fractionated lignin

The physicochemical properties of the fractionated lignin after SSF of the pretreated and post-hydrolyzed wheat straw (ELPP), and the pretreated wheat straw at 175 °C with sulfuric acid dosage of 25 mg/g DM (ELP25) were characterized by the GPC and NMR spectra, which were compared with a typical ethanol organosolv lignin (EOL).

The relative molecular weights (Mn, Mw and PDI) of the above lignin samples were shown in Table 2. PDI of ELPP and ELP25 were wider than that of EOL, as shown by PDI(Mw/Mn) > 4.0. The Mn and Mw of ELPP (4890 Da and 20145 Da) were very close to those of EOL (5283 Da and 17355 Da). This fact suggested that the DAP at mild conditions coupled with post-hydrolysis might like the ethanol organosolv pretreatment only caused a slight change of lignin (Huijgen et al., 2014). By contrast, ELP25 showed the highest Mw (29,600 Da), the lowest Mn (3270 Da) and wide PDI, which was most likely the cleavage of inter-unit linkages generated the small fragment of lignin, and further recondensed. This was consistent with the previous researches on the dilute acid and hydrothermal pretreatment (Jensen et al., 2017; Sun et al., 2016).

The side-chain and aromatic regions of the above lignin samples were characterized by 2D-HSQC NMR (Fig. 4, Table 2). For the side-chain region, ELPP presented more lignin functionalities and interunit linkages than those left in ELP25, but less than those in the EOL. Generally, the enhancement in pretreatment severity promoted the lignin depolymerization. For the aromatic region, the signals of G and S units gradually disappeared and the condensed S units increased with the pretreatment severity increasing, leading to the S/G ratio increased from 1.2 in EOL, to 3.8 in ELPP and 5.3 in ELP25, which was consistent with previously reported studies (Cai et al., 2020). G units contain more reactive sites and can be easily reacted with other groups to form condensation (Islam et al., 2021). ELPP showed higher amount of G units than those of ELP25, which depended on the pretreatment severity. In addition, *p*CA and FA are the major acylated lignin units in wheat straw, which significantly decreased in ELPP and ELP25 with the pretreatment severity increasing.

The above results indicated that the obtained lignin by upgrading DAP with post-hydrolysis preserved more active structure such as β-O-4 linkages than that from the current DAP method, which was consistent with the report that lignin molecular weight is directly proportional to the amount of β-O-4 linkages in the lignin (Wang et al., 2017). The less condensation in the lignin residue obtained from the upgrading DAP might be another reason for improvement of enzymatic hydrolysis (Fig. 3). An association between the condensed degree of lignin and its

Table 2
Structural characteristics (interunit linkages, aromatic units, and relative molecular weight) of wheat straw lignin.

Samples	$S_{2/6}$	$S'_{2/6}$	S_{Cond}	G	pCA	FA	S/G	β-O-4	β-5	β-β	Mn	Mw	PDI
EOL	39.5	6.0	19.2	35.2	15.7	6.8	1.2	68.8	6.9	15.8	5283	17,355	3.28
ELPP	40.8	6.7	36.8	15.7	13.6	1.4	3.8	45.2	1.6	13.5	4890	20,145	4.11
ELP25	34.3	5.9	47.1	12.7	9.3	1.2	5.3	32.2	1.5	7.6	3270	29,600	8.23

EOL is the ethanol organosolv lignin from wheat straw, ELPP is the enzymatic hydrolysis residual lignin from the pretreated and post-hydrolyzed wheat straw, ELP25 is the enzymatic hydrolysis lignin from the wheat straw pretreated at 175 °C with sulfuric acid dosage of 25 mg/g DM.
G, guaiacyl; S, syringyl; pCA, p-coumarate; FA, ferulate; S_{Cond} represents the condensed $S_{2/6}$.
Mw = weight-average molecular weight, Mn = number-average molecular weight, PDI is the polydispersity index, and equals to Mw/Mn.

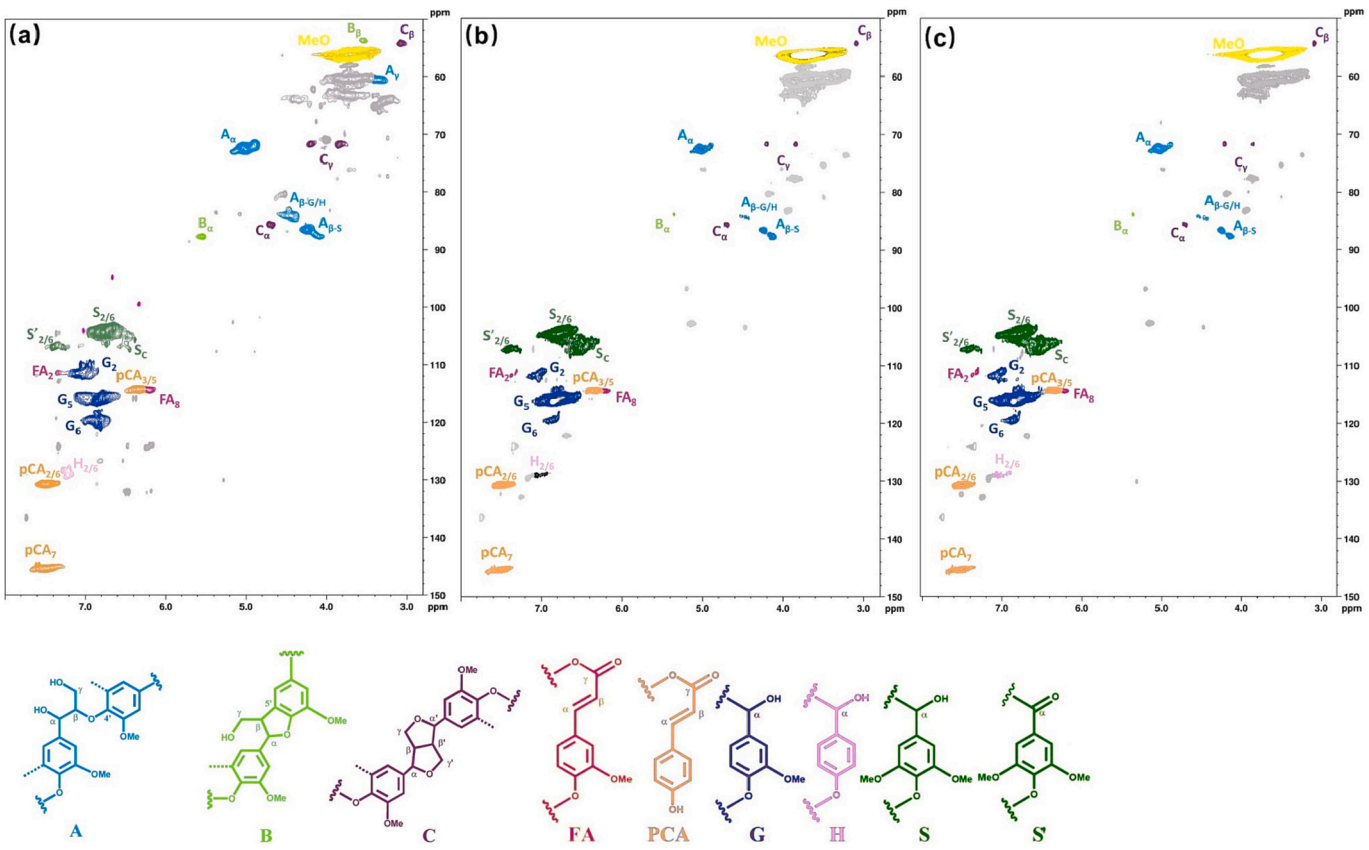


Fig. 4. 2D ^{1}H–^{13}C HSQC NMR spectra (lignin side-chain/polysaccharide and aromatic regions) of (a) Ethanol organosolv lignin of wheat straw (EOL), (b) enzymatic hydrolysis lignin residue from the pretreated and post-hydrolyzed wheat straw, and (c) enzymatic hydrolysis lignin residue from pretreated wheat straw at 175 °C with sulfuric acid dosage of 25 mg/g DM. Correlation signals are categorized and color-coded by the type of aromatic units. A, β-O-4-aryl ether; B, phenylcoumaran (β-5); C, resinol (β-β); G, guaiacyl; H, p-hydroxyphenyl; S, syringyl; pCA, p-coumarate; FA, ferulate.

inhibition on enzymatic hydrolysis has been reported by the previous research (Sun et al., 2016). Moreover, the higher β-O-4 content in lignin would lead to the higher yield of phenolic monomers in depolymerization (Deuss et al., 2017). Therefore, the upgrading DAP method might provide a potential for the high value-added phenolic compounds production from the lignin residue.

3.6. Mass balance and carbon efficiency of the process

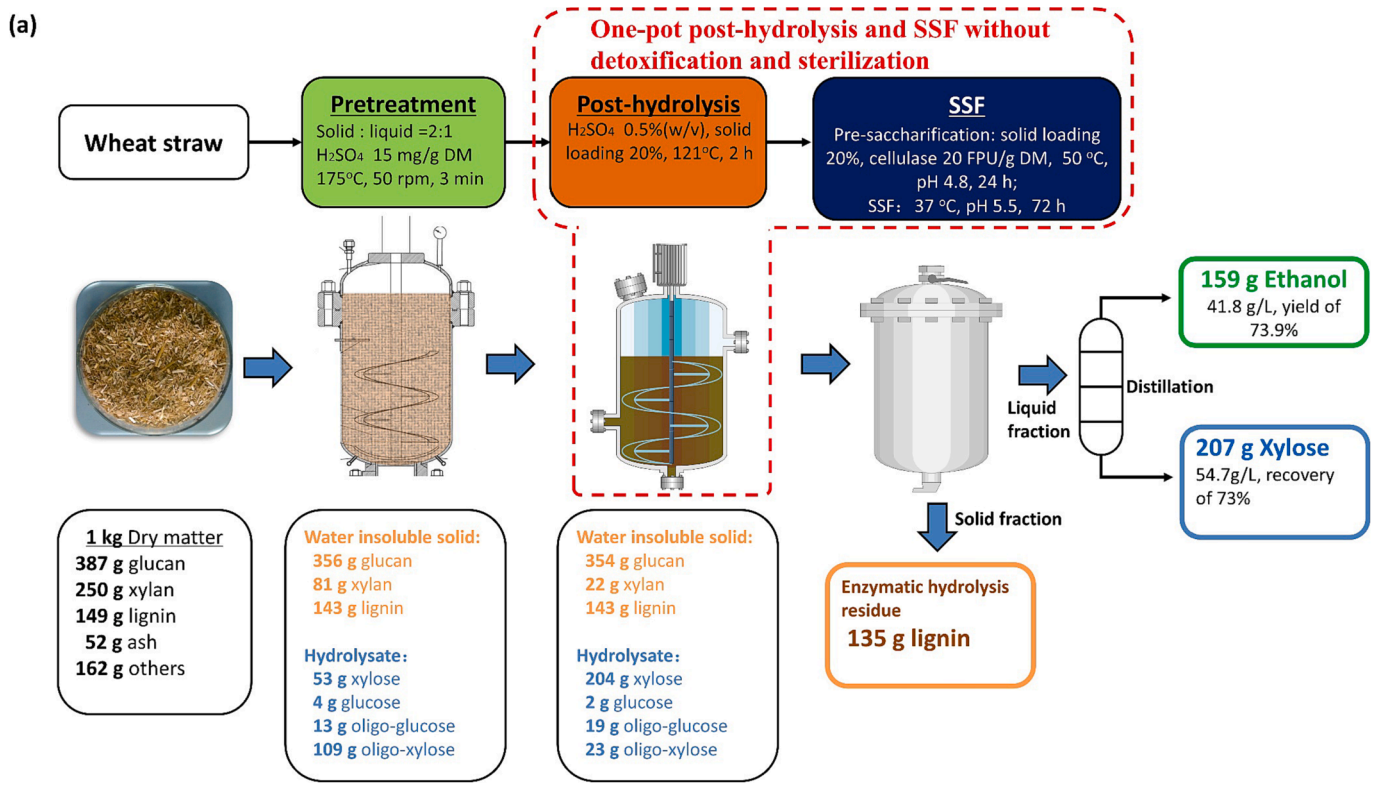
The mass balance of the proposed overall process for co-production of ethanol, xylose and reactive lignin from lignocellulose were investigated based on 1 kg dry matter (Fig. 5a). The DAP and one-pot of the post-hydrolysis and SSF without detoxification and sterilization was performed for comprehensive utilization of lignocellulose. After the DAP, 870 g dry matter was recovered, in which the glucan and lignin (356 g and 143 g) almost kept constant, while xylan (81 g) significantly decreased and converted to xylose and *xylo*-oligomers. After the post-hydrolysis, over 80 % of xylan was hydrolyzed to 204 g xylose and 23 g *xylo*-oligomers. After SSF, 159 g ethanol, 207 g xylose and 135 g lignin were harvested from 1 kg wheat straw by this fractionation process at the optimal condition. Compared with previous reported various pretreatment and fractionation lignocellulose methods, the upgrading DAP strategy for complete utilization of lignocellulose presented the highest total recovery of ethanol, xylose and reactive lignin (see supplementary material).

The carbon flow of biorefinery processes were showed in Fig. 5b. After the general DAP and SSF, 15.6 %, 13.6 % and 26.4 % of wheat straw carbon was converted to ethanol, xylose and residual lignin. However, this carbon presented in the residual lignin was higher than that of the initial lignin (22.7 %) in wheat straw, which probably the condensed and/or pseudo lignin and could only be used for low value applications, such as combustion for power generation. Carbon efficiency of the upgrading strategy of DAP with post-hydrolysis and SSF was significantly increased to 66 %, including the higher amount of bioethanol (22.3 %), xylose (22.3 %) and reactive lignin (21.4 %) in the product stream. These yields are close to those obtained by other strategies, such as the recent reported integrated biorefinery processes for phenolics, oligomers and pulp (76 %) or ethanol, xylose and adhesive (79 %)(Liao et al., 2020; Pang et al., 2021).

Based on the comparison, although the fractionation process in this study did not show the highest yield of the three main components, it presented the advantages including comprehensive utilization of lignocellulose, protection of lignin structure, lower wastewater generation (high solids loading) and avoiding detoxification.

4. Conclusion

An upgrading DAP strategy with post-hydrolysis was demonstrated to be effective for carbon efficient conversion and comprehensive application of lignocellulose. DAP at lower severity predominantly degraded hemicellulose, improved enzymatic hydrolysis of cellulose, and produced minor inhibitors. Subsequently, post-hydrolysis at 20 % solids loading efficiently converted the xylan and xylooligosaccharides to xylose and kept inhibitor at low level. SSF of the pretreated and post-hydrolyzed WS at 20 % solid loading without the detoxification process provided a high xylose recovery and ethanol concentration. The obtained lignin residue indicated the relatively intact natural lignin structural characteristics.



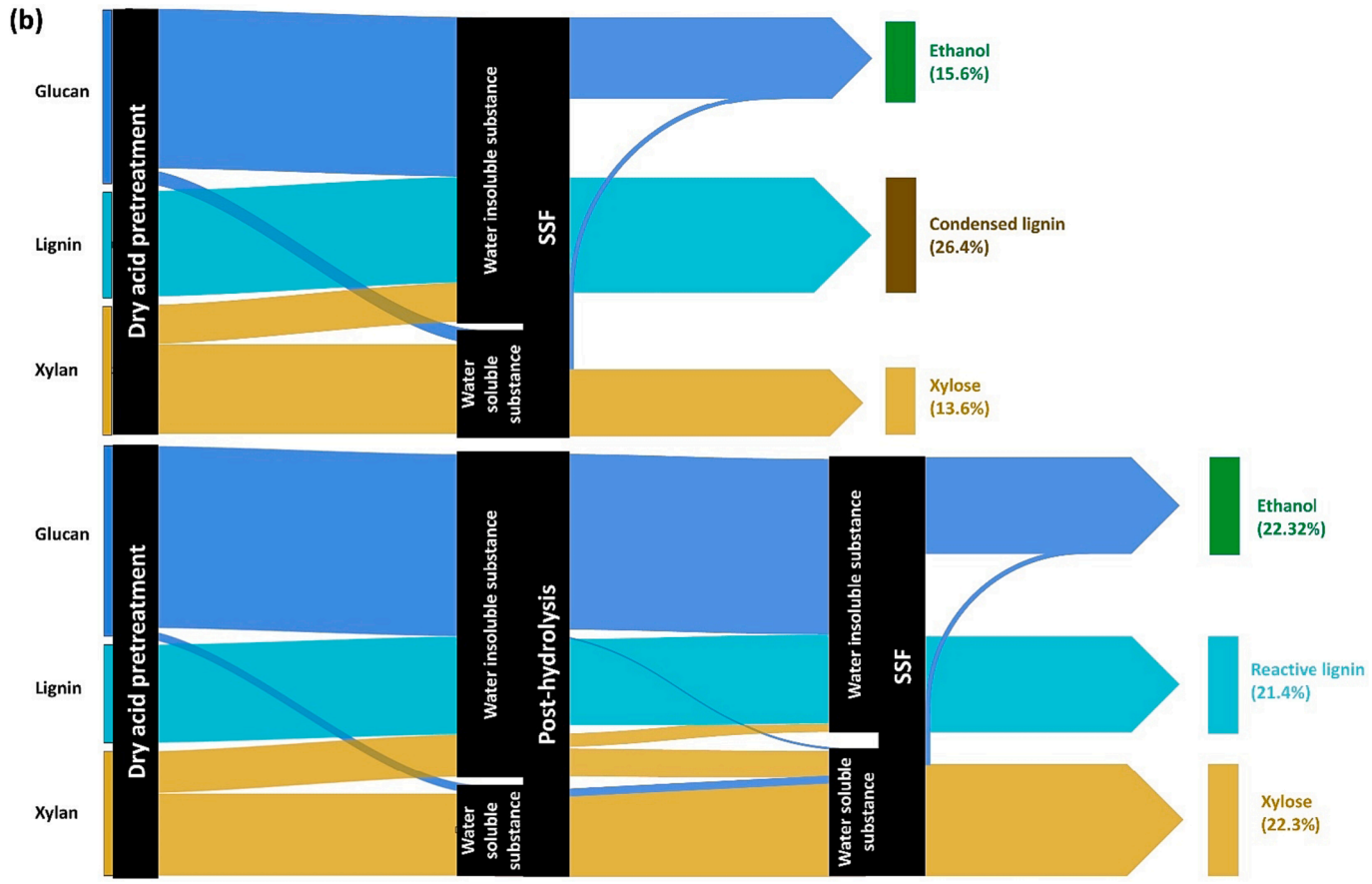


Fig. 5. Schematic diagram of mass balance of wheat straw lignocellulose fractionation process.

CRediT authorship contribution statement

Hanqi Gu: Writing – original draft, Investigation, Formal analysis, Data curation. **Xushen Han:** Investigation. **Jian Zhang:** Methodology. **Jie Bao:** Writing – review & editing, Methodology.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2023.130261.

References

Adney, B., Baker, J., 2008. Measurement of Cellulase Activities. National Renewable Energy Laboratory. NREL/TP-510-42628.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254.
Cai, C., Hirth, K., Gleisner, R., Lou, H., Qiu, X., Zhu, J.Y., 2020. Maleic acid as a dicarboxylic acid hydrotrope for sustainable fractionation of wood at atmospheric pressure and ≤100°C: mode and utility of lignin esterification. Green Chem. 22 (5), 1605–1617.
Deuss, P.J., Lancefield, C.S., Narani, A., de Vries, J.G., Westwood, N.J., Barta, K., 2017. Phenolic acetals from lignins of varying compositions via iron(iii) triflate catalysed depolymerisation. Green Chem. 19 (12), 2774–2782.
Ec, V.D.P., Bakker, R.R., Baets, P., Eggink, G., 2014. By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio) chemicals and fuels. Appl. Microbiol. Biotechnol. 98 (23), 9579–9593.
Gu, H., Zhang, J., Bao, J., 2015. High tolerance and physiological mechanism of Zymomonas mobilis to phenolic inhibitors in ethanol fermentation of corncob residue. Biotechnol. Bioeng. 112 (9), 1770–1782.
Gu, H., An, R., Bao, J., 2018. Pretreatment refining leads to constant particle size distribution of lignocellulose biomass in enzymatic hydrolysis. Chem. Eng. J. 352, 198–205.
Han, X., Bao, J., 2018. General method to correct the fluctuation of acid based pretreatment efficiency of lignocellulose for highly efficient bioconversion. ACS Sustain. Chem. Eng. 6 (3), 4212–4219.
Han, X., Li, L., Wei, C., Zhang, J., Bao, J., 2019. Facilitation of l-lactic acid fermentation by lignocellulose biomass rich in vitamin B compounds. J. Agr. Food Chem. 67 (25), 7082–7086.
Hansen, M.A.T., Kristensen, J.B., Felby, C., Jørgensen, H., 2011. Pretreatment and enzymatic hydrolysis of wheat straw (Triticum aestivum L.) – The impact of lignin relocation and plant tissues on enzymatic accessibility. Bioresour. Technol. 102 (3), 2804–2811.
He, N., Jia, J., Qiu, Z., Fang, C., Lidén, G., Liu, X., Bao, J., 2022. Cyclic l-lactide synthesis from lignocellulose biomass by biorefining with complete inhibitor removal and highly simultaneous sugars assimilation. Biotechnol. Bioeng. 119 (7), 1903–1915.
He, Y., Zhang, J., Bao, J., 2014a. Dry dilute acid pretreatment by co-currently feeding of corn stover feedstock and dilute acid solution without impregnation. Bioresour. Technol. 158, 360–364.
He, Y., Zhang, L.P., Zhang, J., Bao, J., 2014b. Helically agitated mixing in dry dilute acid pretreatment enhances the bioconversion of corn stover into ethanol. Biotechnol. Biofuels 7.
He, Y., Zhang, J., Bao, J., 2016. Acceleration of biodetoxification on dilute acid pretreated lignocellulose feedstock by aeration and the consequent ethanol fermentation evaluation. Biotechnol. Biofuels 9 (1), 19.
Huijgen, W.J.J., Telysheva, G., Arshanitsa, A., Gosselink, R.J.A., de Wild, P.J., 2014. Characteristics of wheat straw lignins from ethanol-based organosolv treatment. Ind. Crop. Prod. 59, 85–95.
Islam, M.K., Rehman, S., Guan, J., Lau, C.-Y., Tse, H.-Y., Yeung, C.S., Leu, S.-Y., 2021. Biphasic pretreatment for energy and carbon efficient conversion of lignocellulose into bioenergy and reactive lignin. Appl. Energ. 303, 117653.
Jensen, A., Cabrera, Y., Hsieh, C.-W., Nielsen, J., Ralph, J., Felby, C., 2017. 2D NMR characterization of wheat straw residual lignin after dilute acid pretreatment with different severities. Holzforschung 71 (6), 461–469.
Kim, Y., Ximenes, E., Mosier, N.S., Ladisch, M.R., 2011. Soluble inhibitors/deactivators of cellulase enzymes from lignocellulosic biomass. Enzyme. Microb. Technol. 48 (4), 408–415.
Kim, Y., Kreke, T., Ladisch, M.R., 2013. Reaction mechanisms and kinetics of xylo-oligosaccharide hydrolysis by dicarboxylic acids. AlChE J. 59 (1), 188–199.
Li, N., Li, Y., Yoo, C.G., Yang, X., Lin, X., Ralph, J., Pan, X., 2018. An uncondensed lignin depolymerized in the solid state and isolated from lignocellulosic biomass: a mechanistic study. Green Chem. 20 (18), 4224–4235.
Liao, Y., Koelewijn, S.-F., Van den Bossche, G., Van Aelst, J., Van den Bosch, S., Renders, T., Navare, K., Nicolaï, T., Van Aelst, K., Maesen, M., Matsushima, H., Thevelein, J.M., Van Acker, K., Lagrain, B., Verboekend, D., Sels, B.F., 2020. A sustainable wood biorefinery for low–carbon footprint chemicals production. Science 367 (6484), 1385–1390.
Lima, M.A., Lavorente, G.B., da Silva, H.K.P., Bragatto, J., Rezende, C.A., Bernardinelli, O.D., deAzevedo, E.R., Gomez, L.D., McQueen-Mason, S.J., Labate, C. A., Polikarpov, I., 2013. Effects of pretreatment on morphology, chemical composition and enzymatic digestibility of eucalyptus bark: a potentially valuable source of fermentable sugars for biofuel production – part 1. Biotechnol. Biofuels 6 (1), 75.
Liu, G., Bao, J., 2017. Maximizing cellulosic ethanol potentials by minimizing wastewater generation and energy consumption: Competing with corn ethanol. Bioresour. Technol. 245, 18–26.
Luterbacher, J.S., Rand, J.M., Alonso, D.M., Han, J., Youngquist, J.T., Maravelias, C.T., Pfleger, B.F., Dumesic, J.A., 2014. Nonenzymatic sugar production from biomass using biomass-derived gamma-valerolactone. Science 343 (6168), 277–280.
Pang, B., Sun, Z., Wang, L., Chen, W.-J., Sun, Q., Cao, X.-F., Shen, X.-J., Xiao, L., Yan, J.-L., Deuss, P.J., Yuan, T.-Q., Sun, R.-C., 2021. Improved value and carbon footprint by complete utilization of corncob lignocellulose. Chem. Eng. J. 419, 129565.
Qing, Q., Yang, B., Wyman, C.E., 2010. Xylooligomers are strong inhibitors of cellulose hydrolysis by enzymes. Bioresour. Technol. 101 (24), 9624–9630.
Selig, M., Weiss, N., Ji, Y., 2008. Enzymatic Saccharification of Lignocellulosic Biomass. National Renewable Energy Laboratory. NREL/TP-510-42629.
Shen, X.J., Wang, B., Huang, P.L., Wen, J.L., Sun, R.C., 2016. Effects of aluminum chloride-catalyzed hydrothermal pretreatment on the structural characteristics of lignin and enzymatic hydrolysis. Bioresour. Technol. 206, 57–64.
Shevchenko, S.M., Chang, K., Robinson, J., Saddler, J.N., 2000. Optimization of monosaccharide recovery by post-hydrolysis of the water-soluble hemicellulose component after steam explosion of softwood chips. Bioresour. Technol. 72 (3), 207–211.
Shuai, L., Yang, Q., Zhu, J.Y., Lu, F.C., Weimer, P.J., Ralph, J., Pan, X.J., 2010. Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. Bioresour. Technol. 101 (9), 3106–3114.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008. Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples. National Renewable Energy Laboratory. NREL/TP-510-42623.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2011. Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory. NREL/TP-510-42618.
Sun, S., Huang, Y., Sun, R., Tu, M., 2016. The strong association of condensed phenolic moieties in isolated lignins with their inhibition of enzymatic hydrolysis. Green Chem. 18 (15), 4276–4286.
Tjeerdsma, B.F., Militz, H., 2005. Chemical changes in hydrothermal treated wood: FTIR analysis of combined hydrothermal and dry heat-treated wood. Holz Als Roh- Und Werkstoff 63 (2), 102–111.
Wang, H.-M., Wang, B., Wen, J.-L., Yuan, T.-Q., Sun, R.-C., 2017. Structural characteristics of lignin macromolecules from different eucalyptus species. ACS Sustain. Chem. Eng. 5 (12), 11618–11627.
Wang, L., Zhang, R., Li, J., Guo, L., Yang, H., Ma, F., Yu, H., 2018. Comparative study of the fast pyrolysis behavior of ginkgo, poplar, and wheat straw lignin at different temperatures. Ind. Crop. Prod. 122, 465–472.
Yang, G., Jahan, M.S., Liu, H., Ni, Y., 2012. Acid hydrolysis of prehydrolysis liquor produced from the kraft-based dissolving pulp production process. Ind. Eng. Chem. Res. 51 (43), 13902–13907.
Zhang, J., Bao, J., 2012. A modified method for calculating practical ethanol yield at high lignocellulosic solids content and high ethanol titer. Bioresour. Technol. 116, 74–79.
Zhang, J., Chu, D., Huang, J., Yu, Z., Dai, G., Bao, J., 2010. Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor. Biotechnol. Bioeng. 105 (4), 718–728.
Zhang, T., Kumar, R., Tsai, Y.D., Elander, R.T., Wyman, C.E., 2015. Xylose yields and relationship to combined severity for dilute acid post-hydrolysis of xylooligomers from hydrothermal pretreatment of corn stover. Green Chem. 17 (1), 394–403.
Zhang, J., Wang, X., Chu, D., He, Y., Bao, J., 2011. Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production. Bioresour. Technol. 102 (6), 4480–4488.
Zhou, P.-P., Meng, J., Bao, J., 2017. Fermentative production of high titer citric acid from corn stover feedstock after dry dilute acid pretreatment and biodetoxification. Bioresour. Technol. 224, 563–572.