Bioresource Technology 289 (2019) 121708

Contents lists available at ScienceDirect

Bioresource Technology

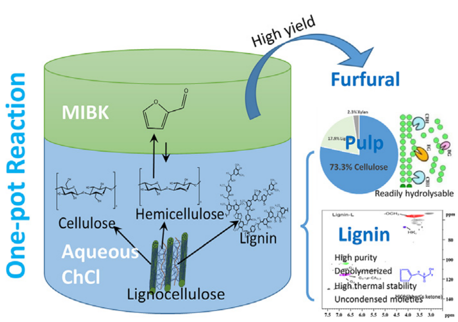
journal homepage: www.elsevier.com/locate/biortech

One-pot selective conversion of lignocellulosic biomass into furfural and co-products using aqueous choline chloride/methyl isobutyl ketone biphasic solvent system

Zhu Chen[a], Xianglan Bai[b], Lusi A[b], William A. Jacoby[a], Caixia Wan[a,*]

[a] Department of Biomedical, Biological, and Chemical Engineering, University of Missouri, 1406 East Rollins Street, Columbia, MO 65211, USA
[b] Department of Mechanical Engineering, Iowa State University, 2529 Union Drive, Ames, IA 50011, USA

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Choline chloride
Biphasic solvent system
Furfural
Lignin
Cellulose digestibility

ABSTRACT

This study investigated simultaneous lignocellulose fractionation and conversion in a one-pot reaction using an aqueous choline chloride/methyl isobutyl ketone (ChCl/MIBK) biphasic solvent system. Under the optimized condition (170 °C, 60 min, 0.6 wt% H_2SO_4, 10.7 wt% solid loading), the biphasic solvent solubilized 96% xylan in raw switchgrass, which was simultaneously converted to furfural with a yield of 84.04%. The biphasic solvent was also able to selectively extract lignin, which had a high purity (93.1%), and uncondensed moieties (i.e., Hibbert's ketone), as well as decreased molecular weight and polydispersity index. The resultant pulp was enriched with cellulose (73.3%), which can be completely hydrolyzed into glucose within 48 h via enzymatic hydrolysis. Aqueous ChCl was successfully recycled and reused for atleast three cycles with similar performance in switchgrass fractionation. This study demonstrated that aqueous ChCl/MIBK biphasic system was an effective solvent system for co-production of furfural, high quality technical lignin and digestible cellulose for further upgrading.

1. Introduction

Lignocellulosic biomass is a major category of renewable feedstock. Replacement of petroleum source with lignocellulosic biomass for the production of fuels and chemicals helps to address concerns over climate change and environmental pollution. In the past decade great research efforts have been devoted to the conversion of lignocellulosic biomass into various platform chemicals (Araji et al., 2017; Mika et al., 2017). Furfural is one of top value-added platform chemicals derived from biomass (Bozell and Petersen, 2010), and has a broad spectrum of

* Corresponding author.
E-mail address: wanca@missouri.edu (C. Wan).

https://doi.org/10.1016/j.biortech.2019.121708
Received 5 May 2019; Received in revised form 25 June 2019; Accepted 26 June 2019
Available online 27 June 2019

industrial application such as solvents, plastics, and pharmaceuticals (Cai et al., 2014; Zhang et al., 2013). Recently proved furfural-to-distillate fuel pathway opens a new avenue to its application, which would further drive furfural market growth (Bond et al., 2014).

Unlike most other biomass-based platform chemicals, there is no synthetic route available to produce furfural from petroleum source. Xylan-rich lignocellulosic biomass is almost the exclusive feedstock for furfural production (Peleteiro et al., 2016; Zhang et al., 2013). Furfural production involves xylan hydrolysis into xylose followed by xylose dehydration into furfural (Mamman et al., 2008). Current production technology primarily employs aqueous sulfuric acid-based reaction system integrated with simultaneous furfural removal by steam stripping. However, this process suffers from low furfural yields (about 50%), high sulfuric acid usage (about 20% of furfural output), and high energy consumption (20–50 tons of steam per ton of furfural) (Mittal et al., 2017). Another major issue is the generation of waste solid residues at a large quantity. It is estimated that about 23 million tons of solid residues are produced annually by China, the largest furfural producer (Chen et al., 2014; Mamman et al., 2008). Such solid residues are rich in cellulose and lignin, but have low valorization potential and thus commonly combusted as a low-grade solid fuel for power supply (Mamman et al., 2008). Post-treatment can make them more usable, but complicates the overall process and requires extra investments (Chen et al., 2014; Moghaddam et al., 2017; Yu et al., 2014). All the shortcomings with current production technology thus limit the sustainable growth of furfural industry.

From the perspective of sustainable biorefinery, current biorefinery operations face a major challenge of improving its cost competitiveness. Co-producing bioproducts with established and emerging markets from non-cellulosic streams (i.e., hemicellulose, lignin) offers the opportunity to overcome this challenge (Alonso et al., 2017). However, in a conventional biorefinery primarily targeting cellulose conversion, the valorization potential of hemicellulose and lignin is often sacrificed. For instance, dilute acid pretreatment leads to lignin condensation and xylose degradation while improving cellulose digestibility. Especially under severe conditions, uncontrolled xylose degradation during pretreatment can lead to the formation of undesired pseudolignin and humin (Cheng et al., 2018). Lignin with condensed structure and untailored properties is often not readily valorizable. Therefore, new pretreatment strategies should be developed to maximize the feedstock utilization for increased revenue of lignocellulosic biomass. One strategy is simultaneous biomass fractionation and xylan conversion in one pot, which would not only minimize xylose degradation into unwanted products, but also simplify the overall processes for valuable coproducts. Some recent studies reported such an integrated strategy, but only concerned digestible pulp and furfural production (Wang et al., 2018; Zhang et al., 2017). Although decent glucose and furfural yields can be obtained, there is much room to synchronize and improve both glucose and furfural yields. Extraction of valorizable lignin should also become an integral part of one-pot reaction system.

Biphasic solvent system comprised of an aqueous reactive phase and an organic extraction solvent phase has been investigated for high-yield furfural production from lignocellulosic biomass. Compared to monophasic system, biphasic system allows for rapid extraction of furfural into organic phase to avoid its degradation, leading to significantly improved furfural yields (Zhang et al., 2014; Zhang et al., 2017). Methyl isobutyl ketone (MIBK) is one of the most commonly used extraction solvents in a biphasic system as it has a high partition coefficient with furfural (Antonyraj et al., 2014; Mittal et al., 2017). In terms of aqueous reactive phase, the presence of halide salts and co-solvents have been shown to promote xylose transformation into furfural (Carrasquillo-Flores et al., 2013; Jiang et al., 2018; Motagamwala et al., 2016; Qing et al., 2017). Among various salts/co-solvents reported in literature, choline chloride (ChCl) is emerging for biomass processing, and has demonstrated impressive beneficial effects in acidic aqueous environment (Chen et al., 2018b; Jérôme and De Oliveira Vigier, 2017; Jiang et al., 2018). For instance, it played a pivotal role in converting concentrated pure xylose solution (as high as 50 wt%) to furfural with up to 75% yield using MIBK as an extraction phase (Jiang et al., 2018). Compared to sodium chloride, ChCl facilitates rapid xylose-to-furfural reaction due to the formation of reactive choline xyloside intermediate (Jiang et al., 2018). Our prior study also demonstrated for the first time that aqueous ChCl was an excellent solvent for biomass fractionation, leading to markedly improved cellulose digestibility and efficient extraction of lignin with reactive ether bond preserved to a great degree (Chen et al., 2018b). Furthermore, ChCl has shown good recyclability and reusability without noticeable degradation (Chen et al., 2018b; Jiang et al., 2018).

In this study, we explored for the first time aqueous ChCl/MIBK biphasic system for simultaneous switchgrass fractionation and furfural production. It aimed to address the aforementioned major challenges with current cellulosic biofuel and furfural production processes. Specially, highly digestible pulp and furfural as well as high-quality lignin were yielded through the proposed one-pot reaction. Reaction condition and biphasic solvent system were optimized. Cellulose-rich pulp was analyzed for its major component distribution and also evaluated for its digestibility. Lignin was characterized via a series of analytical techniques in terms of detailed chemistry, molecular weight distribution, and thermal stability and volatility, as well as aromatic monomer production via fast pyrolysis. Moreover, the recyclability and reusability of aqueous ChCl were investigated.

2. Materials and methods

2.1. Materials

Switchgrass was collected from the Bradford Farm at the University of Missouri in Columbia, Missouri, USA. Raw switchgrass was air-dried, ground through 40-mesh screen, and stored in an airtight container. Hydrolytic enzymes (Cellic® CTec2) were kindly provided by Novozyme (Franklinton, NC, USA). All the chemicals were purchased from Fisher Scientific and used as received (Hampton, NH, USA).

2.2. One-pot reaction

Furfural production was conducted by adding 0.5 g switchgrass and 5 g aqueous ChCl (70 wt% ChCl) into a glass pressure tube (Ace Glass # 8648-27, Ace glass, Inc., Vuneland, NJ), unless otherwise stated. MIBK (5 mL) was added to form a biphasic reaction system. The pressure tube was then placed in a preheated oil bath for a predetermined time. Different reaction conditions were tested, including pretreatment temperature (150, 160, 170 °C), acid concentration (0.4, 0.5, 0.6 wt%), solid loading (9.1, 10.7, 12.3 wt%), and reaction time (30, 45, 60 min). The slurry was continuously mixed with a magnetic stirring bar at 600 rpm throughout the reaction. Upon completion of reaction, the pressure tube was taken out of the oil bath immediately and cooled down to room temperature. Thereafter, the upper layer (MIBK) was separated from the slurry by decanting it into a glass vial carefully. The bottom layer was reaction slurry to which 10 mL acetone: water (1:1, v/v) was added followed by vacuum filtration. The solid was collected, washed with 10 mL acetone: water (1:1, v/v) twice, and stored at −20 °C prior to further analysis. All the filtrate was combined and vacuum-evaporated to remove acetone, resulting in lignin precipitation. The precipitated lignin was collected by centrifugation at 12,000*g* for 5 min and washed by using deionized (DI) water 4 times. After drying in an oven at 45 °C for 12 h, lignin was then stored in an airtight glass vial prior to further use. It should be noted that the repeated centrifugation and washing steps here were to minimize impurities which would interfere with the succeeding characterization of lignin. For one-pot with solvent recycling and reuse, the liquid collected after lignin precipitation was condensed by evaporating excess water in a convection oven at 65 °C, and then added with 5 mL fresh MIBK and 0.2 wt% acid (offsetting consumed acid) for a new cycle. All the new one-pot reaction cycles were conducted under the optimized reaction condition.

2.3. Enzymatic hydrolysis

Untreated or one-pot treated switchgrass was added at 2 wt% solid loading to citrate buffer (50 mM, pH 5.5) in a glass vial. Cellic® CTec2 was loaded at 20 mg protein/g solid. After 48 h hydrolysis, the slurry was centrifuged at 17,000g for 2 min, and the supernatant was then collected for further analysis.

2.4. Analytical methods

Compositional analysis of untreated and pretreated switchgrass was conducted following two-stage acid hydrolysis as previously reported (Sluiter et al., 2008). In brief, dry samples were hydrolyzed with 72% sulfuric acid at 30 °C for 60 min followed by 4% sulfuric acid hydrolysis at 121 °C for 60 min. Acid insoluble lignin was determined gravimetrically, while acid soluble lignin was determined spectrophotometrically by measuring the filtrate absorbance at 320 nm. Sugar monomers were analyzed by high performance liquid chromatography (HPLC) Agilent 1200 series (Agilent Technologies, Inc., USA) equipped with a refractive index detector (RID) and a HPX-87P column (Bio-Rad, USA). The temperatures of RID and column were maintained at 35 and 80 °C, respectively, and the mobile phase was HPLC grade water, eluting at 0.6 mL/min. Furfural titer was determined using the same HPLC system equipped with a C18 column (ZOBRAX), and 20 vol% methanol as the mobile phase, eluting at 1 mL/min. The temperatures of column and RID detector were maintained at 30 and 35 °C, respectively.

Furfural yield was calculated as the molar ratio of furfural produced to xylose solubilized from raw switchgrass. Lignin yield was calculated as recovered lignin with respect to solubilized lignin in a single cycle, and cumulative lignin yield was calculated based on the cumulative pretreatment cycles. Glucose yield was calculated as the percentage of theoretical glucose yield of raw and pretreated switchgrass. All the analyses were conducted in at least duplicate, and the averaged values were reported.

2D HSQC NMR analysis of both recovered lignin and cellulolytic enzyme lignin (CEL) was conducted following the method described in our previous study (Chen et al., 2018a). CEL has a structure close to native lignin and is used as a comparison. To prepare CEL, extractive-free switchgrass was ball-milled and subjected to enzymatic hydrolysis for 48 h. The solid residues after enzymatic hydrolysis were collected and extracted via mild acidolysis using dioxane:water (96:4, v/v) mixture containing 0.04 N HCl for 24 h. The extract was collected by centrifugation and neutralized with sodium bicarbonate. Then, it was added dropwise to cold DI water, resulting in the precipitation of crude lignin. The crude lignin was collected and further purified by dissolving in acetic acid:water mixture (90:10, v/v), followed by precipitation in cold DI water. The precipitated lignin was freeze-dried and then dissolved in 1,2-dichloroethane:ethanol mixture (2:1, v/v) followed by precipitation using diethyl ether. The precipitated lignin was collected and dried in a vacuum oven overnight to obtain CEL. NMR spectra were acquired on a Bruker AVIII 800 MHz spectrometer using Bruker supplied pulse sequence named hsqcetgp at 50 °C throughout the NMR acquisition. The spectral width was 15.4 ppm for ^{1}H and 170.0 ppm for ^{13}C. A total of 2048 (^{1}H) complex points were acquired with 256 (^{13}C) time increments. The $^{1}J_{CH}$ was set to 145 Hz which is the average one-bond C–H coupling constant. The number of scans was 48 and the repetition delay was 1.5 s. The ^{13}C dimension was zero-filled to 2048 points before the data was subjected to a sine-squared window function (shifted 90°) and Fourier transformation. The chemical shift axes were calibrated with respect to the solvent signals (2.49 ppm for residual proton and 39.5 ppm for ^{13}C).

Fourier transform infrared (FTIR) analysis was conducted using a Thermo Scientific Nicolet iS10 (Thermo Fisher Scientific Inc., Waltham, MA) equipped with a Smart iTR accessory. The wave numbers ranged from 750 to 4000 cm^{-1}, and each sample was scanned 32 times at a resolution of 4 cm^{-1} and an interval of 1 cm^{-1}.

Molecular weight distribution of lignin was measured using Dionex Ultimate 3000 series HPLC equipped with a Shodex Refrative Index detector and Diode Array Detector. Two Agilent PLgel 3 µm 100 Å 300 × 7.5 mm (p/n PL1110-6320) were connected in series, and tetrahydrofuran (THF) with a flow rate of 1 mL/min at 25 °C was the eluent. Polystyrene was used as calibration standard and its molecular weight ranged from 162 to 45120 g/mol. The lignin molecules were detected using ultraviolet wavelength of 254 nm. Prior to the GPC analysis, lignin was acetylated using a previously described method in order to increase its solubility in THF (Zhou et al., 2016).

Thermal stability of lignin and its inorganic impurity content were measured using Mettler Toledo thermogravimetric analysis system (TGA/DSC 1 STAR^{e} system). First, lignin was pyrolyzed using nitrogen to 105 °C at 10 °C/min and then held for 40 min. It was then continuously pyrolyzed up to 900 °C. Finally, air was introduced to combust the solid residue.

Fast pyrolysis of lignin was carried out using a Frontier Lab Tandem micropyrolyzer (Tx-3050 TR) as described in our prior study (Zhou et al., 2016). In brief, about 0.5 mg of lignin was pyrolyzed in a furnace maintained at 500 °C. The pyrolysis vapor leaving the furnace was carried into an online Agilent 7890B GC/MS-FID by helium gas. Helium was also used as the purge gas in the GC, and its flow rate was 156 mL/min and the split ratio at the GC front inlet was 50:1. The GC oven temperature was kept at 40 °C for 3 min, and then increased to 280 °C at a heating rate of 6 °C/min. The GC oven was further held at 280 °C for 4 min. Phenomenex ZB 1701 (60 m × 0.250 mm × 0.250 µm) columns were used in the FID and MS. The MS was used to identify vapor composition, and the product quantification was conducted based on FID signals using external calibration. Five different concentrations of each identified compound were pre-injected into the GC to create calibration curves. Lignin pyrolysis was triplicated, and the averaged values were reported.

3. Results and discussion

3.1. One-pot reaction for simultaneous biomass fractionation and furfural production

3.1.1. Furfural production

Different reaction conditions were studied for their effects on furfural production (Fig. 1a). We compared furfural titers and yields in MIBK since more furfural is partitioned into MIBK and furfural in MIBK is easier to be recovered than in aqueous phase. Following one-factor-at-a-time method for process optimization, we first tested temperature effect since it is a critical factor affecting furfural production. In general, high temperature favors furfural production (Zhang et al., 2013, 2017). When the temperature increased from 150 to 160 °C, furfural titer and yield in MIBK were doubled. Further increasing the temperature to 170 °C led to more furfural production but to a lesser degree. Acid also plays a crucial role in furfural production as acidic proton catalyzes the hydrolysis of hemicellulose into pentose and further dehydration into furfural. For the tested acid concentrations (0.4–0.6 wt%), furfural production increased with acid concentration, reaching 6.32 g/L titer and 38.62% yield in MIBK at the acid concentration of 0.6%. We also tested different solid loading in order to achieve a high solid loading. It was found that increasing solid loading from 9.1 to 10.7% had a minor effect on furfural yield, but led to a higher furfural titer due to a higher loading. Further increasing solid loading to 12.3% led to reduced furfural production in MIBK. This decrease could be largely attributed to inefficient mixing at high solid loading, which increased the probability of side reactions among furfural, xylose and the intermediates (Yemiş and Mazza, 2011). Based on

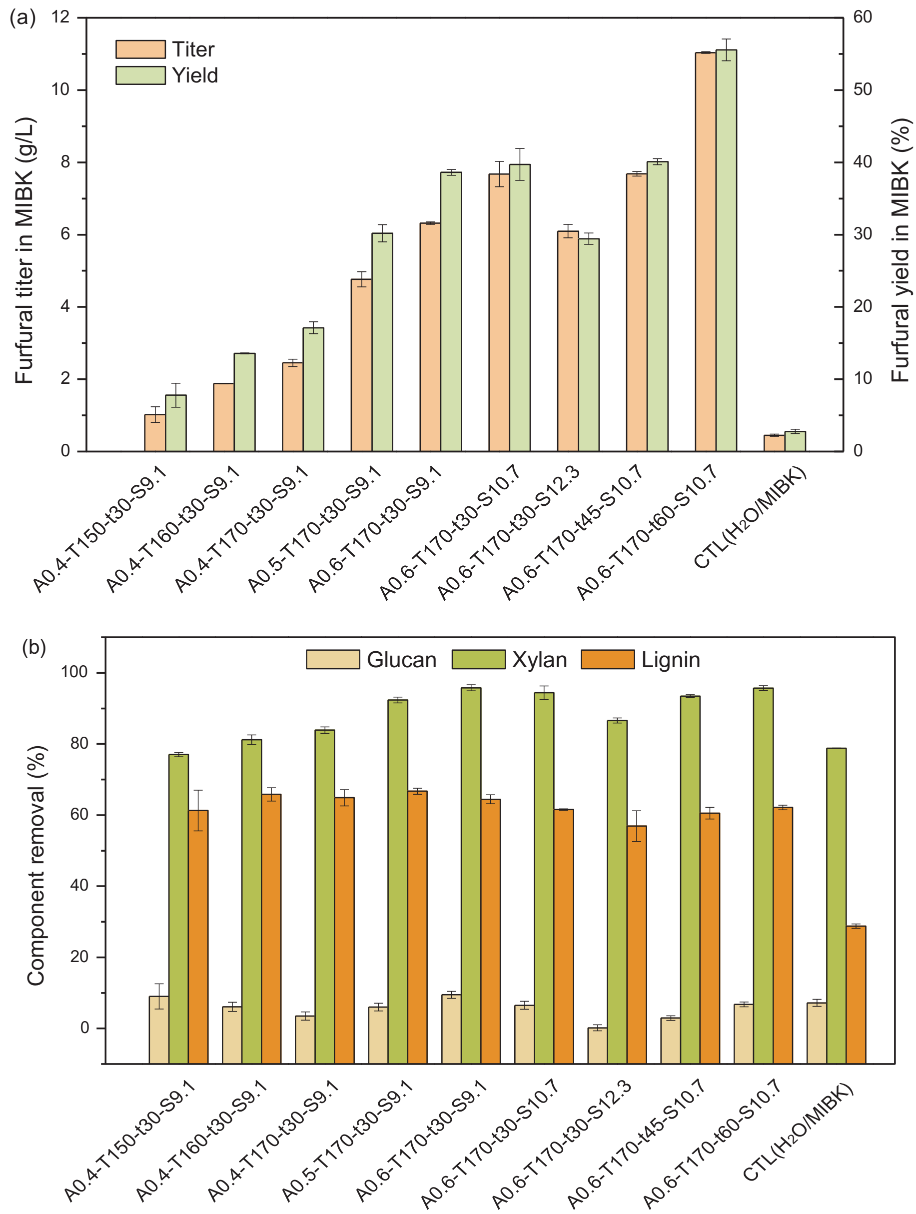


Fig. 1. Effects of one-pot reaction conditions in aqueous ChCl/MIBK on furfural production and biomass fractionation. (a) Furfural titer and yield in MIBK; and (b) Major component removal in response to different reaction conditions. A: acid loading (wt% H_2SO_4); T: temperature (°C); t: reaction time (min); S: solid loading (wt %). CTL: control using a H_2O/MIBK biphasic solvent system under the reaction condition of A0.6-T170-t60-S10.7.

the above optimized parameters we further tested different reaction times (i.e., 30, 45, 60 min), and found that 60 min led to 15% higher furfural yield in MIBK than 30 min. Among all the tested conditions, the reaction conducted at A0.6-T170-t60-S10.7 (i.e., 0.6% H_2SO_4, 170 °C, 10.7% solid loading, and 60 min) led to the highest furfural titer and yield in MIBK of 11.04 g/L and 55.57%, respectively.

3.1.2. *Compositional change of switchgrass*

To understand the lignin and cellulose fates under different reaction conditions, the compositional distribution of solid residue resulting from furfural production was examined. About 60–66% lignin was removed under all the tested conditions except the one with 12.3% solid loading (Fig. 1b). In contrast, xylan was more susceptible to condition variation. Increasing temperature from 150 to 170 °C led to 7% more xylan removal at the acid concentration of 0.4%. At 170 °C, increasing acid from 0.4% to 0.6% led to 12% more xylan removal. Up to 96% xylan was removed from raw switchgrass in one-pot reaction. Under all the tested conditions, more than 90% glucan was preserved in the solid residue. These results indicated that aqueous ChCl selectively removed most of xylan and a large fraction of lignin from switchgrass while preserving most cellulose in switchgrass. As a result, cellulose in the pretreated switchgrass was markedly enriched, reaching as high as 73.3%. Taking into consideration both furfural production and removal of non-cellulosic components, the condition of A0.6-T170-t60-S10.7 (i.e., 0.6% H_2SO_4, 170 °C, 10.7% solid loading, 60 min) was selected as the optimal condition for one-pot furfural production from switchgrass.

3.2. *Performance comparison of aqueous ChCl/MIBK and H_2O/MIBK*

As depicted in Fig. 2a, the H_2O/MIBK biphasic system (control) had a very limited capability of converting xylan to furfural compared to its counterpart aqueous ChCl/MIBK under the same condition (A0.6-T170-t60-S10.7). For aqueous ChCl/MIBK, an overall furfural yield of 84.04% was achieved, with a yield of 55.57% in MIBK and 28.47% in aqueous phase, corresponding to 11.04 and 5.66 g/L furfural titer, respectively (Fig. 2a). The appreciable amount of furfural retained in the aqueous

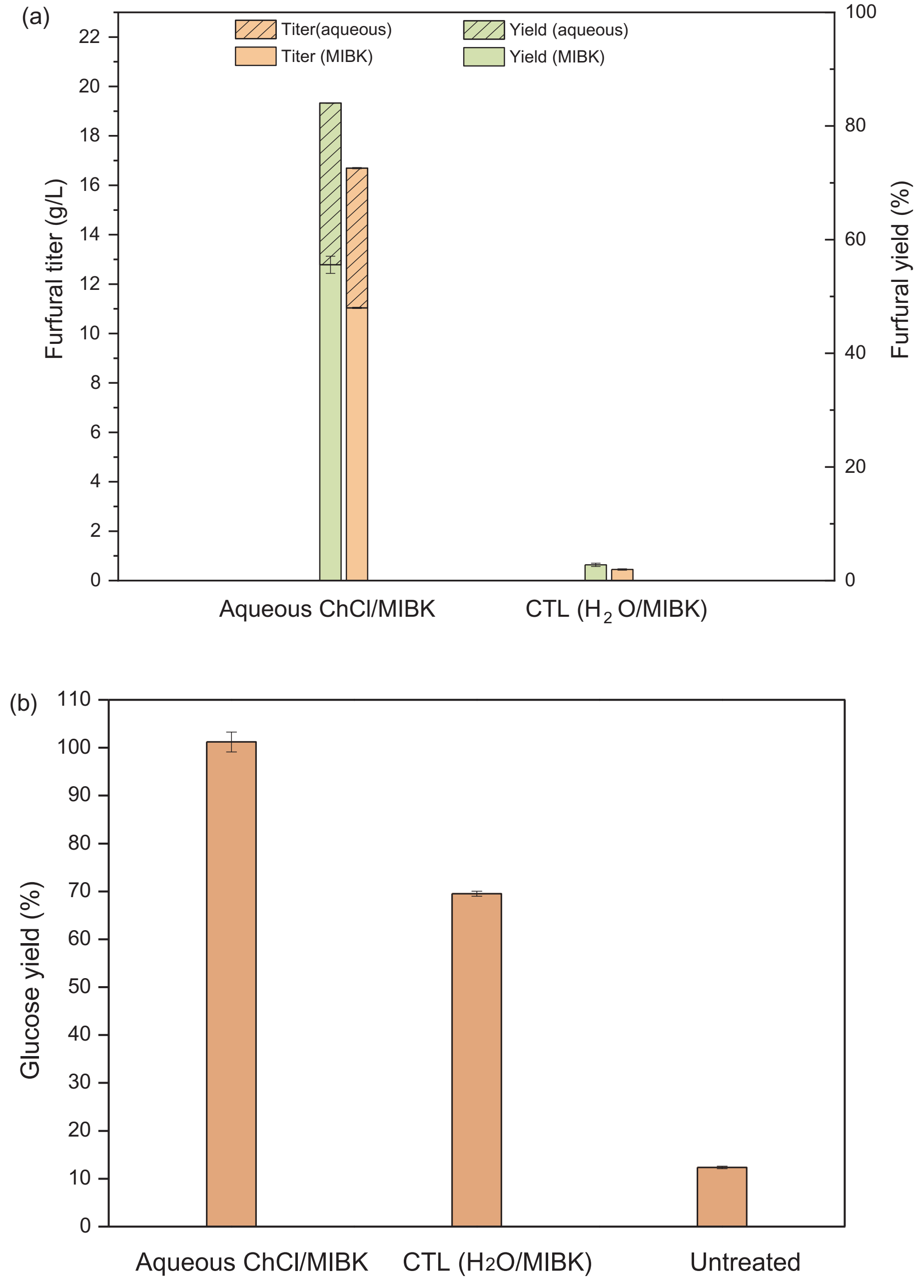


Fig. 2. Comparison of aqueous ChCl/MIBK and CTL (H_2O/MIBK) biphasic systems for one-pot reaction. (a) Furfural production; and (b) Glucose yield of the cellulose pulp. CTL: control using a H_2O/MIBK biphasic solvent system. The reaction condition for both biphasic systems: A0.6-T170-t60-S10.7.

phase could be a result of high viscosity of slurry, which limited the transfer of furfural from aqueous phase to organic phase when only magnetic stirring was used. For such viscous mixture, mechanical stirring may provide better mass transfer and will be explored in our further study. In contrast, with H_2O/MIBK biphasic system, only 0.45 g/L furfural was obtained in MIBK while negligible furfural was detected in aqueous phase (Fig. 2a). As a result, the overall furfural yield from H_2O/MIBK was only 3% (Fig. 2a). Compared to H_2O/MIBK, aqueous ChCl/MIBK removed 17% more xylan and 34% more lignin, while glucan removal was similar in both solvent systems (Fig. 1b). Overall, aqueous ChCl appeared to be a better solvent for xylan and lignin dissolution, and also accelerating the xylose dehydration into furfural. Fractionation of lignin-polysaccharide complex under acidic condition necessitates the cleavage of various bonds (e.g., ether bonds, glycosidic bonds), in which active acidic protons are involved. With water as a solvent, acidic protons can be solvated by water molecule under a mild acidic condition and thus become less reactive (Mellmer et al., 2014). In contrast, the presence of ChCl could help release the solvated protons, and make them more available to reactions. Similar solvent effects have also been observed with some organic solvent systems (e.g., γ-valerolactone, dioxane, THF) (Mellmer et al., 2014). In addition, ChCl can react with xylose to form a choline xyloside intermediate, which has higher reactivity than xylose toward furfural formation under acidic condition (Jiang et al., 2018). As a result, a higher furfural titer and yield can be obtained in aqueous ChCl/MIBK than H_2O/MIBK. Enzymatic hydrolysis of cellulose pulp revealed that the pulp resulting from aqueous ChCl/MIBK was completely digestible, which gave 30% higher glucose yield than that resulting from H_2O/MIBK (Fig. 2b). The much higher cellulose digestibility of the one-pot pulp could be attributed to its lower lignin and xylan contents, which rendered better access of cellulase to cellulose (Xu et al., 2016). Since the one-pot delignification just reached about 62%, it suggested that that residual lignin in the pulp

Table 1
Lignin purity and molecule weight distribution.

Lignin[a]	Purity (%)	Mn	Mw	PD[b]
CEL	90.2	1951	9545	4.89
Lignin-L	93.1	1294	4227	3.27

[a] CEL: cellulolytic enzyme lignin. Lignin-L: lignin recovered from one-pot reaction (1st cycle) under the optimized reaction condition (A0.6-T170-t60-S10.7).
[b] PD stands for polydispersity.

was not a limiting factor for sugar release. This finding is consistent with prior studies on ionic liquid pretreatment or alkaline pretreatment that reported that switchgrass with about 60% delignification gave over 95% glucose yields (Karp et al., 2015; Li et al., 2010).

Overall, the aqueous ChCl/MIBK outperformed H_2O/MIBK in terms of simultaneous furfural production and lignocellulose fractionation as well as resulting cellulose digestibility. Zhang et al. reported formic acid-catalyzed aqueous NaCl/MIBK biphasic system for furfural production with 82.0% yield from *Eucalyptus* (Zhang et al., 2017). However, the corresponding cellulose digestibility was only 58%, and the cellulose removal was nonselective as 43% cellulose was decomposed during the biomass fractionation by the same solvent system (Zhang et al., 2017). Using another biphasic system composed of ChCl:oxalic acid/MIBK with $AlCl_3$ as the catalyst, cellulose in *Eucalyptus* were better preserved and more digestible, but the furfural yield was decreased to 70.3% (Wang et al., 2018). Lignin fate and properties remained unclear for either solvent system. Compared with these biphasic systems, the proposed aqueous ChCl/MIBK system demonstrated even better performance in terms of furfural yield, and cellulose retention and digestibility. Moreover, as discussed in the latter section, lignin extracted by aqueous ChCl/MIBK had high purity and valorizable potential.

3.3. Recyclability of aqueous ChCl

Recycling solvent could potentially lower the cost associated with waste stream management and chemical consumption. Thus, the recyclability of aqueous ChCl was investigated using the optimized condition of A0.6-T170-t60-S10.7. The furfural yield and titer in MIBK decreased at the second cycle, reaching near 50% and 11 g/L. Further decrease in furfural production was observed with the 3rd cycle. Nevertheless, the furfural yield over 40% in MIBK was still achieved. The compositional analysis revealed that xylan removal was not affected by solvent recycling, while lignin removal gradually decreased over the increased cycles. Cumulative lignin yield gradually increased, reaching about 68%. Unrecovered lignin could react with sugar-derived compounds and form pseudo-lignin, which would in turn affect furfural production (Dussan et al., 2016). A gradual decrease in sugar yield of the resulting pulp was also observed with the recycling of aqueous ChCl, which could be due to the increased lignin content in the pulp. Impurities, such as unrecovered lignin and compounds from sugar degradation, can cause the decrease of performance (Liu et al., 2013). It is worth maximizing lignin recovery and conditioning recycled aqueous ChCl to remove interfering impurities in the future study. Purification of ChCl via ethanol extraction has been proposed to improve the performance of recycled ChCl (Liu et al., 2013). In addition, membrane-based filtration can be adopted for better lignin recovery and less energy consumption, and therefore, improved performance of recycled ChCl (Arkell et al., 2014).

3.4. Lignin characterization

After one-pot process, lignin solubilized in the pretreatment liquor, termed Lignin-L, can be recovered via precipitation. Since lignin upgrading routes largely depend on lignin properties (Kai et al., 2016; Linger et al., 2014; Xie et al., 2017), detailed physicochemical properties of lignin would inform lignin upgrading.

3.4.1. 2D HSQC NMR

2D HSQC NMR was used to understand chemical transformation of lignin. The main ^{13}C–^{1}H cross-signals in the HSQC spectra of switchgrass lignin were assigned according to previous studies (Samuel et al., 2010, 2011). The results indicated that lignin (Lignin-L) extracted by aqueous ChCl experienced a complete cleavage of β-O-4 linkage (A). Interestingly, a peak corresponding to Hibbert's ketone (HK) was clearly shown in Lignin-L. The presence of uncondensed moiety (i.e., HK) corroborated the cleavage of β-O-4 linkages. HK was also observed with lignin isolated by pretreatment solvents using ChCl:lactic acid and acidified lithium bromide trihydrate (Alvarez-Vasco et al., 2016; Li et al., 2018). Uncondensed or less condensed structure is desired for many applications, especially aromatic monomer production (Li et al., 2018). A prior study also suggested that lignin containing HK structure is a good source for novel aromatic monomer production when the HK structure is adjacent to a cleavable linkage (Miles-Barrett et al., 2016). In addition, there was no apparent signal in the carbohydrate anomeric regions (δ_C/δ_H 90–105/3.9–5.4) (Yuan et al., 2011), suggesting that Lignin-L was free of carbohydrate. Purity analysis further confirmed that this lignin had a purity of 93.1% with no carbohydrate detected (Table 1). Lignin with high purity may find its applications in many fields, such as antioxidant, resin synthesis, and polyurethane synthesis (Lauberts et al., 2017; Lora and Glasser, 2002). Minor amounts of β-5 and β-β linkages were detected in CEL but not in Lignin-L.

Syringyl (S), guaiacyl (G), and *p*-coumarate (*p*-CA) units were the dominant peaks across the aromatic regions in the lignin samples. However, the peaks for S and G units in Lignin-L showed a weak signal compared with CEL. This was likely due to the reactions between S/G units and reactive intermediates formed upon the cleavage of β-O-4 linkages in Lignin-L (Chen et al., 2017). Ferulate (FA) was detected in CEL, but not in Lignin-L. Lignin-polysaccharide interlinkages were formed via cross-linking of ferulic acid with lignin monomers/oligomers (Kim and Ralph, 2010). The absence of FA in Lignin-L suggested the cleavage of lignin-polysaccharide in one-pot reaction, which facilitated the solubilization of lignin and xylan into aqueous ChCl.

3.4.2. FTIR results

The structural and functional groups of lignin were further investigated based on FTIR results. In the FTIR spectra of CEL, a broad peak appearing at 3000–3600 cm^{-1} represents O—H stretching (both phenolic OH and aliphatic OH), and the peak at 2842–3000 cm^{-1} is for C—H stretch in methyl and methylene groups. The peak centered at 1700 cm^{-1} is for C═O stretch in carbonyls, and 1600 cm^{-1} is for aromatic skeletal vibrations plus C═O stretch. Also, the peak at 1500 cm^{-1} represents aromatic skeletal vibrations, the peak at 1230–1240 cm^{-1} is for C—O—C stretching for aryl-alkyl ether linkage, and 1166 cm^{-1} is for conjugated C═O in ester group. In addition, 1120 cm^{-1} stands for aromatic C—H in-plane deformation plus secondary alcohols, and 1030 cm^{-1} is for C—O deformation in primary alcohols and unconjugated C═O stretch.

The spectra of Lignin-L and CEL had many similarities. However, the peak of C—H stretch (2842–3000 cm^{-1}) was not as sharp as it was observed with CEL. The peak intensities at 1460 cm^{-1} (—CH_3 and —CH_2—) and 1430 cm^{-1} (C—H in-plane deformation plus aromatic skeletal vibration) also decreased, which suggested that the content of alkyl linkages decreased in Lignin-L. The peak at 1230–1240 cm^{-1} was easily observed, suggesting Lignin-L still contains a good amount of aryl-arkyl ether linkages. However, its peak intensity compared to the peak intensity for aromatic ring vibration (1500 cm^{-1}) was lower compared to that with CEL, due to cleavage of β-O-4 linkages. In addition to above, the peak at 1030 cm^{-1} (for primary alcohols) also decreased noticeably in Lignin-L. On the other hand, the peaks for O—H stretch and carbonyl C═O (1700 cm^{-1}) increased in Lignin-L compared

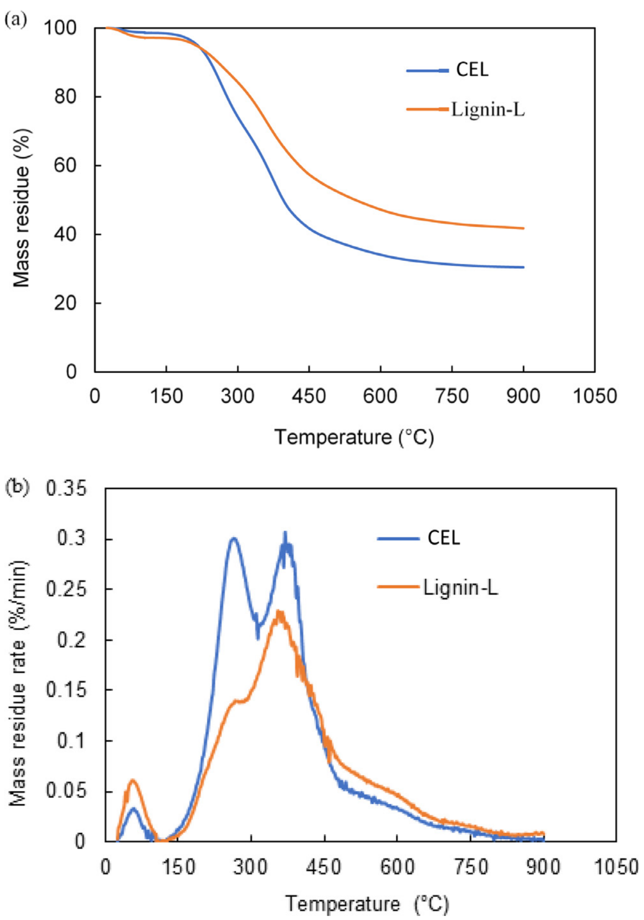


Fig. 3. TGA (a) and DTG (b) profiles of CEL and lignin-L. CEL: Cellulolytic enzyme lignin. Lignin-L: lignin recovered from one-pot reaction (1st cycle) under the optimized reaction condition (A0.6-T170-t60-S10.7).

to that in CEL, which is likely a result of lignin fragmentations and associated bond cleavages during the one-pot process.

3.4.3. GPC analysis

The average molecular weights and polydispersity (PD) of Lignin-L obtained from this study are compared with that of CEL (Table 1). The weight average molecular weights (Mw) of Lignin-L was 4227 g/mol compared to 9545 g/mol with CEL, confirming lignin fragmentation and bond cleavages during the switchgrass pretreatment.

3.4.4. TGA analysis results

TGA profiles were used to determine thermal stability and volatility of lignin (Fig. 3). Lignin-L was much more stable than CEL as it gradually decomposed with increasing temperatures. There was 41.7% solid residue when pyrolysis temperature was 900 °C, which is significantly higher than 30.4% with CEL. Higher thermal stability and low volatility of the lignin are usually associated with the types of lignin linkages that require different levels of dissociation energies. The complete cleavage of β-O-4 linkages during the one-pot aqueous ChCl extraction not only reduced Mw in Lignin-L, but also increased the relative content of thermally stable C—C and C—O linkages. Some lignin structures like phenylcoumaran, stilben and biphenol are thermally resistant even during pyrolysis (Bai et al., 2014), and thus they are unlikely destructed during the biomass pretreatment. Also, radical couplings of lignin fragments during the pretreatment could also newly form 4-O-5 and 5-5 linkages in the extracted lignin to increase its thermal stability. Although pyrolysis at high temperatures could promote side chain cracking in lignin, the remaining solid is more easily turned into polyaromatic structure. From the combustion of the solid residue at 900 °C, it appeared that Lignin-L was relatively free of inorganic impurities (0.78% ash).

Table 2
Yields of phenolic monomers from lignin via fast pyrolysis.

Compounds/yield (%)	CEL[a]	Lignin-L[a]
Phenol	0.19 ± 0.03	0.30 ± 0.01
Guaiacol	0.33 ± 0.03	0.39 ± 0.01
o-Cresol	0.06 ± 0.01	0.05 ± 0.00
p-Cresol	0.21 ± 0.04	0.29 ± 0.01
p-Methylguaiacol	0.40 ± 0.07	0.48 ± 0.01
4-Ethylphenol	0.05 ± 0.02	0.11 ± 0.01
4-Ethylguaiacol	0.33 ± 0.09	0.87 ± 0.05
4-Vinylphenol	5.03 ± 0.32	2.21 ± 0.13
4-Vinylguaiacol	5.26 ± 0.30	3.84 ± 0.05
Eugenol	0.03 ± 0.00	0.01 ± 0.00
Syringol	0.37 ± 0.02	0.27 ± 0.01
Isoeugenol	0.09 ± 0.02	0.04 ± 0.00
trans-Isoeugenol	0.25 ± 0.02	0.09 ± 0.00
1,2,4-Trimethoxybenzene	0.41 ± 0.06	0.35 ± 0.02
Vanillin	0.28 ± 0.03	0.06 ± 0.00
Apocynin	0.15 ± 0.01	0.09 ± 0.00
3′,5′-Dimethoxyacetophenone	0.34 ± 0.03	0.25 ± 0.01
2,6-Dimethoxy-4-allylphenol	0.56 ± 0.11	0.56 ± 0.03
Acetophenone, 4′-hydroxy-3′,5′-dimethoxy-	0.24 ± 0.12	0.06 ± 0.01
Total phenolic monomers	14.58 ± 1.31	10.12 ± 0.38

[a] CEL: cellulolytic enzyme lignin. Lignin-L: lignin recovered from one-pot reaction (1st cycle) under the optimized reaction condition (A0.6-T170-t60-S10.7).

3.4.5. Aromatic monomer production via pyrolysis

The yields of phenolic monomers produced from fast pyrolysis of lignin are given in Table 2. There were no variations on the types of phenolic monomers produced from Lignin-L and CEL. Two vinylphenols (4-vinylphenol and 2-methoxyl-4-vinylphenol) were dominant among the monomers, which are associated with coumarate and ferulate groups present in herbaceous biomass. The total monomer yield was lower with Lignin-L compared to CEL (10.12% vs. 14.58%), due to the cleavage of β-O-4 linkages and other structural modifications occurred during the aqueous ChCl extraction. Although incomparable with CEL, the total monomer yield produced from Lignin-L was similar to or higher than the yields obtained from the lignin extracted in our previous studies using acidified, aqueous ChCl:EG solutions at lower pretreatment temperatures (Chen et al., 2018a).

4. Conclusions

Aqueous ChCl/MIBK biphasic system showed effective biomass fractionation and furfural production in a single-step reaction. A maximum furfural yield of 84.04% was obtained. More than 90% cellulose was preserved in the cellulose pulp, which can be completely hydrolyzed into glucose. Lignin experienced partial depolymerization during one-pot process, evidenced by decreased Mw and Mn as well as PD. One-pot technical lignin also showed other attractive physicochemical properties for further upgrading, including high purity (93.1%), uncondensed Hibbert's ketone moieties, high thermal stability, and high aromatic monomer yield. This study demonstrated an efficient and waste-less biphasic solvent system for value-added lignocellulose conversion.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

This work is partially supported by the National Science Foundation

(Grant No. 1605034 and 1706046). The authors also thank Dr. Yuan Xue for his contribution to lignin characterization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.121708.

References

Alonso, D.M., Hakim, S.H., Zhou, S., Won, W., Hosseinaei, O., Tao, J., Garcia-Negron, V., Motagamwala, A.H., Mellmer, M.A., Huang, K., 2017. Increasing the revenue from lignocellulosic biomass: Maximizing feedstock utilization. Sci. Adv. 3, e1603301.
Alvarez-Vasco, C., Ma, R., Quintero, M., Guo, M., Geleynse, S., Ramasamy, K.K., Wolcott, M., Zhang, X., 2016. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization. Green Chem. 18, 5133–5141.
Antonyraj, C.A., Kim, B., Kim, Y., Shin, S., Lee, K.-Y., Kim, I., Cho, J.K., 2014. Heterogeneous selective oxidation of 5-hydroxymethyl-2-furfural (HMF) into 2,5-diformylfuran catalyzed by vanadium supported activated carbon in MIBK, extracting solvent for HMF. Catal. Commun. 57, 64–68.
Araji, N., Madjinza, D.D., Chatel, G., Moores, A., Jerome, F., De Oliveira Vigier, K., 2017. Synthesis of maleic and fumaric acids from furfural in the presence of betaine hydrochloride and hydrogen peroxide. Green Chem. 19, 98–101.
Arkell, A., Olsson, J., Wallberg, O., 2014. Process performance in lignin separation from softwood black liquor by membrane filtration. Chem. Eng. Res. Des. 92, 1792–1800.
Bai, X., Kim, K.H., Brown, R.C., Dalluge, E., Hutchinson, C., Lee, Y.J., Dalluge, D., 2014. Formation of phenolic oligomers during fast pyrolysis of lignin. Fuel 128, 170–179.
Bond, J.Q., Upadhye, A.A., Olcay, H., Tompsett, G.A., Jae, J., Xing, R., Alonso, D.M., Wang, D., Zhang, T., Kumar, R., Foster, A., Sen, S.M., Maravelias, C.T., Malina, R., Barrett, S.R.H., Lobo, R., Wyman, C.E., Dumesic, J.A., Huber, G.W., 2014. Production of renewable jet fuel range alkanes and commodity chemicals from integrated catalytic processing of biomass. Energy Environ. Sci. 7, 1500–1523.
Bozell, J.J., Petersen, G.R., 2010. Technology development for the production of biobased products from biorefinery carbohydrates—the US Department of Energy's "Top 10" revisited. Green Chem. 12, 539–554.
Cai, C.M., Zhang, T., Kumar, R., Wyman, C.E., 2014. Integrated furfural production as a renewable fuel and chemical platform from lignocellulosic biomass. J. Chem. Technol. Biotechnol. 89, 2–10.
Carrasquillo-Flores, R., Kaeldstroem, M., Schueth, F., Dumesic, J.A., Rinaldi, R., 2013. Mechanocatalytic depolymerization of dry (ligno)cellulose as an entry process for high-yield production of furfurals. ACS Catal. 3, 993–997.
Chen, C.-Z., Li, M.-F., Wu, Y.-Y., Sun, R.-C., 2014. Integration of ambient formic acid process and alkaline hydrogen peroxide post-treatment of furfural residue to enhance enzymatic hydrolysis. Ind. Eng. Chem. Res. 53, 12935–12942.
Chen, L., Dou, J., Ma, Q., Li, N., Wu, R., Bian, H., Yelle, D.J., Vuorinen, T., Fu, S., Pan, X., 2017. Rapid and near-complete dissolution of wood lignin at ≤80 °C by a recyclable acid hydrotrope. Sci. Adv. 3, e1701735.
Chen, Z., Bai, X., Lusi, A., Wan, C., 2018a. High-solid lignocellulose processing enabled by natural deep eutectic solvent for lignin extraction and industrially relevant production of renewable chemicals. ACS Sustainable Chem. Eng. 6, 12205–12216.
Chen, Z., Reznicek, W.D., Wan, C., 2018b. Aqueous choline chloride: a novel solvent for switchgrass fractionation and subsequent hemicellulose conversion into furfural. ACS Sustainable Chem. Eng. 6, 6910–6919.
Cheng, B., Wang, X., Lin, Q., Zhang, X., Meng, L., Sun, R.-C., Xin, F., Ren, J., 2018. New understandings of the relationship and initial formation mechanism for pseudo-lignin, humins, and acid-induced hydrothermal carbon. J. Agric. Food Chem. 66, 11981–11989.
Dussan, K., Girisuta, B., Lopes, M., Leahy, J.J., Hayes, M.H., 2016. Effects of soluble lignin on the formic acid-catalyzed formation of furfural: A case study for the upgrading of hemicellulose. ChemSusChem 9, 492–504.
Jérôme, F., De Oliveira Vigier, K., 2017. Catalytic conversion of carbohydrates to furanic derivatives in the presence of choline chloride. Catalysts 7, 218.
Jiang, S., Verrier, C., Ahmar, M., Lai, J., Ma, C., Muller, E., Queneau, Y., Pera-Titus, M., Jérôme, F., De Oliveira Vigier, K., 2018. Unveiling the role of choline chloride in furfural synthesis from highly concentrated feeds of xylose. Green Chem. 20, 5104–5110.
Kai, D., Tan, M.J., Chee, P.L., Chua, Y.K., Yap, Y.L., Loh, X.J., 2016. Towards lignin-based functional materials in a sustainable world. Green Chem. 18, 1175–1200.
Karp, E.M., Resch, M.G., Donohoe, B.S., Ciesielski, P.N., O'Brien, M.H., Nill, J.E., Mittal, A., Biddy, M.J., Beckham, G.T., 2015. Alkaline pretreatment of switchgrass. ACS Sustainable Chem. Eng. 3, 1479–1491.
Kim, H., Ralph, J., 2010. Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6/pyridine-d5. Org. Biomol. Chem. 8, 576–591.
Lauberts, M., Sevastyanova, O., Ponomarenko, J., Dizhbite, T., Dobele, G., Volperts, A., Lauberte, L., Telysheva, G., 2017. Fractionation of technical lignin with ionic liquids as a method for improving purity and antioxidant activity. Ind. Crops Prod. 95, 512–520.
Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A., Singh, S., 2010. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresour. Technol. 101, 4900–4906.
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, 4224–4235.
Linger, J.G., Vardon, D.R., Guarnieri, M.T., Karp, E.M., Hunsinger, G.B., Franden, M.A., Johnson, C.W., Chupka, G., Strathmann, T.J., Pienkos, P.T., 2014. Lignin valorization through integrated biological funneling and chemical catalysis. Proc. Natl. Acad. Sci. U.S.A. 111, 12013–12018.
Liu, F., Audemar, M., Vigier, K.D.O., Cartigny, D., Clacens, J.-M., Gomes, M.F.C., Pádua, A.A., De Campo, F., Jérôme, F., 2013. Selectivity enhancement in the aqueous acid-catalyzed conversion of glucose to 5-hydroxymethylfurfural induced by choline chloride. Green Chem. 15, 3205–3213.
Lora, J.H., Glasser, W.G., 2002. Recent industrial applications of lignin: a sustainable alternative to nonrenewable materials. J. Polym. Environ. 10, 39–48.
Mamman, A.S., Lee, J.M., Kim, Y.C., Hwang, I.T., Park, N.J., Hwang, Y.K., Chang, J.S., Hwang, J.S., 2008. Furfural: Hemicellulose/xylosederived biochemical. Biofuels. Bioprod. Biorefin. 2, 438–454.
Mellmer, M.A., Sener, C., Gallo, J.M.R., Luterbacher, J.S., Alonso, D.M., Dumesic, J.A., 2014. Solvent effects in acid-catalyzed biomass conversion reactions. Angew. Chem. Int. Ed. 53, 11872–11875.
Mika, L.S.T., Cséfalvay, E., Németh, A.R., 2017. Catalytic conversion of carbohydrates to initial platform chemicals: chemistry and sustainability. Chem. Rev. 118, 505–613.
Miles-Barrett, D.M., Neal, A.R., Hand, C., Montgomery, J.R.D., Panovic, I., Ojo, O.S., Lancefield, C.S., Cordes, D.B., Slawin, A.M.Z., Lebl, T., Westwood, N.J., 2016. The synthesis and analysis of lignin-bound Hibbert ketone structures in technical lignins. Org. Biomol. Chem. 14, 10023–10030.
Mittal, A., Black, S.K., Vinzant, T.B., O'Brien, M., Tucker, M.P., Johnson, D.K., 2017. Production of furfural from process-relevant biomass-derived pentoses in a biphasic reaction system. ACS Sustainable Chem. Eng. 5, 5694–5701.
Moghaddam, L., Rencoret, J., Maliger, V.R., Rackemann, D.W., Harrison, M.D., Gutierrez, A., del Río, J.C., Doherty, W.O., 2017. Structural characteristics of bagasse furfural residue and its lignin component. An NMR, Py-GC/MS, and FTIR study. ACS Sustainable Chem. Eng. 5, 4846–4855.
Motagamwala, A.H., Won, W., Maravelias, C.T., Dumesic, J.A., 2016. An engineered solvent system for sugar production from lignocellulosic biomass using biomass derived [gamma]-valerolactone. Green Chem. 18, 5756–5763.
Peleteiro, S., Rivas, S., Alonso, J.L., Santos, V., Parajó, J.C., 2016. Furfural production using ionic liquids: a review. Bioresour. Technol. 202, 181–191.
Qing, Q., Guo, Q., Zhou, L., Wan, Y., Xu, Y., Ji, H., Gao, X., Zhang, Y., 2017. Catalytic conversion of corncob and corncob pretreatment hydrolysate to furfural in a biphasic system with addition of sodium chloride. Bioresour. Technol. 226, 247–254.
Samuel, R., Pu, Y., Raman, B., Ragauskas, A.J., 2010. Structural characterization and comparison of switchgrass ball-milled lignin before and after dilute acid pretreatment. Appl. Biochem. Biotechnol. 162, 62–74.
Samuel, R., Foston, M., Jiang, N., Allison, L., Ragauskas, A.J., 2011. Structural changes in switchgrass lignin and hemicelluloses during pretreatments by NMR analysis. Polym. Degrad. Stab. 96, 2002–2009.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. National Renewable Energy Laboratory, Technical Report NREL/TP-510-42618.
Wang, Z.-K., Shen, X.-J., Chen, J.-J., Jiang, Y.-Q., Hu, Z.-Y., Wang, X., Liu, L., 2018. Lignocellulose fractionation into furfural and glucose by $AlCl_3$-catalyzed DES/MIBK biphasic pretreatment. Int. J. Biol. Macromol. 117, 721–726.
Xie, S., Sun, Q., Pu, Y., Lin, F., Sun, S., Wang, X., Ragauskas, A.J., Yuan, J.S., 2017. Advanced chemical design for efficient lignin bioconversion. ACS Sustainable Chem. Eng. 5, 2215–2223.
Xu, G.-C., Ding, J.-C., Han, R.-Z., Dong, J.-J., Ni, Y., 2016. Enhancing cellulose accessibility of corn stover by deep eutectic solvent pretreatment for butanol fermentation. Bioresour. Technol. 203, 364–369.
Yemiş, O., Mazza, G., 2011. Acid-catalyzed conversion of xylose, xylan and straw into furfural by microwave-assisted reaction. Bioresour. Technol. 102, 7371–7378.
Yu, H., Xing, Y., Lei, F., Liu, Z., Liu, Z., Jiang, J., 2014. Improvement of the enzymatic hydrolysis of furfural residues by pretreatment with combined green liquor and ethanol organosolv. Bioresour. Technol. 167, 46–52.
Yuan, T.-Q., Sun, S.-N., Xu, F., Sun, R.-C., 2011. Characterization of lignin structures and lignin–carbohydrate complex (LCC) linkages by quantitative 13C and 2D HSQC NMR spectroscopy. J. Agric. Food. Chem. 59, 10604–10614.
Zhang, L., Yu, H., Wang, P., Dong, H., Peng, X., 2013. Conversion of xylan, d-xylose and lignocellulosic biomass into furfural using $AlCl_3$ as catalyst in ionic liquid. Bioresour. Technol. 130, 110–116.
Zhang, L.-X., Yu, H., Yu, H.-B., Chen, Z., Yang, L.J.C.C.L., 2014. Conversion of xylose and xylan into furfural in biorenewable choline chloride–oxalic acid deep eutectic solvent with the addition of metal chloride. Chin. Chem. Lett. 25, 1132–1136.
Zhang, X., Bai, Y., Cao, X., Sun, R., 2017. Pretreatment of Eucalyptus in biphasic system for furfural production and accelerated enzymatic hydrolysis. Bioresour. Technol. 238, 1–6.
Zhou, S., Xue, Y., Sharma, A., Bai, X., 2016. Lignin valorization through thermochemical conversion: comparison of hardwood, softwood and herbaceous lignin. ACS Sustainable Chem. Eng. 4, 6608–6617.