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Pyridinium protic ionic liquids: Effective solvents for delignification of wheat straw

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ABSTRACT

Lignocellulosic biomass covers a vast area of the globe and contains many valuable components that can be envisaged for numerous renewable products. The valorization of these components is hindered by its cumbersome isolation due to the recalcitrant nature of lignocellulosic biomass. Acidic ionic liquids (AILs) have emerged in this field as effective pretreatment solvents. The unique property of disrupting lignin-cellulose-hemicellulose bonds and dissolving lignin makes these ILs significant for the separation of lignin and cellulosic constituents. In this study three protic ionic liquids based on pyridinium cation and hydrogen sulfate anion (HSO_4^-) have been synthesized; [PyH][HSO_4] (IL_1), [PyH][HSO_4. (H_2SO_4)] (IL_2), and [PyH][HSO_4 . (H_2SO_4)$_3$] (IL_3) and utilized for the isolation of lignin from wheat straw. The synthesized ILs were characterized by NMR and FTIR analyses. The lignin yield was optimized with respect to reaction temperature, time, biomass loading, and type of IL. The lignin removal efficacy of the ionic liquid with highest ratio of acid [PyH][HSO_4.(H_2SO_4)$_3$] (IL_3) is indicated by high delignification (79%) and lignin recovery (77%) under mild conditions (60 °C, 2 h). The cellulose rich material (CRM) and isolated lignin were characterized by FTIR, SEM, TGA, GPC, and HSQC NMR to assess their structure, molecular weight and stability. The cellulosic part was further hydrolyzed enzymatically to evaluate the capability of the recovered pulp to generate glucose. An appreciable yield of reducing sugars (85%) substantiates high lignin removal.

1. Introduction

Lignocellulosic biomass is a widely investigated material mainly for the sake of the generation of energy and partially for many useful and renewable chemicals having numerous applications in industry and daily life [1]. The remarkable products which can be obtained from economical biomass sources are bioethanol, bio-gas, bio-hydrogen, hydroxymethylfurfural (HMF), levulinic acid (LA), and lignin-based products. The fascination of lignocellulosic biomass materials lies in their ubiquitous and economical availability in nature and huge stocks from waste materials such as crop residues and municipal waste. Lignocellulosic biomass is largely composed of three components known as cellulose, lignin, and hemicellulose in varying amounts [2]. The composition is strictly dependent on the type, region, and season of growth of specific lignocellulosic biomass source. While cellulose and hemicellulose are mostly considered for their usefulness as a source of energy and other materials of industrial and daily life importance; lignin is also very significant. Lignin can be used as a low-value heating fuel, binder and dispersant [3], also it may become a generous source of value-added compounds, such as adhesives, polyurethane, polyesters, biofilms and biologically active polyphenols (aromatics) [4–9]. Lignin contains a variety of aromatic compounds that are currently obtained from fossil fuels and it, therefore, may be anticipated as the most competitive candidate for this purpose [4] owing to large deposits of lignocellulosic materials in nature.

The utilization of this potential of lignin and ultimately lignocellulosic biomass is hindered by the adamant nature of biomass polymeric structure. Fractionating lignocellulosic biomass into its major fractions is therefore indispensable for developing an economically viable biorefinery process. Particularly, an effective and low-cost method for extraction of lignin from biomass is.

essential for facile access to carbohydrate fraction and the generation of significant products from lignin [4]. Previously the most frequent commercial method for lignin extraction from biomass has been Kraft

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method (for paper and pulp). However, this process involves high temperatures and environmentally hazardous chemicals that need to be tackled to develop eco-friendly strategies.

Ionic liquids are green chemicals having amazing features with solvating ability being the most prominent. The solvent and catalytic abilities of ILs in the field of biomass processing are gaining growing attention. The isolation of lignin from biomass is accomplished using different ionic liquids; the most common among which are protic ionic liquids (PILs) [10–14]. Protic ILs are the ionic liquids that possess acidic functionality either in cation or anion (or both) [15]. Significance of PILs lies in their facile synthesis; relatively mild biomass processing and the valorization of biomass they also afford through efficacious removal of lignin. The lignin obtained from pretreatment with PILs undergoes very little change in its structure, thus further processing for bio-refinery products from lignin is rendered simple and efficient. Achhinivu et al. [13] utilized protic ILs for the extraction of lignin from corn stover. In this study Pyrrolidinum acetate ([Pyrr][Ac]) exhibited good lignin extraction (75%) at 90 °C for a pretreatment time of 24 h. Recently Gschwend and coworkers [16] used ultra-low cost Brosted acidic ionic liquid for highly efficient lignin removal (80%). In addition to high lignin removal, the ionic liquid was recovered proficiently (>99%) and reused successfully. Hu et al. [17] succeeded in high lignin removal from Coir and Poplar biomass using [BMIM] [HSO_4] in aqueous alcohols. As a result of pretreatment with this system, CRM (cellulose rich material) containing very low lignin content was obtained (0.95%). The lignin extraction was characterized with almost the same structure as that in milled wood from the above two biomass sources. Other examples where PILs have been employed for pretreatment and delignification may also be mentioned [18–22].

Here we describe the use of protic acidic ionic liquids (PAILs) based on pyridinium cation and clusters on HSO_4^- anion (HSO_4^-. $(H_2SO_4)_{x\text{-}1}$ where X = 1, 2, 4) for lignin extraction from wheat straw. The synthesized ionic liquids, after characterization with NMR and FT-IR, were used for the pretreatment of wheat straw to separate the lignin and cellulosic constituents. The study exhibits that anion-clusters based PILs act as promising solvents in extracting lignin from wheat straw. The lignin extraction has been optimized regarding temperature, time, and type of IL. To study the effect of pretreatment, the pulp and extracted lignin were characterized through compositional analysis (NREL protocol), FT-IR, SEM, GPC, HSQC, and TGA. Furthermore, the raw biomass and acquired delignified cellulose pulps were subjected to saccharification catalyzed by cellulase enzymes (Novozymes). The release of glucose from the saccharification of biomass samples was determined using the HPLC method employing the aminex HPX-87P column.

2. Experimental

2.1. Materials

The analytical grade chemicals i.e. Pyridine (Riedel-de Haën) and 72% sulfuric acid (Sigma) were purchased and used without further purification. Wheat straw biomass was purchased from the local market in Lahore Pakistan. All the solvents i.e. ethanol, methanol, acetone, etc. were distilled before use.

2.2. Synthesis of ILs

Ionic liquids containing clusters of hydrogen sulfate anion and molecular H_2SO_4 (HSO_4^- . (x-1)H_2SO_4) were synthesized following the method of synthesis of imidazolium ionic liquids already reported in the literature [23]. One mole of pyridine was reacted with a different number of moles (1, 2, and 4 mol) of 72% H_2SO_4 initially at low temperature (dropwise addition of the acid at 0–5 °C) and then at room temperature for one hour. The reaction was monitored by TLC using a solvent mixture of n-hexane and ethyl acetate (1:1). Very good yields (80–95%) were obtained from all the synthetic reactions using fixed amount of pyridine and varying number of moles of H_2SO_4. The reaction showing the synthesis of ILs is given in the following scheme (Scheme 1).

2.2.1. NMR data of PILs

The H NMR data of the synthesized ILs is given below. Notations H_1, H_2, H_3 and H_4 refer to Fig. 2.

^1H NMR (DMSO-D_6, 400 MHz): IL1: δ 8.1–8.3 (2H, m, H_4), 8.5–8.7 (1H, m, H_3), 8.8–8.9 (2H, m, H_2), 10.2 (5H, br, H_1); **IL2:** δ 8.01–8.1 (1H, m, H_4); 8.51–8.71 (2H, m, H_3); 8.87–8.89 (2H, m, H_2); 10.2 (9H, br, H_1) **IL3:** δ 6.98–7.99 (1H, m, H_4); 8.48–8.53; 8.7 (2H, m, H_3); 9.48 (20H, br, H_1)

2.2.2. Pretreatment

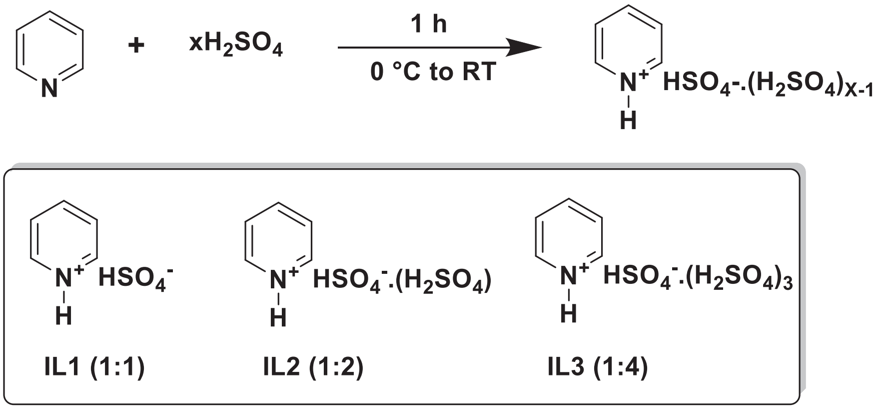
The biomass was crushed and ground and suitable particle size was obtained using mesh sieves. The subsequent method for pretreatment and separation of lignin is given in the supplementary information.

2.2.3. Compositional analysis

Compositional analysis of the untreated and pretreated pulp samples was carried out using the protocol established at NREL [24]. The detailed method is provided in the supplementary information.

2.3. Characterization

The acid to the base ratio in the synthesized ILs was precisely maintained using Mettler-Toledo Compact Titrator G20S. The FT-IR analyses of the ILs, untreated biomass, pretreated samples (CRM), and extracted



Scheme 1. Synthesis of ionic liquids with different mole ratios of H_2SO_4 and different synthesized ionic liquids with their designations

lignin were conducted using the Agilent Cary 630 FTIR instrument. The spectra were recorded within the range 4000–650 cm^{-}. The NMR spectra of the ionic liquids were recorded on Avance AV-400 MHz using DMSO-d_6 as a solvent. Thermogravimetric analysis (TGA) of the original biomass (untreated), pretreated biomass (using PILs), and lignin samples were carried out using Perkin Elmer TGA Pyris Diamond USA. The change in mass (%) during TGA analysis was observed within the temperature range 65–960 °C with a heating rate of 10 °C/min and the flow of nitrogen gas at 100 mL/min. HSQC of the lignin samples was done using Bruker 600 MHz spectrometer (pulse sequence hsqcetgpsi2, the spectral width of 10 ppm in F2 (^{1}H) having 2048 data points and for ^{13}C 160 ppm in F1 with 256 data points, 16 scans and 1 s interscan delay). For GPC measurements an Agilent 1260 Infinity instrument was used which had a Viscotek column set (AGuard, A6000M, and A3000M). For the detection of the signals, the Agilent 1260 Infinity RID detector was used. For sample preparation, 20 mg of lignin samples were dissolved in a mixture of GPC grade DMSO and an aqueous solution of LiBr (eluent, 1 g/L) and filtered through 0.2 μm syringe filter. The sample was run at a flow rate of 0.4 mL/min at 60 °C. Ten pullulan standards (Agilent calibration kit, $180 < Mp < 780{,}000$) were used for the calibration of the instrument. SEM images were obtained using a Scanning electron microscope (Model VEGA TESCON) with varying pressure. Gold sputtering was used and images with different magnification (200, 3000 x) were scanned.

3. Results and discussion

3.1. FT-IR analysis of ionic liquids

FT-IR spectra of the synthesized ionic liquids are given in Fig. 1. There are certain peaks in these spectra which correspond to the anionic part of ILs which is of real interest. Concentrated sulfuric acid bears both the molecular H_2SO_4 and anion HSO^- therefore, it might contain two types of frequencies [25]. The single bond S-O frequencies are found within the range 800–950 cm^{-1}, the double bond S=O stretches appear from 950 to1300 cm^{-1} whereas the S-OH bonds fall in the range 1300–1450 cm^{-1} [25,26]. In IL_1, the peak at 862 cm^{-1} corresponds to S=O vibration and at 1029 cm^{-1} belongs to S=O stretching in the corresponding HSO_4^-. The peaks at about 1150 cm^{-} exhibit various vibrations of SO_3 where the most intense vibration peak appears at 1143 cm^{-1} [25]. In the other two ILs (IL_2, IL_3) the peaks corresponding to S=O are featured with weak intensities and are shifted towards lower wavenumber which is a consequence of strong hydrogen bonds with H_2SO_4 molecules for the formation of clusters centered on the anion. The strong hydrogen bond takes the oxygen atom away from sulfur and thus wavenumber is reduced. As seen in the spectra of the latter two ILs (IL_2 and IL_3), there are peaks in the range 1370–1430 cm^{-1} which are weak in case of IL_1. These peaks are in fact due to hydrogen bond (O-H) formation between hydrogen atoms of H_2SO_4 molecules and oxygen atoms of HSO_4^- anion resulting in cluster formation as shown in Fig. 2. The peaks assigned to different bonds are listed in Table S1 in the supplementary information.

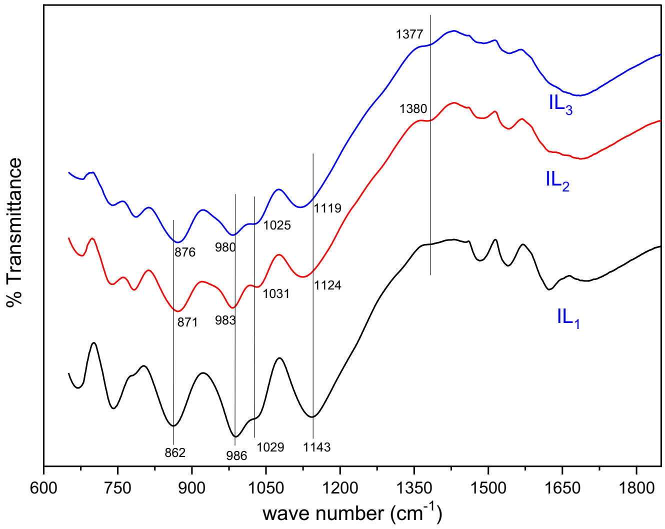


Fig. 1. Comparison of FT-IR spectra of the three ionic liquids

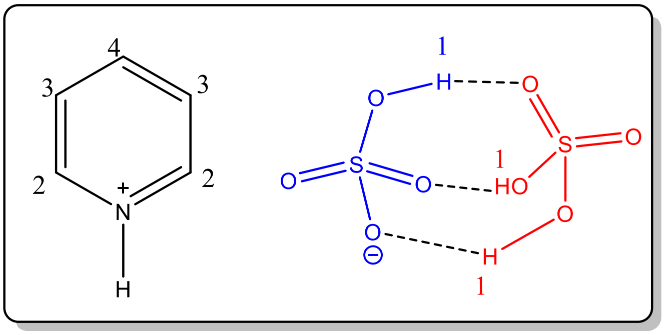


Fig. 2. Designation of different protons in the general structure of ionic liquids.

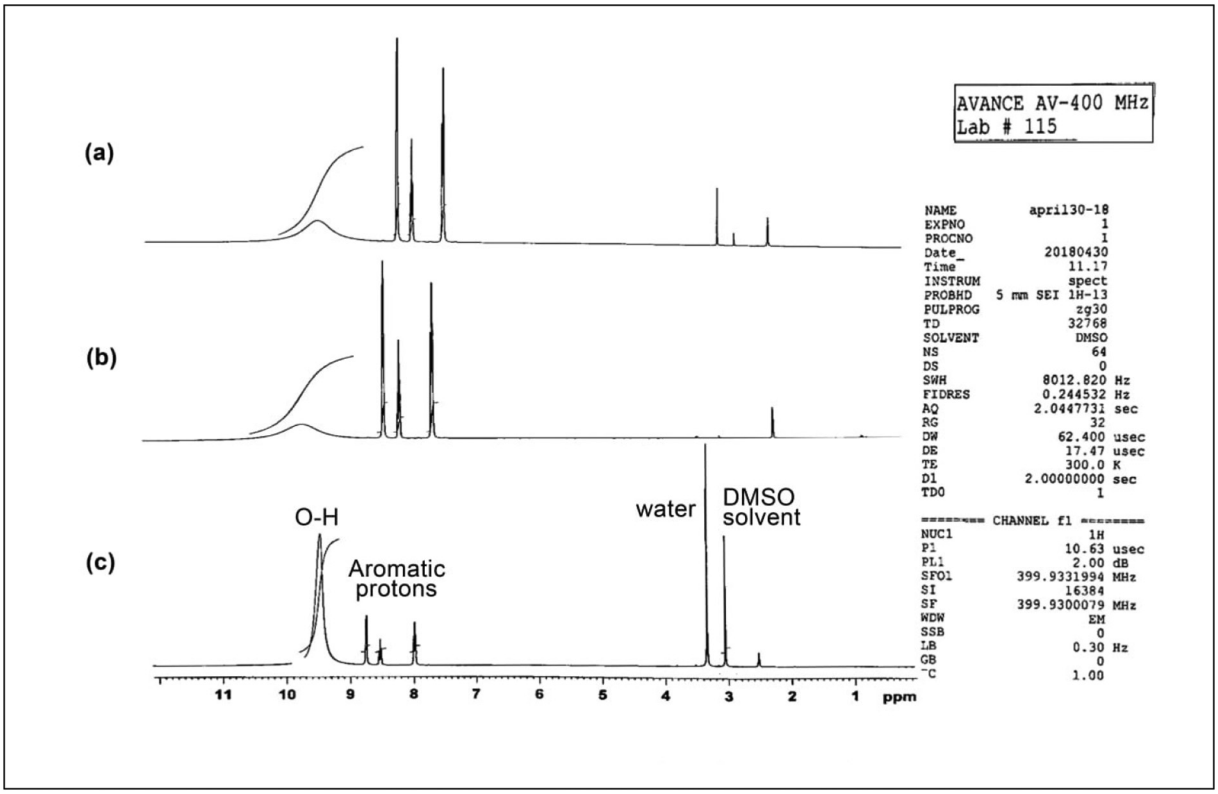
3.2. NMR analysis of ILS

Fig. 3 contains the comparison of different peaks in the ^{1}H NMR spectra of the ILs used in this study. For IL_1 (Fig. 3a), the most downfield signal (δ=10.22) corresponds to HSO_4^- proton and or nitrogen proton. The spectrum shows that the aromatic protons do not give a well-defined coupling pattern so these are being termed as multiplets. The multiplet centered at δ8.3 is assigned to two ring protons denoted as proton 4 in the pyridinium ring (Fig. 2). The signal for proton at position 3 (centered at 8.60) and 2 (at 8.06) each is a multiplet but the more downfield among these two is the one which is closer to ring nitrogen (at position 2 in Fig. 2).

For IL_2 (Fig. 3b), the peak for HSO_4^- has shifted slightly upfield (δ= 10.20) because of hydrogen bond formation of an additional mole of H_2SO_4 (1:2) with the anion HSO_4^-. The signal centered at 8.61 is a multiplet and it corresponds to the proton at position 4 whereas the peak at 8.078 is again a triplet and is assigned to protons at position 3. In the same line the NMR spectrum for IL_3 (Fig. 4c) exhibits peaks for protons at positions 2 (multiplet at δ = 8.723), 3 (multiplet at δ = 7.96) and 4 (multiplet δ= 8.50). The most interesting part of the spectrum for IL_3 is the peak at 9.48 which is assigned to protons around HSO_4^- anion. Its integration shows almost four times the number of protons of HSO_4^- anion in IL_1. It means that adding four mole equivalents of H_2SO_4 increases the number of hydrogen bonds around the anion. However, the δ value of this proton in IL_3 shows it to be far down filed compared to that in IL_2 (where it is 10.20) implying that further increase in a number of protons around HSO_4^- results in relatively weaker hydrogen bonds. This is in accordance the findings in the literature [23,27].

4. Compositional analysis and biomass characteristics

The composition of original(untreated) wheat straw and that of pretreated wheat straw samples was determined from the compositional analysis method following the NREL protocol [24]. It was found that the wheat straw contains 37.18% glucans, 23.65% xylans. The lignin is 21.86% as a sum of acid soluble lignin (ASL) and acid insoluble lignin (AIL). The whole composition of the raw wheat straw as determined from the compositional analysis is given in the following diagram (Fig. 4). The amounts of different components are shown in percent of the total.



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Date_           20180430
Time               11.17
INSTRUM            spect
PROBHD   5 mm SEI 1H-13
PULPROG             zg30
TD                 32768
SOLVENT             DMSO
NS                    64
DS                     0
SWH           8012.820 Hz
FIDRES        0.244532 Hz
AQ           2.0447731 sec
RG                    32
DW              62.400 usec
DE               17.47 usec
TE               300.0 K
D1          2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1               10.63 usec
PL1               2.00 dB
SFO1       399.9331994 MHz
SI                 16384
SF         399.9300079 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
¯C                  1.00
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Fig. 3. ^{1}H NMR spectra of different pyridinium ionic liquids a) IL_1, b) IL_2, and c) IL_3 (using DMSO-d_6 as a solvent)

The composition of the biomass samples pretreated under optimized conditions is provided in the following Table 1 which shows the amount of different components as a percentage of the total.

Different characteristics of the pretreated biomass and change in their amounts are depicted by the graphs in Fig. 5. All the quantities are expressed in percent *w*/w of the theoretical maximum (amount in untreated biomass). Percentage of pulp recovery was calculated from gravimetrically measured amounts of pulp compared to the initial amount of biomass. The percent of lignin was determined by weighing the extracted lignin and comparing it with the amount of lignin in the original biomass. The percentages of hemicellulose removal and delignification were calculated using the relative amounts of hemicellulose and lignin in untreated biomass and pretreated samples from the compositional analysis method. The detailed procedure of the two methods is described previously.

It is clear from the graph that with the increase in time of pretreatment the pulp recovery is lower as a consequence of delignification and removal of hemicellulose. The least pulp recovery was obtained in case of pretreatment with IL_3 which is obvious in low lignin content and hemicellulose component of the pretreated biomass as given in the above table (Table 2).

The removal of hemicellulose from the biomass as a result of pretreatment is more prominent compared to the delignification. In all the graphs it is obvious that hemicellulose content lowers with

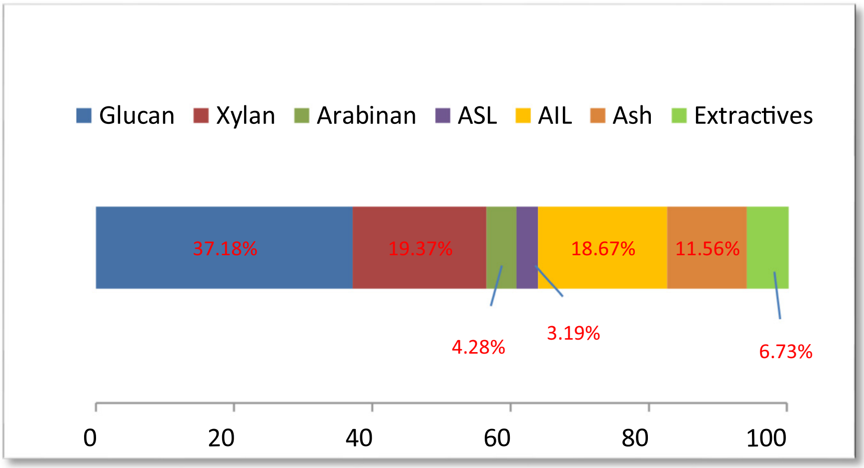

Fig. 4. Composition of raw wheat straw used in this study. ASL: acid soluble lignin, AIL: acid insoluble lignin.

Table 1
Composition of pretreated biomass samples determined by compositional analysis; for IL_1, IL_2 at 100 °C, and for IL_3 at 60 °C for 2 h.

Ionic Liquid	Glucan (%)	Xylan (%)	Arabinan (%)	ASL (%)	AIL (%)	Ash (%)	Mass Loss (%)
IL1	55.8	8.15	1.24	1.16	7.84	7.2	18.61
IL2	59.6	6.39	1.06	1.29	5.87	7.1	18.31
IL3	61.5	3.45	0.82	0.95	2.84	9.8	21.64

increasing time of processing. But the more prominent increase is seen after 1 h in all the cases. The recovery of hemicellulose is not possible from the solvent mixture, unlike lignin. Which may be due to the formation of other products from hemicellulose e.g. furfural [28].

In previous studies, those ILs are reported for the extraction of lignin which has higher hydrogen bond basicity [29,30]. This is indicated here as well when we compare our results with the ILs used in this study. With more H_2SO_4 content the ability of the IL to make hydrogen bonds with the biomass components is also enhanced; as a consequence, more delignified pulp having less hemicellulose content is obtained. The delignification is also more effective as the time of pretreatment progresses where the peak delignification in the case of IL_1 (59%) and IL_2 (69%) is achieved at 100 °C for 2 h and in case of IL_3 (79%) at 60 °C for 2 h. Thus ionic liquid IL_3 removes the lignin from wheat straw much more effectively as compared to the other two ILs. The efficiency of this IL owes to the clusters of the anion HSO_4^- and H_2SO_4 molecules which are capable of extensive hydrogen bonding with the constituents of biomass with; IL_1 and IL_2 at 100 °C for 2 h; IL_3 at 60 °C for 2 h

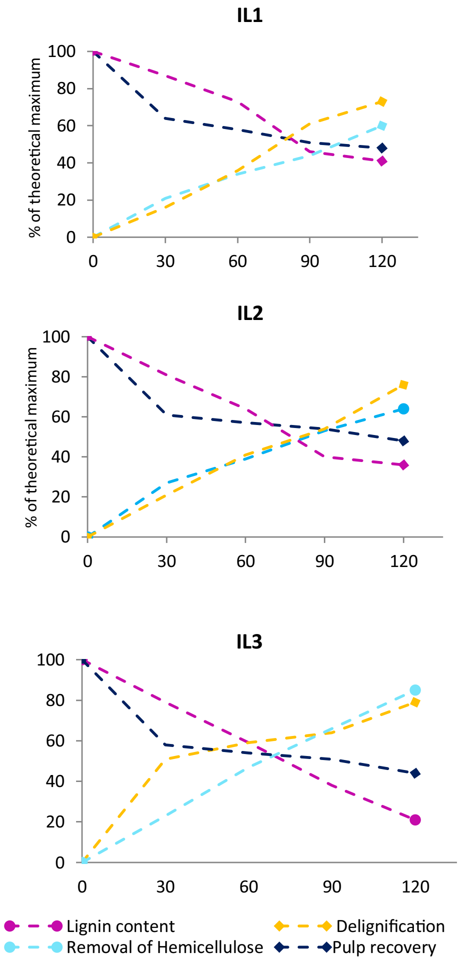

Fig. 5. Main indicators of processing of wheat straw after pretreatment.

Table 2
Molecular weight and polydispersity index of isolated lignin using IL_1, IL_2 at 100 °C and IL at 60 °C (2 h, 5% loading).

Ionic Liquid	M_n (Da)	M_w (Da)	PDI
IL_1	1688	8855	5.2
IL_2	1486	7900	5.3
IL_3	1414	7001	4.9

M_w: average molecular mass, M_n: number average molecular mass, PDI: polydispersity index.

5. Effect of factors on lignin extraction

The pretreatment of the wheat straw samples was carried out under different conditions of time, temperature, particle size, and type of ionic liquid. The percentage yields of lignin and cellulose rich material (CRM) have been calculated by comparing the amounts of isolated lignin and CRM after pretreatment with the amounts of these components present in the untreated biomass (theoretical maximum).

The percent yield of extracted lignin was calculated relative to the amount of lignin in the original biomass (theoretical maximum) using the following formula.

$$\boldsymbol{Y_l} = \frac{M_l}{\frac{M_o \times M_{ODW}}{100}} \times 100\%$$

where Y_l is the percent yield of lignin to be calculated, M_l is the amount of lignin extracted from the biomass sample, M_o is the percent of lignin in the original biomass and M_{ODW} is the oven dried amount of biomass sample initially taken.

A discussion of the effect of different pretreatment conditions is hereby elaborated under the following sub-sections.

5.1. Effect of temperature

As expected, the extent of pretreatment and the lignin extraction was enhanced with the increase in temperature. Out of the three types of ionic liquids, IL_1 ([Py] [HSO_4]) and IL_2 ([Py] [$HSO_4.H_2SO_4$] were used for the range 40–100 °C. The third ionic liquid IL_3 ([Py] [$HSO_4.3H_2SO_4$] was used at 40 °C, 50 °C, and 60 °C. The reason why this IL was employed at lower temperatures is blackening (charring) of the biomass at higher temperatures. As shown in Fig. 6 the maximum lignin recovery was achieved at 60 °C with IL_3 (77%) while lignin yield with IL_1 and IL_2 came to be 59% and 69% respectively. The observed amounts of extracted lignin at different temperatures are coherent with the results in different studies [12,13]. These studies exhibit that

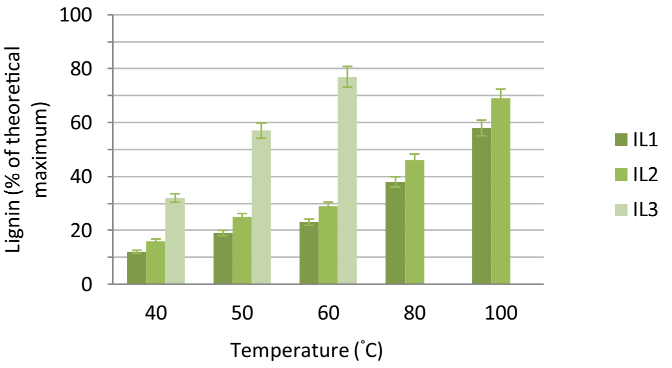

Fig. 6. Percentage of lignin extracted from biomass using ionic liquids at.

the amount of extracted lignin increases with elevating temperature. This owes to the higher vibrations of the bonds between lignin and celluloses [12]. The ionic liquid IL$_3$ extracted higher amounts of lignin even at low enough temperature (60 °C) which is due to corresponding higher acidity (more hydrogen bond basicity); a significant characteristic for appreciable lignin extraction. The following figure exhibits the amounts (%*w*/w of total lignin in the initial biomass) of lignin extracted by employing different ionic liquids Fig. 6 different temperatures (180–800 μm, 5%, 2 h).

5.2. Effect of time

Pretreatment of the wheat straw was carried out for various time durations (0.5 h to 2 h). The ionic liquids IL$_1$ and IL$_2$ took relatively more time to appreciably extract lignin; however, IL$_3$ appeared to be more effective. Fig. 7 shows the amounts of lignin extracted (% of theoretical maximum) using different ILs for varying time durations. Increasing the time of pretreatment improves the efficiency of the process. However, beyond a pretreatment time of 2 h the biomass blackening occurred and very low or no yield of lignin resulted. It may also be noted that the ionic liquid expected to have the highest acidity (IL$_3$) produces the best result (77%) in coherence with the reported literature [13,14]. In the previous studies [11,13,28] the pretreatment time for extraction was more extensive (up to 24 h) and the higher lignin yields were attained at relatively higher temperatures compared to the present study temperatures (5% biomass load,60 °C for IL$_{3,}$ and 100 °C for IL$_1$ and IL$_2$).

5.3. Effect of biomass loading

Previous studies, regarding pretreatment of biomass and lignin extraction, show that low biomass loading is more effective than higher biomass loading. This is because the contact of IL ions and the biomass particles increases when small weight is loaded [11,31]. In this case, too, the PILs gave the results accordingly in favor of low biomass loading. But in our study, the maximum yield is obtained using IL$_3$ at pretty mild conditions (60 °C, 2 h). This is attributed to the higher hydrogen bond-forming content of this IL. As exhibited by the graph in Fig. 8, the removal of lignin is more efficiently achieved in case of low loading of biomass. Here biomass loading of 5% (77%), gave the highest amount of lignin followed by 10% (64%), and the least lignin extraction was realized with 15% (53%) biomass loading. The low biomass loading allows the ions of IL and the biomass particles to interact more freely because of small sized particles [31]. Another probable reason might be the increased density of the reaction mixture in case of heavier biomass loading.

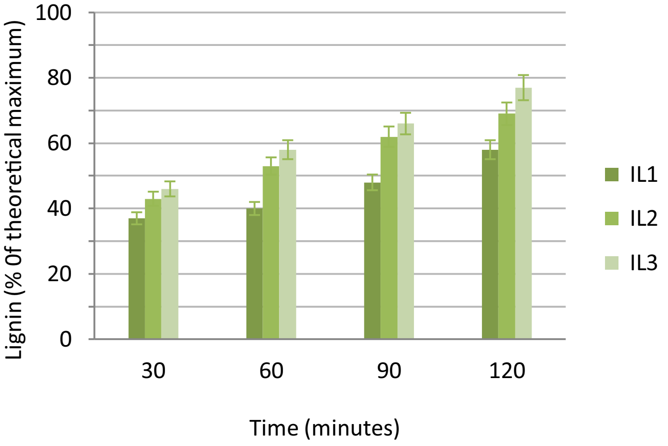

Fig. 7. Percentage of lignin extracted from biomass using ionic liquids at different.

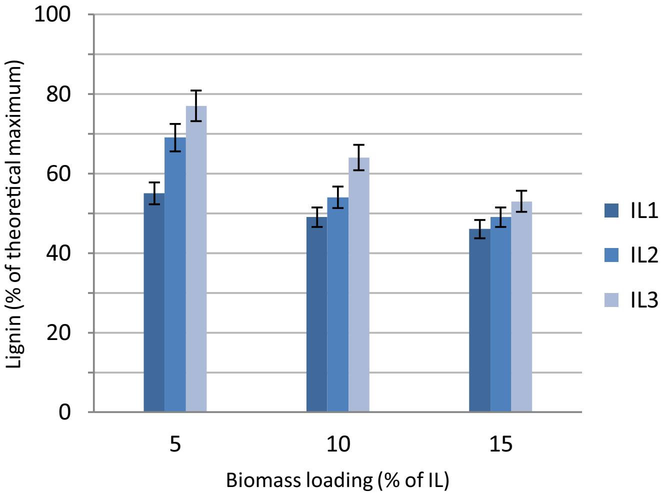

Fig. 8. Effect of biomass loading (%) on pretreatment and lignin extraction (60 °C, 2 h)

5.4. Process optimization

In this study, three ionic liquids, denoted by IL$_1$, IL$_{2,}$ and IL$_{3,}$ were used for the pretreatment of wheat straw. The ILs were produced by treating a fixed amount of pyridine (base) with different mole ratios of 72% sulfuric acid. Many parameters were tested as discussed above and the most efficient pretreatment was observed in the case of ionic liquid IL$_3$**.** A possible justification of the highest efficiency of this IL is probably its higher acidity (hydrogen bond basicity) which is expected to be higher compared to the other two ionic liquids [32]. The graph in Fig. 9 indicates that the maximum amount of lignin was isolated from pretreatment with IL$_3$. The optimum yield (77%) was obtained by pretreatment at 60 °C for 2 h. The optimized lignin yield with IL$_1$ and IL$_2$ was found to be 58% and 69% after 2 h at 100 °C. The previous findings reported in the literature show that ILs with higher acidities or more ability to make hydrogen bonds can provide better dissolution and hence effective isolation of lignin [13,14,33]. Here we see that increasing the number of mole equivalents of acid in ionic liquid improves the delignification of biomass and hence renders it more viable for further processing. Our delignification results reveal that pyridinium based PILs containing clusters (dimers and trimers) on anion are more efficient as compared to the monomeric ILs, formed by equal mole equivalents of acid and base, used previously of lignin extraction (Particle size:180-800 μm, biomass loading 5%, time: 2 h).

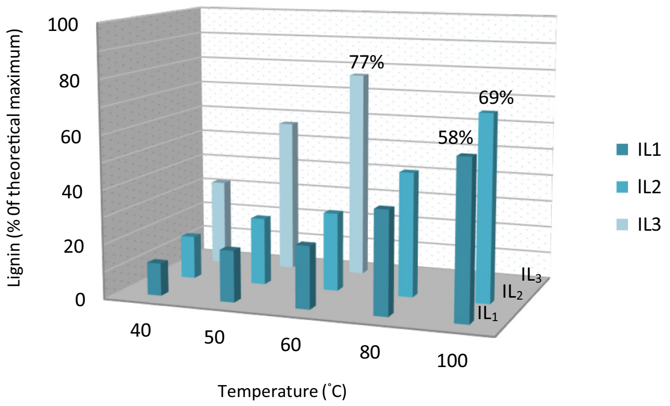

Fig. 9. Comparison of different ionic liquids used in this study for their efficiency.

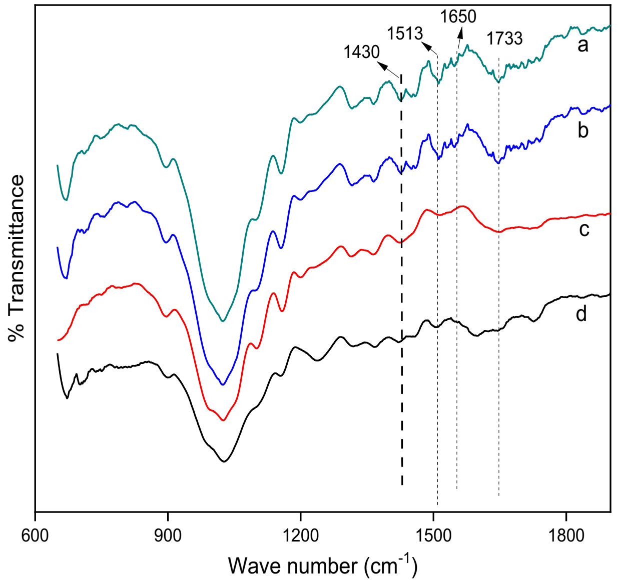

Fig. 10. FT-IR spectra of the biomass samples; a) untreated, b) IL_1 treated (100 °C, 2 h), c) IL_2.

6. Analysis of biomass

6.1. FT-IR analysis

The following diagram (Fig. 10) exhibits a comparative overlap of the FT-IR spectra of untreated biomass and IL-treated biomass samples. The peaks at 1430, 1371, 1318, 1162, 1119, 1025, and 897 cm^{-1} are associated with cellulose. The signal at 897 cm^{-1} is assigned to the glycosidic linkage (at C_1) and the peak at 1025 cm^{-} corresponds to the C-O-C stretching in glucopyranose ring [34]. This peak is obvious in all the samples but in the case of biomass sample pretreated using IL_3 (mole ratio 4:1) it is relatively weaker. This is ascribed to some loss of carbohydrates during pretreatment which may be possibly due to higher acidity of the PIL. The peak at about 1160 cm^{-} is attributed to C-O antisymmetric stretching. The CH_2 symmetric bending is represented by the peak at 1430 cm^{-} [35]. The signal intensity of this band has reduced which is an indication of less crystalline cellulose in the IL-pretreated samples of biomass [35]. The peak at 1319 cm^{-} is due to CH bending in carbohydrates and 1365 cm^{-} is for CH bending in cellulose. The peak at 1513 cm^{-} is assigned to the aromatic ring conjugated bonds which is a characteristic of lignin. This peak (1513 cm^{-}) has significantly reduced intensity verifying appreciable removal of lignin from the biomass after pretreatment. The peak at 1650 cm^{-} is also characteristic of phenolic OH bonds in lignin. This peak has also been rendered weak in the pretreated biomass. Out of all the ionic liquids used, the IL_3-pretreated sample exhibits the most pronounced reduction in peak intensity. This is in coherence with the yields of lignin as stated above treated (100 °C, 2 h) and d) IL_3 treated (60 °C, 2 h, 5%)

6.2. Thermo-gravimetric Analysis (TGA)

Thermogravimetric analysis was done to assess the stability variation caused by pretreatment. Thermographs of untreated and pretreated biomass is furnished in Fig. 11. It is evident that there are two plateaus for each curve; the initial plateau indicates a phase of moisture removal loosely bound on the surface after which a steep fall occurs as a result of a loss of mass from biomass due to degradation [36]. Finally, the curve straightens and goes horizontal up to the end; and in this phase, the graph corresponds to the charring of carbohydrates [37]. The difference in the stability of the untreated and treated biomass samples implies the effectiveness of pretreatment. Thermograph shows reduced stabilities of the biomass samples after treatment with different ILs. The biomass sample treated with IL_3 (d) is the least stable; this is an outcome of the most reduced crystallinity, which shows the efficacy of this IL in pretreatment and delignification of wheat straw. The overall sequence of stability of treated and untreated biomass samples is: untreated > IL_1 > IL_2 > IL_3.

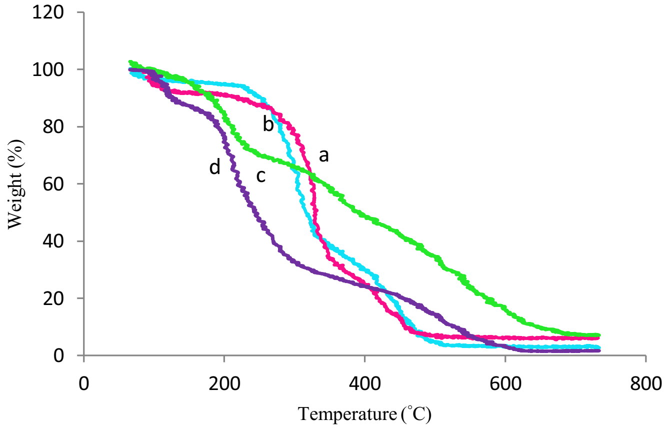

Fig. 11. Thermogravimetric analysis (TGA) of different biomass samples a) untreated, b) IL_1 treated (100 °C, 2 h), c) IL_2 treated (100 °C, 2 h) and d) IL_3 treated (60 °C, 2 h, 5%)

This is based on the shape and the onset temperatures of TGA curves. The onset temperature of the untreated biomass sample is 332 °C whereas, for the biomass samples treated by $IL_{1,}$ $IL_{2,}$ and IL_3, it is 310 °C, 220 °C, and 205 °C respectively. The given results can be justified based on the change in morphology of the biomass after pretreatment. The untreated biomass is more recalcitrant owing to the highly crystalline network of cellulose, hemicellulose, and lignin. After pretreatment, the appreciable removal of lignin makes the biomass more amorphous compared with the original (untreated). The removal of lignin occurs as different types of bonds; lignin-cellulose and lignin-hemicellulose (responsible for highly crystalline nature), are broken. Along with the removal of lignin, some amount of cellulose and hemicellulose is also lost resulting in a further reduction instability.

6.3. Scanning Electron Microscopy (SEM)

The original biomass and regenerated CRM were subjected to scanning electron microscopy (SEM) to verify the effect of pretreatment on the morphology of the polymeric network of biomass. Previous studies on the utilization of protic ILs in biomass valorization demonstrate that an increase in acidity disrupts the morphology [13]. This was expected in the case of our study also and it is clear from the SEM images (Fig. 12) that the PILs used in this study have affected the morphological arrangement of biomass. The SEM image of untreated biomass shows a flat structure that undergoes disruption when treated with the PILs. This accords with the results obtained in the previous studies [13,35]. The flakiest structure is obtained in the case of ionic liquid IL_3. This is in agreement with the percentage of extracted lignin (highest in case of IL_3) and FT-IR spectra of the biomass samples (100 °C, 2 h), c) IL_2 treated (100 °C, 2 h) and d) IL_3 treated (60 °C, 2 h, 5%)

7. Saccharification

To evaluate the effect of delignification and potential of recovered pulp from untreated and treated biomass enzymatic saccharification was performed at 50 °C and 250 rpm for 7 days. The amount of sugars was determined using the HPLC technique equipped with an aminex HPX-87P column. The graph (Fig. 13) exhibits the variation in quantity

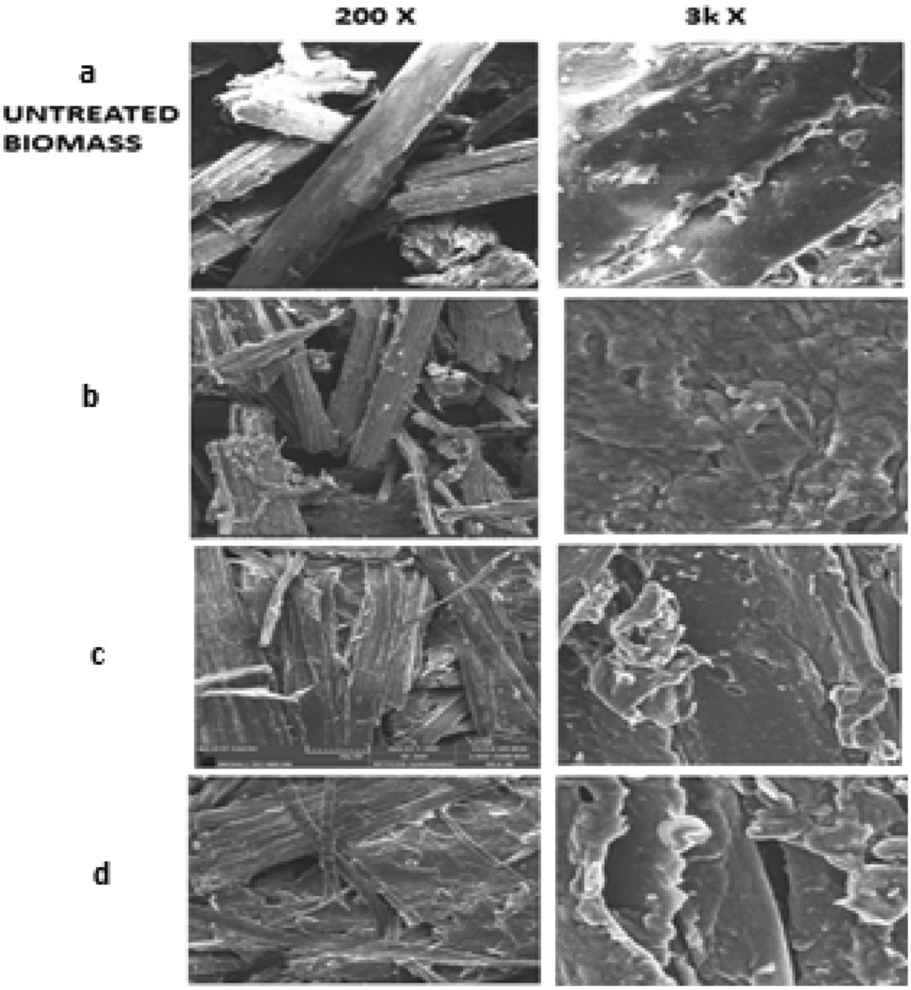

Fig. 12. SEM images of different biomass samples; a) untreated, b) IL_1 treated.

glucose from the untreated wheat straw and optimized pretreated samples after different time durations. The raw biomass sample was run without any ionic liquid-treatment and it yielded only 21% glucose of the theoretical maximum. It was found that the extent of yield was enhanced with increasing time of pretreatment at respective temperatures. It is apparent that the sample obtained after pretreatment with IL_1 gave the lowest yield of reducing sugars at all time-durations. The amount of TRS obtained in this study is appreciably comparable and even better than the yields in previous studies on PILs found in the literature [35,38]. The biomass sample treated by IL_2 provides the highest TRS value (84%) after pretreatment at 100 °C for 2 h. While under the same conditions, the yield of glucose from the IL_1 treated sample was 67%. This is in accordance with the previous studies where the increase in saccharification yield with increasing extent of delignification is reported [28,35,38]. However, for IL_3 highest delignification does not produce the highest amount of TRS. This may be attributed to the deformation of the cellulosic structure of pulp due to carbonization which in turn may be resulted from higher acidic content of the IL_3 from enzymatic saccharification after 7 days (50 °C, 250 rpm).

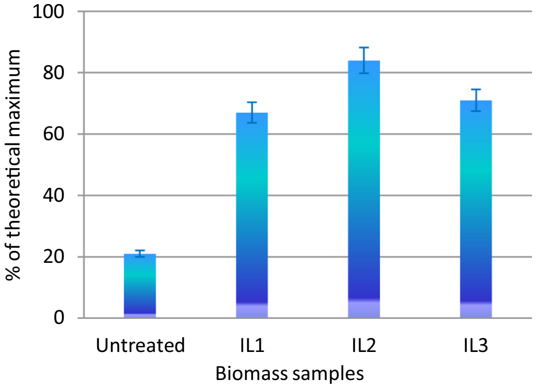

Fig. 13. Yield of Glucose of untreated and pretreated wheat straw samples.

8. Analysis of extracted lignin

8.1. HSQC NMR

Fig. 14 depicts structures of different fractions having relative positions marked as α, β, and γ. The wheat straw lignins obtained through different methods have slightly different ratios of various fractions. But a general inference is that it has a higher Guiacyl component than Syringyl fraction [39]. This is also true about the lignin extracted using IL_3 under optimum conditions in this study.

Fig. 15 shows the prominent peaks in HSQC NMR within the range δ1- δ9 (H NMR) and δ50 to δ130 (C NMR). The peaks of major components are labeled respectively. The Guiacyl (G) unit is the most abundant followed by Syringyl (S), Coniferyl (C), and other units, Tricin

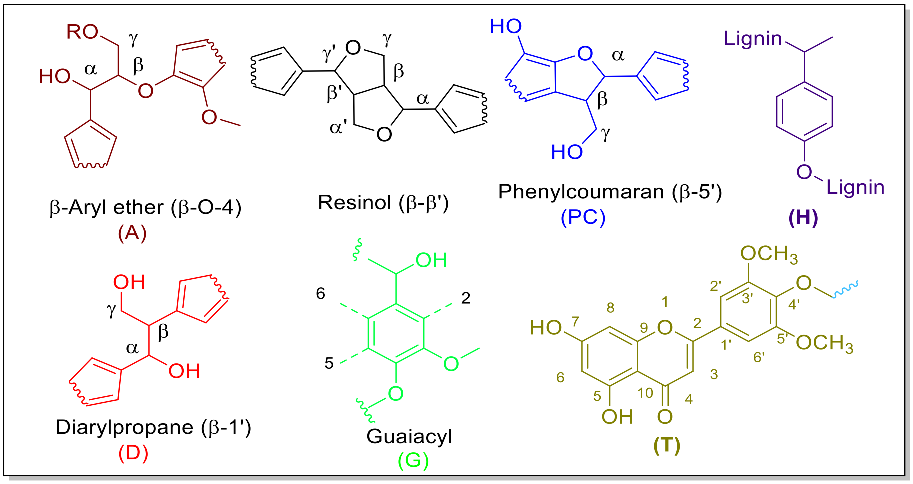

Fig. 14. Significant bonds and subunits of lignin.

(T) and other less common units. The β-O-4 linkage (A_β) has the highest percentage of all the ether linkages which is an indication that there is not much structural disruption in the structure of lignin recovered from wheat straw under mild conditions (60 °C, 2 h). The aromatic portion of the HSQC NMR spectrum (δ_C/δ_H: 90–150/6.0–7.7) is given in Fig. 16. The peaks assigned to different positions of Guiacyl fraction of lignin are shown. It is reported in the previous literature that peak for G_2 can be taken as the reference peak for other signals (G_2cond., G5 and G_6) [40]. This is because it mostly does not undergo any modification or condensation during the extraction. However, G_2 may be shifted to G_2 cond. (condensed form) due to modification in the G_5 and G_6 positions. From the areas of contours in the spectrum, it was found that the intensity of G_5 and G_6 is lower than G_2 and there is a prominent peak of G2 cond. (H NMR 7.0–7.9; and 110–114C NMR). Thus it shows a moderate condensation of lignin during the process. Some previous studies indicate more severe degradation and condensation of the lignin extracted using protic ILs [21,28]. In this case, we also found condensation of polymer which is obvious from the appearance of $G_{2\ \mathrm{cond.}}$ The peak in the spectrum, but the extent of condensation is lesser comparatively.

8.2. Gel Permeation Chromatography (GPC)

The Gel Permeation Chromatography was conducted for the lignin extracted from wheat straw by pretreatment using the three ILs. The GPC determined values of number average molecular weight (M_n), average molecular weight (M_w) and polydispersity of different lignin samples are given in Table.2. It is known previously that the organosolve lignin mostly bears higher molecular mass compared to ionosolve lignin [41,42]. Previous studies suggest that disruption of lignin side chain linkages is caused by IL anions specifically protic ILs [43]. In the present study, the molecular mass of the lignin decreases with the increasing ability of IL to make hydrogen bonds and thus disrupt the lignin linkages. We can see that the lignin from ionic liquid IL_1 has the highest molecular mass and that from IL_3 has the lowest while lignin from IL_2 being intermediate. The polydispersity index (PDI) of lignin also differs by varying the ionic liquid. IL_1 and IL_2 extracted lignin shows higher PDI compared to the lignin isolated using IL_3; thus showing more uniform structural features of lignin extracted with the first two ionic liquids [44]. Thus the above observations indicate that clustered ionic liquids extract

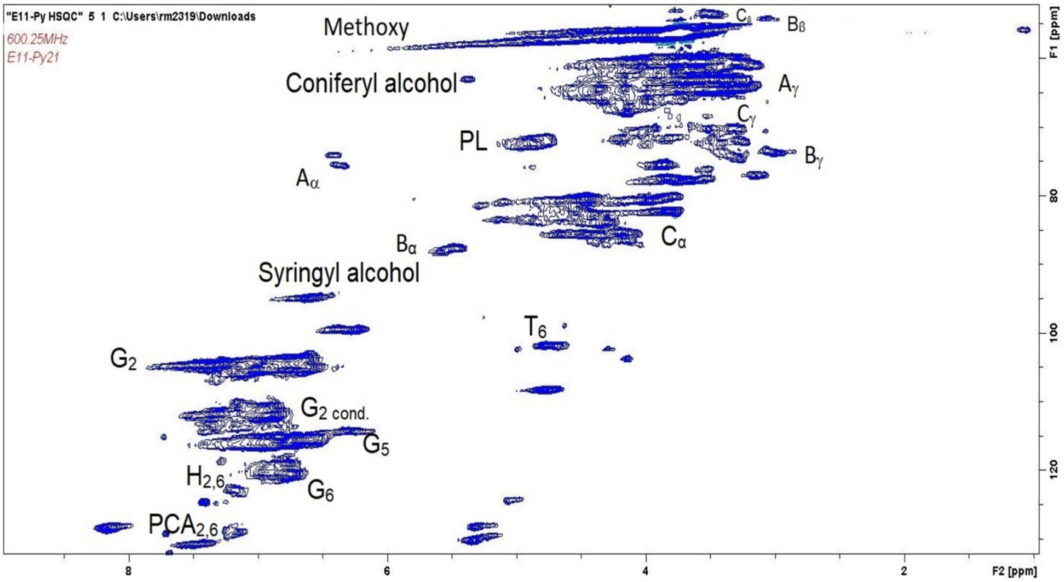

Fig. 15. HSQC NMR spectrum of the lignin extracted from wheat straw using IL_3 at 60 °C for 2 h.

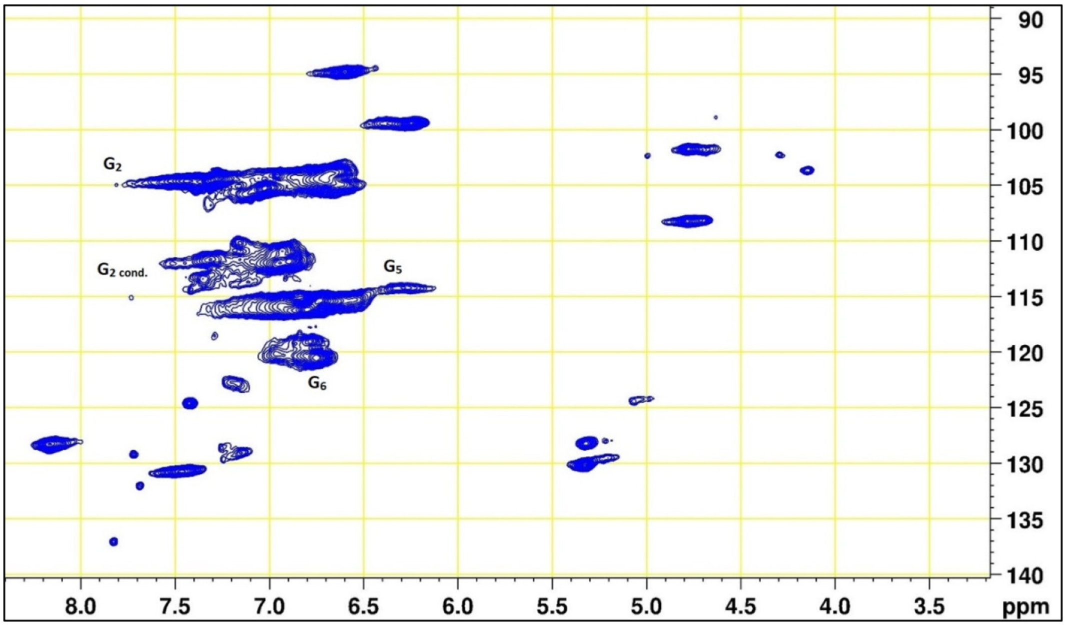

Fig. 16. The relative intensities of different peaks of Guaiacyl unit in HSQC NMR of the lignin

lignin having fairly high molecular weight with better uniformity of structure.

8.3. FT-IR analysis of lignin

FT-IR spectra of lignin fractions isolated after pretreatment with ILs are given in the following diagram (Fig. 17). The peaks in the FT-IR spectrum which are generally assigned to lignin vary depending upon the type of lignin (e.g. hardwood lignin, softwood lignin). But there are some common peaks which may be generalized to describe the spectra. Some important peaks are being considered here for our purpose, which has been assigned the wavenumber values from previous literature [3,45].

The band at 1730 cm^{-1} is for unconjugated C=O stretching and that at about 1660 cm^{-1} for conjugated C=O stretching. The peaks between 1500 and 1600 cm^{-1} correspond to aromatic skeletal vibrations and are robust evidence for the presence of lignin. The absorption band from 1400 to 1500 cm^{-1} characterizes aromatic skeletal vibrations combined with C-H symmetric stretching. The peak at about 1370 cm^{-1} pertains to phenolic OH and that at about 1330 cm^{-1} exhibits C=O stretching of syringyl unit. Absorption band at 1220 cm^{-} characterizes C-C stretching combined with C-O and C=O stretching. The peaks at 1140 cm^{-1} and 1116 cm^{-1} are the frequencies absorbed by C-H in plane vibrations and ether linkage (C-O-) respectively. The absorption bands within 800–850 cm^{-1} correspond to C-H out of plane vibrations of Guaicyl units. A minor degradation effect can be observed in the case of IL$_3$ in which some peaks have reduced intensities. This owes to the high acidity of this IL as pointed out by previous studies on lignin extraction using PILs [14,43].

8.4. Thermogravimetric analysis of extracted lignin

Thermograms of the extracted lignins after pretreatment with ILs are furnished in Fig. 18. The change in mass (%) was observed within the temperature range 60–960 °C. Different types of lignins have been analyzed and reported using thermogravimetric analysis in literature [46,47]. According to these studies, the major degradation of organosolv

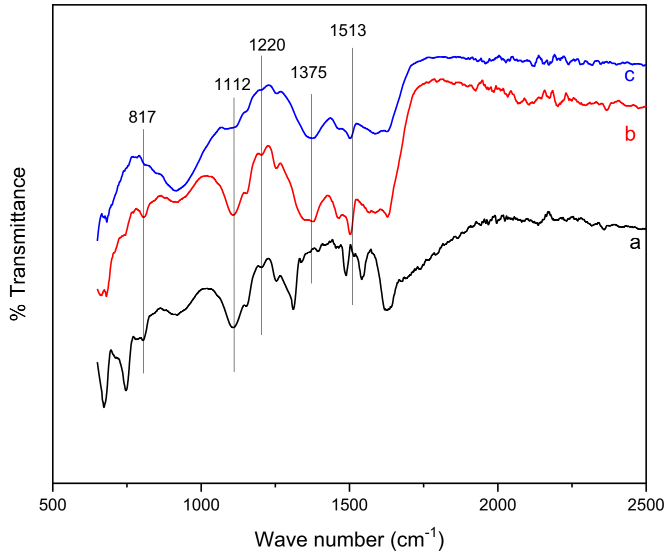

Fig. 17. FT-IR spectra of lignin extracted from wheat straw using a) IL$_1$, b) IL$_2$, c) IL$_3$.

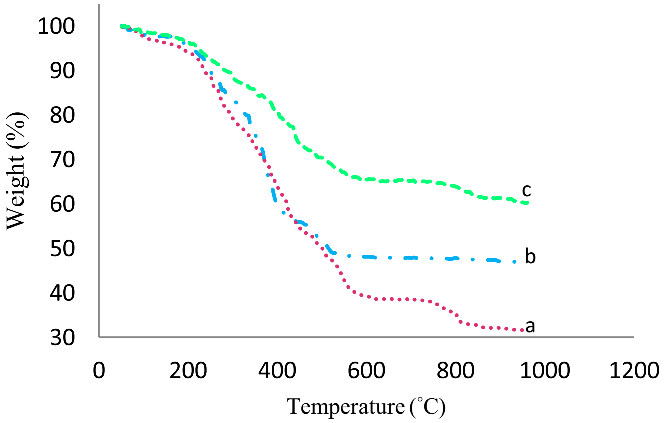

Fig. 18. TGA curves of the three samples of lignin from a) IL$_1$, b) IL$_2$ and c) IL$_3$.

lignin occurs between the range 250–400 °C. It is also known that wheat straw gives relatively higher char (about 40%) after decomposition [47]. As shown in Fig. 15, there are two plateaus for each curve. The initial plateau indicates a phase of water removal after which a steep fall occurs as a result of the loss of mass from biomass. Finally, the curve straightens and goes horizontal up to the end. The two lignin samples (IL_1 and IL_2) show a comparative degradational pattern but the lignin extracted from IL_3 shows much resistance under the same conditions. This may, perhaps, be due to carbonization (charring) or ash content.

9. Conclusion

Three ionic liquids have been synthesized from pyridine and H_2SO_4 and employed for pretreatment of biomass and consequent extraction of lignin. The clustered protic ionic liquids are based on pyridine and sulfuric acid in varying mole ratios. Results depict that the ILs are efficient enough to extract lignin from wheat straw under mild conditions. FT-IR of biomass samples supports the lignin removal which is further strengthened by good TRS values and reduced stability evidenced by TGA. The optimum TRS value (85%) could be furnished by the biomass samples after pretreatment at 100 °C for 2 h with 5% biomass loading. The optimum amount of extracted lignin (77%) was achieved by pretreatment with IL_3, the ionic liquid containing 4 mol ratio of sulfuric acid. The optimum conditions for lignin removal are 60 °C, 2 h, with 5% biomass loading. The characterization of the isolated lignin was done using HSQC NMR, Gel Permeation Chromatography, FT-IR, and TGA. The HSQC analysis showed some condensation of lignin during the extraction process. The GPC results showed high molecular weights and polydispersity indicating the less degraded structure with fair uniformity. Thermogravimetric analysis (TGA) further substantiated the presence of stable lignin samples.

Author statement

Azmat Mehmood Asim did experimental work as well as paper write up, Sadia Naz also helps in paper writing and did some characterization of the samples. Maliha Uroos and Nawshad Muhammad conceive the idea and supervised the project.

Declaration of Competing Interest

The authors have no conflict of interest (financial or otherwise).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2020.115013.

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