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ORIGINAL ARTICLE

# Extraction of lignin, structural characterization and bioconversion of sugarcane bagasse after ionic liquid assisted pretreatment

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## Abstract

The primary focus of this work was to recover lignin and investigate the structural changes in sugarcane bagasse after pretreatment with ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM]oAc). 90% lignin recovery was achieved while bagasse was treated with [EMIM]oAc at 140 °C, 120 min reaction time and 1:20 bagasse to the ionic liquid ratio (w/w). The impact of ionic liquid pretreatment on bagasse was confirmed by qualitative analysis of untreated and pretreated bagasse. Scanning electron microscopy analysis exhibited the porous and irregular structure of bagasse after pretreatment. X-ray powder diffraction analysis verified a decrease in crystallinity as a result of the pretreatment process by showing a 14.7% reduction of Crystallinity index after ionic liquid treatment. The efficacy of [EMIM]oAc on bagasse treatment was also examined by enzymatic hydrolysis which manifested an increase in reducing sugar yield as a result of pretreatment. Maximum yield of 54.3% reducing sugar was obtained after 72 h enzymatic hydrolysis of pretreated bagasse. Recovered lignin was analyzed qualitatively. 1D NMR spectroscopy of lignin revealed the presence of essential functional groups whereas 2D NMR spectroscopy showed the dominance of etherified syringyl unit. Further ionic liquid recovery and reuse were substantiated by Gel permeation chromatography analysis of lignin. Weight average molecular weight ($M_w$) of lignin extracted by fresh [EMIM]oAc was obtained as 1769 g/mol (in the previous study) while lignin recovered by recycled [EMIM]oAc showed almost equal $M_w$ 1765 g/mol in this study. Thus, the current investigation corroborated satisfactory performance of [EMIM]oAc in lignocellulose processing which further enhanced enzymatic hydrolysis in the subsequent step.

**Keywords** Sugarcane bagasse · Pretreatment · Ionic liquid recycle · Lignin recovery · Enzymatic hydrolysis

## Abbreviations

| | |
|---|---|
| SCB | Sugarcane bagasse |
| [EMIM]oAc | 1-Ethyl-3-methylimidazolium acetate |
| IL | Ionic liquid |
| RTIL | Room temperature ionic liquid |
| FTIR | Fourier transform infrared spectrophotometer |
| NMR | Nuclear magnetic resonance |
| GPC | Gel Permeation chromatography |
| TGA | Thermogravimetric analysis |
| NREL | National Renewable Energy Laboratory |

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## Introduction

Bioethanol, derived from biomass has established itself as one of the leading biofuels in the global market as numerous countries primarily Brazil and USA are now shifting their interest on the renewable resources of energy to encourage sustainable development (Sarkar et al. 2012). In this context, lignocellulosic biomass has proved to be a cost-effective and most abundant renewable resource which is non-polluting agricultural residue and potential to be converted into biofuel. The main components of lignocelluloses are lignin, and cross-linked polysaccharides, i.e., cellulose and hemicellulose. The cellulosic part which occurs in the highest percentage is used to synthesize biofuels such as ethanol. Cellulose, a homopolymer of successive glucose units which are attached by β-1,4 glycosidic linkage. Hemicellulose is a

heteropolymer of pentose and hexose sugars (Balat 2011). In the biofuel industry, during the processing of lignocelluloses to bioethanol, lignin is obtained as the main byproduct. It is a heterogeneous copolymer, combines cellulose and hemicellulose by hydrogen bonds and ester linkage, respectively. This cross-linked aromatic polymer contains three monolignols: sinapyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol which are present in lignin as three units: syringyl unit, guaiacyl alcohol, and *p*-hydroxyphenyl. Lignin is also a source of polyurethanes and polyesters, surfactant, plastics, carbon-fibers, etc. (Moghaddam et al. 2014). Lignin can also be used as a substitute of phenol for resin synthesis (Tymchyshyn and Xu 2010). Different analytical techniques such as FTIR, NMR, GPC, and TGA have been used to investigate the fundamental lignin properties such as composition, molecular weight, thermostability (Moghaddam et al. 2014). Thus during processing of lignocelluloses biomass to biofuel, as an alternate of lignin removal, recovery of lignin can emphasize the process to be commercially viable. Therefore, prime steps involved in the production of biofuel from lignocellulosic biomass are: (1) pretreatment of biomass for lignin recovery followed by liberation of celluloses and hemicelluloses, (2) enzymatic hydrolysis of the cellulose and hemicelluloses to produce different fermentable sugars such as glucose, mannose, xylose, etc., (3) microbial fermentation and product separation (Adsul et al. 2011). Pretreatment of lignocelluloses is the most crucial step as it removes lignin, depolymerize hemicelluloses and reduces the crystallinity of cellulose to enhance enzymatic hydrolysis (Yoon et al. 2012). Cellulose is not easily dissolved in conventional solvents owing to the tight packing of chains by intermolecular and intramolecular hydrogen bonds. The structure of lignin impacts an effect on the efficiency of the pretreatment method employed (Sidik et al. 2013). Major limitations of conventional physical, chemical, physicochemical pretreatment processes are the use of irradiation, the formation of toxic by-products and high energy consumption. As an alternate, ionic liquid has come up with great success as a pretreatment solvent as it can dissolve either or both lignin and cellulose and can separate lignin and reduce cellulose crystallinity (Tan and Lee 2012). Ionic liquids (ILs) are molten salt consist of organic anions and cations with a melting temperature of less than 100 °C. These are non-volatile, non-flammable and can dissolve biomass and polymer such as cellulose under favorable condition (Olivier-Bourbigou et al. 2010; Swatloski et al. 2002; Dadi et al. 2006). Significant advantages of ionic liquid as a pretreatment solvent are low viscosity, the requirement of small quantity, easy to recycle and shorter reaction time (Fu and Mazza 2011). However, the only limitation is its high price which can be overcome by recovery and reuse of ionic liquid. This has been recognized as a green solvent and investigated in the last few years as an alternative to a conventional organic solvent for lignocellulose fractionation (Sidik et al. 2013). Recently, lignocellulose pretreatment using ionic liquid has grabbed the attention of the researcher towards the investigation of eco-friendly pretreatment process. Commercial imidazolium ionic liquids have been successfully applied for lignocelluloses dissolution for lignin recovery in pure form (Yuan et al. 2013; Casas et al. 2012) and to enhance enzymatic hydrolysis (Bahrani et al. 2015; Trinh et al. 2015; Qiu and Aita 2013; Qiu et al. 2012). Lignin recovery from various lignocellulosic biomass with the aid of different ionic liquid was reviewed by Saha et al. (2017a, b). Moghaddam et al. (2014) recovered 90% pure lignin from [BMIM]Cl and [BMIM][$CH_3SO_3$] treated sugarcane bagasse using HCl as a catalyst. Fractionation of wheat straw by [EMIM]oAc leads to a recovery of highly pure cellulose and lignin as studied by Lopes et al. (2013). 5.8% lignin was recovered when commercial [EMIM]oAc was used for poplar wood pretreatment (Kim et al. 2011), and lignin yield can be enhanced if lignocelluloses biomass can be treated with ionic liquid along with organic solvent (Sun et al. 2013). Xu et al. investigated pretreatment of eucalyptus by six different imidazolium ionic liquid followed by extraction of lignin using alkaline ethanol solution. They achieved the highest yield of 35% lignin while eucalyptus was treated by 1-allyl-3-methylimidazolium chloride ([AMIM]Cl). Lignin recovered by the combined effect of ionic liquid and alkaline ethanol pretreatment contained higher etherified linkage and showed higher thermal stability as compared to milled wood lignin and alkaline ethanol lignin (Xu et al. 2015). 52.64% of original lignin was recovered from sugarcane bagasse while fractionated by 1-butyl-3-methylimidazolium chloride treatment followed by acetone/water precipitation and NaOH aided extraction. Herein ionic liquid was recycled by treating with acetonitrile, and the recyclability was verified by NMR analysis of fresh and recycled ionic liquid (Lan et al. 2011). Tan et al. synthesized ionic liquid 1-ethyl-3-methylimidazolium alkylbenzenesulfonate ([$C_2$mim][ABS]) and used to extract lignin from sugarcane bagasse at elevated temperature and ambient pressure. Lignin was recovered by precipitation, and the ionic liquid was recycled. 93% lignin yield was achieved with 2220 g/mol molecular weight (Tan et al. 2009). Ionic liquid was proved as a green solvent for the dissolution of lignin. Pu et al. studied the dissolution of 20% lignin in imidazolium ionic liquid and indicated that the nature of anion influence lignin solubility (Pu et al. 2007). A recovery of 60.48% original lignin, conversion of 92.55% cellulose and a yield of 97.77% reducing sugar were observed by Zhang et al. (2015) while treating corn stover with synthesized [HMIM]Cl.

Bahrani et al. (2015) observed 70.38% fermentable sugar conversion after hydrolysis of 1,3-dimethylimidazolium dimethyl phosphate ([Mmim][DMP]) treated sugarcane bagasse for 48 h. Reducing sugar of 5.48 mg/10 mg

pretreated wheat straw was obtained when wheat straw was processed with 1-ethyl-3-methyl imidazolium diethyl phosphate ([EMIM]DEP) (Li et al. 2009). Recycle and reuse of ionic liquid without disrupting its structure, property and pretreatment efficiency, validated ionic liquid mediated biomass processing as sustainable and eco-friendly (Sun et al. 2013; Pinkert et al. 2011).

One of the most available lignocelluloses in tropical countries such as India and Brazil are sugarcane bagasse as sugarcane is the most cultivated crops in these countries. Almost 336.15 million tons sugarcane was produced in India according to the harvest year of 2012/2013 (Solomon 2014), and the production rate in Brazil is 652 millions of metric tons in 2013/2014 harvest year (Antunes et al. 2014). As per the GAIN report, sugarcane production is expected to rise to 415 million metric ton (MMT) in marketing year 2018/2019 (Aradhey 2018). Ionic liquid 1-ethyl-3-methylimidazolium acetate ([EMIM]oAc) is an efficient solvent for bagasse processing as compared to other conventional alkali and acid as well as other imidazolium ionic liquid treatment (Yoon et al. 2011). Yoon et al. (2012) examined the structural changes of sugarcane bagasse after [EMIM]oAc pretreatment and observed yield of 69.7% reducing sugar at the optimized condition. Though the efficiency of [EMIM]oAc on bagasse pretreatment was well studied (Lee et al. 2009; Qiu et al. 2012; Qiu and Aita 2013; Yoon et al. 2011, 2012), lignin recovery was not reported.

Thus, current study explored sugarcane bagasse pretreatment using [EMIM]oAc to recover lignin and observe a structural modification of bagasse and subsequent enzymatic hydrolysis of pretreated biomass. [EMIM]oAc was chosen as a solvent for bagasse pretreatment as the chemical structure of lignin is not altered during this [EMIM]oAc mediated pretreatment (Lee et al. 2009). Raw and pretreated bagasse were characterized by Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD) to observe the effect of the ionic liquid on bagasse. Further, the performance of ionic liquid was verified by enzymatic hydrolysis of bagasse before and after pretreatment. The lignin so regenerated was also characterized using nuclear magnetic resonance (NMR). recyclability and reusability of [EMIM]oAc was verified by GPC of lignin extracted with both fresh and recycled IL.

## Materials and methods

### Materials

Sugarcane bagasse (SCB) was supplied by local juice mill of West Bengal, India. At the outset, SCB was cleaned with water, sieved to a size of 1–3 cm and subjected to drying for about a day in a hot air oven. After drying, the size of the bagasse was reduced to fine powder using a grinder (Bajaj Twister Mixer Grinder). Powdered bagasse was screened to a size of 250–500 µm using sieves of respective particle size and stored in a sealed container at room temperature. Chemicals required for pre-treatment like ionic liquid 1-ethyl-3-methylimidazolium acetate (> 95%) was supplied by Io-li-tech, Germany and acetone (> 95%) was purchased from Sigma Aldrich (USA). The chemical composition of SCB was determined as 30% cellulose, 24% hemicelluloses and 22.4% lignin as per the analytical procedure adopted from National Renewable Energy Laboratory (NREL) (Sluiter et al. 2012). For enzymatic hydrolysis commercial cellulase from *Trichoderma reesei* (ATCC 26921), sodium citrate and citric acid were bought from Sigma Aldrich (USA). For the estimation of reducing sugar 3,5-dinitrosalicylic acid (DNS), sodium hydroxide, potassium sodium tartrate, and glucose were obtained from Sigma Aldrich.

### Ionic liquid aided pretreatment of sugarcane bagasse

Sugarcane bagasse pretreatment was carried out for recovering the maximum amount of lignin, taking the optimized pretreatment conditions from the previous study (Saha et al. 2017a, b). Dried bagasse powder was mixed with ionic liquid [EMIM]oAc in a vial. A hot plate equipped with a magnetic stirrer (IKA® RCT Basis Safety Control, Germany) was used to perform reaction homogenization. The reaction was carried out at 140 °C, 600 rpm for 120 min and 1:20 bagasse to the ionic liquid ratio (wt/wt). Upon completion of the reaction, the semisolid sample was cooled down to room temperature and 1:1 (v/v) acetone/ deionized water was supplemented to it. Then it was stirred for 1 h in a magnetic stirrer for cellulosic material regeneration. The resulting solution was filtered using vacuum filtration (AXIVA) with 0.45 µm filter paper to separate cellulosic material as retentate. Pretreated sample or cellulosic material was washed well with acetone/deionized water (1:1) to remove any remaining ionic liquid from it. After washing, the cellulosic material was dried overnight in a hot air oven and kept for characterization. The filtrate was heated to evaporate acetone to precipitate lignin and then subjected to vacuum filtration. The lignin was obtained as retentate. It was dried and kept in airtight container for characterization. Water was evaporated from the filtrate to recover ionic liquid, and it was dried to remove moisture. Lignin recovery was determined according to the equation suggested by Sun et al. (2013).

$$\text{Lignin yield} = \left(\frac{\text{Weight of recovered lignin}}{\text{Weight of acid insoluble lignin}}\right) \times 100\%$$

A schematic of the pretreatment process was shown in Fig. 1.

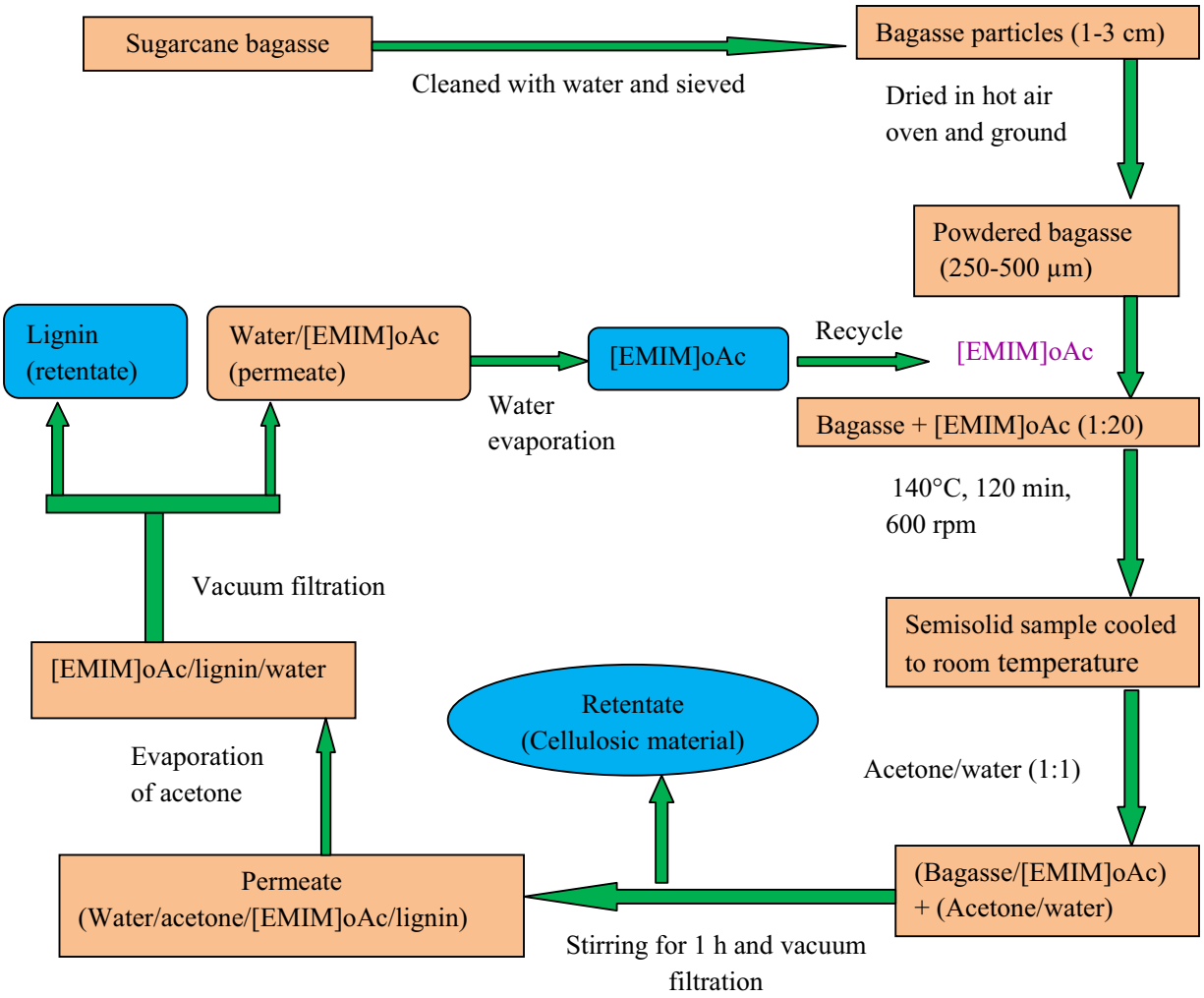



**Fig. 1** Schematic of the pretreatment process

## Characterization of raw and pretreated bagasse

### Scanning electron microscopy (SEM) analysis

Surface morphology of untreated and pretreated sugarcane bagasse was investigated by Scanning Electron Microscope (JEOL JSM-6360, Japan). The dried bagasse samples were coated with gold before analysis, and the images were obtained with 17 kV accelerating voltage. Analysis of SEM images investigated fiber dimension.

### X-ray powder diffraction (XRD) analysis

A high-resolution X-ray diffractometer (X'pert PRO, PANalytical B.V., P.W 3040/60, Netherland) was employed to study the crystallinity of untreated and pretreated sugarcane bagasse. Samples were scanned at 5°/min scanning speed operated at 45 mA, and 40 kV and the radiation was detected in a range of $2\theta = 10°–40°$ with 300 s exposure time and 0.02° step size. The crystallinity index (CrI) was determined according to the equation defined by Segal et al. (1959).

$$\mathrm{CrI} = \{(I_{002} - I_{\mathrm{am}})/I_{002}\} \times 100,$$

where CrI implied the degree of crystallinity. $I_{002}$ represents maximum peak intensity (crystalline region) on 002 lattice plane at almost $2\theta = 22°$, and $I_{\mathrm{am}}$ is the diffraction intensity of amorphous region at $2\theta = 18°$.

## Enzymatic hydrolysis

To assess the pretreatment impact on enzymatic hydrolysis of bagasse, both untreated and pretreated bagasse was subjected to saccharification using commercial cellulase. For the hydrolysis reaction, the reaction mixture was prepared in 0.1 M sodium citrate buffer with 10% w/v bagasse sample and enzyme loading of 20 FPU/g substrate. pH was maintained at 4.8. Hydrolysis was performed at 50 °C and 150 rpm for 72 h in an incubator shaker (Metrex Scientific

Instruments Pvt. Ltd.). The sample was taken at 0, 4, 10, 24, 36, 48, 72 h and centrifuged at 8000 rpm for 10 min. The supernatant was analyzed by the dinitrosalicylic method (DNS) method (Miller 1959) to determine the concentration of reducing sugar. The yield of reducing sugar obtained from the hydrolysis process was determined by the equation as proposed by Yoon et al. (2011).

$$\text{Reducing sugar yield} = \frac{\text{Reducing sugar weight}}{\text{Weight of pretreated bagasse used in hydrolysis}} \times 100\%$$

## Characterization of extracted lignin

### Gel permeation chromatography (GPC) analysis

Gel permeation chromatography (GPC) was employed to investigate average molecular weight of recovered lignin. GPC instrument (Agilent Technologies 1260 infinity) was employed with a PLgel 20 micron (µm) mixed-A column (300 × 7.5 mm) and PLgel 20 µm guard column (50 × 7.5 mm) to determine molecular weight distribution. The chromatogram was recorded using a refraction index (RI) detector. Before analysis 5 mg lignin sample was dissolved in 500 µl *N,N*-dimethylformamide (DMF) and 20 µl sample were injected. DMF was utilized as mobile phase at 1 ml/min flow rate, and 30 °C was maintained as column temperature. Polystyrene with a molecular mass range of 580–6,870,000 Da was used to prepare a calibration curve. Number average molecular weight ($M_n$), weight average molecular weight ($M_w$), and polydispersity ($M_w/M_n$) of lignin were determined after comparing with standard curve.

### 1D and 2D nuclear magnetic resonance (NMR) spectroscopy

30 mg lignin sample was dissolved in 1 ml DMSO-$d_6$ and then transferred to the NMR tube (5 mm). Both 1D ($^{13}$C and $^{1}$H) and 2D (Heteronuclear single-quantum correlation (HSQC)) NMR spectra were acquired by Bruker ADVANCE 600 spectrometer made by Bruker, Germany. Bruker Topspin-NMR software was used to perform data processing. NMR was operated at room temperature from 20,000 scans for $^{13}$C-NMR and 128 scan for $^{1}$H-NMR. For HSQC, the spectral widths were 5000 Hz for $^{1}$H dimension and 20,000 Hz for $^{13}$C dimension. 1024 complex points were collected for $^{1}$H dimension with 1.5 s recycle delay. $^{1}$H-$^{13}$C scalar J-coupling was set to 145 Hz. $^{1}$H signals, $^{13}$C signals, and HSQC cross signals were assigned by comparing with the earlier research reports. The center of solvent (DMSO-$d_6$) peak was used as an internal chemical shift reference point ($\delta_C/\delta_H$ 39.5/2.49).

## Results and discussion

### Characterization of raw and pretreated bagase

The images of sugarcane bagasse before and after pretreatmnet were shown in Fig. 2.

### Scanning electron microscopy analysis

Figure 3 exposed the morphological characteristics of untreated and ionic liquid [EMIM]oAc pretreated sugarcane bagasse as obtained by SEM analysis under magnifications of 500×, 2000×. Raw sugarcane bagasse showed lamellar and smooth surface where cellulose is cross-linked with lignin and hemicellulose. Ionic liquid aided pretreatment disrupted the cellular bond, cracked


**Fig. 2** Image of bagasse **a** before pretreatment, **b** after pretreatment

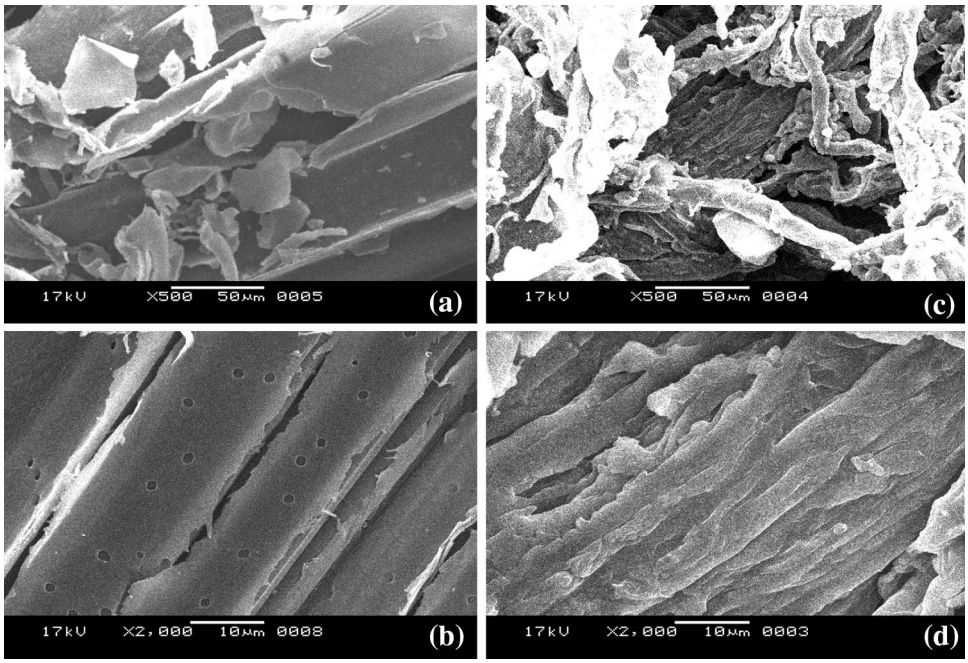



**Fig. 3** **a** SEM image of raw bagasse at magnification: ×500, **b** SEM image of raw bagasse at magnification: ×2000, **c** SEM image of pretreated bagasse; magnification: ×500, **d** SEM image of pretreated bagasse; magnification: ×2000

the surface layer and removed outer amorphous lignin and hemicellulose through depolymerization, thus formed a conglomerate and irregular texture as illustrated in SEM images of pretreated bagasse or regenerated cellulose-rich material. Relatively more irregular and porous structure of pretreated bagasse was observed in higher magnification as compare to lower magnification. This finding agrees with the study of Yoon et al. (2012), who found an almost similar alteration in surface structure when sugarcane bagasse was pretreated with the same ionic liquid.

## XRD analysis of raw and pretreated bagasse

The crystallinity of untreated and ionic liquid pretreated samples were elucidated by XRD as shown in Fig. 4. The strong crystalline structure of cellulose is formed due to the presence of van der Waals force and intermolecular hydrogen bonding between alongside cellulose molecule whereas lignin and hemicellulose are amorphous (Chirayil et al. 2014). Efficient pretreatment method disrupts the bonds and converts the crystalline to an amorphous structure. In this ionic liquid-based study a less crystalline structure and larger amorphous region of the pretreated sample was established from the XRD pattern as it exhibits a substantial reduction

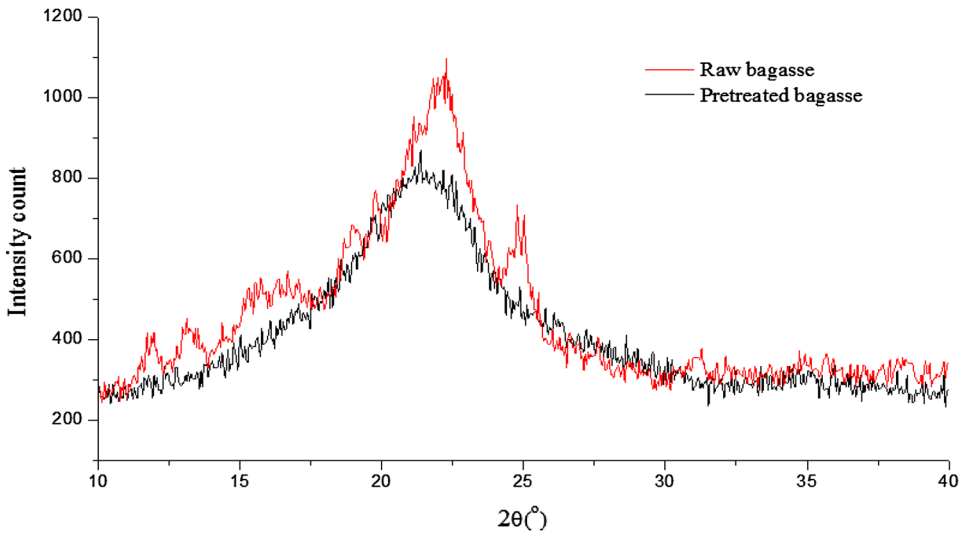



**Fig. 4** XRD analysis of untreated and pretreated bagasse

in peaks intensity as compared to the untreated sample. The reason behind this phenomenon is the partial solubilization and distension of cellulose crystal with [EMIM]oAc due to complexity in cell wall component and structural diversity at moderate pretreatment temperature (Zhang et al. 2014). Crystallinity index (CrI) calculation is a semi-quantitative technique to assess the quantity of crystalline and amorphous cellulosic element in fiber (Park et al. 2010). CrI of untreated bagasse was 51.44 which decreased after pretreatment, and the calculated value of CrI of pretreated bagasse was 36.74. Reduction of the CrI by 14.7% in pretreated biomass implied a rapid decline of crystalline structure and increase of amorphous region after pretreatment. The sharp crystallographic peaks at 18° and 22° of 2$\theta$ inferred 110 and 002 planes which are significant characteristics of native cellulose (Klemm et al. 2005) which indicates that the pretreatment procedure did not alter the crystalline construction type. The XRD pattern of this study is similar to the result obtained by Yoon et al. when they treated sugarcane bagasse with the same ionic liquid (Yoon et al. 2012).

## Enzymatic hydrolysis

The effect of [EMIM]oAc pretreatment on enzymatic saccharification at different hydrolysis time is presented in Table 1. The result showed an increase in enzymatic hydrolysis after the pretreatment of sugarcane bagasse with [EMIM]oAc. The lower quantity of sugar produced from untreated bagasse because the presence of lignin in it which adsorbs cellulase enzyme and interfere with the hydrolysis process (Lee et al. 2009). Besides this, the crystalline structure of cellulose in untreated bagasse was not readily accessible to enzyme whereas after pretreatment crystallinity was reduced and surface area was increased. Regenerated cellulose contained higher β-glucosidic bond fraction and became amorphous which could be efficiently saccharified by an enzyme (Dadi et al. 2006). Thus reducing sugar released from pretreated bagasse was much higher as compared to untreated bagasse. Xu et al. (2015) observed the same phenomenon where saccharification rate and glucose yield increased after pretreatment of eucalyptus with an ionic liquid and also found that efficiency of [EMIM]oAc pretreatment on cellulose conversion was higher than other imidazolium ionic liquid. Another report showed an increase in cellulose digestibility from 4.1 to 87.0% after the pretreatment of energy cane bagasse with [EMIM]oAc (Qiu et al. 2012) Current study revealed that reducing sugar yield increased up to 48 h hydrolysis and after 48 h no significant hydrolysis was observed as shown in Table 1. Reducing sugar yield increased with time as enzyme got more reaction time for hydrolysis (Bahrani et al. 2015). 52.4% reducing sugar yield was obtained after 48 h hydrolysis. This result verified the study of Yoon et al. (2012) where 51.3% yield of reducing sugar was achieved after sugarcane bagasse pretreatment by [EMIM]oAc at 120 °C at 15% solid loading ratio. The study confirmed that [EMIM]oAc pretreatment was effective to alter cellulose structure and enhance enzymatic hydrolysis.

**Table 1** Enzymatic hydrolysis of untreated and pretreated bagasse at different time interval

| Hydrolysis time (h) | Reducing sugar yield (%) | |
|---|---|---|
| | Untreated bagasse | Petreated bagasse |
| 0 | 0 | 0 |
| 4 | 1.5 ± 0.07 | 10.3 ± 0.35 |
| 10 | 3.8 ± 0.28 | 24.1 ± 0.28 |
| 24 | 9.0 ± 0.28 | 33.6 ± 0.42 |
| 36 | 13.0 ± 0.56 | 44.6 ± 0.49 |
| 48 | 20.1 ± 1.34 | 52.4 ± 0.14 |
| 72 | 20.5 ± 0.35 | 54.3 ± 0.07 |

## Characterization of recovered lignin

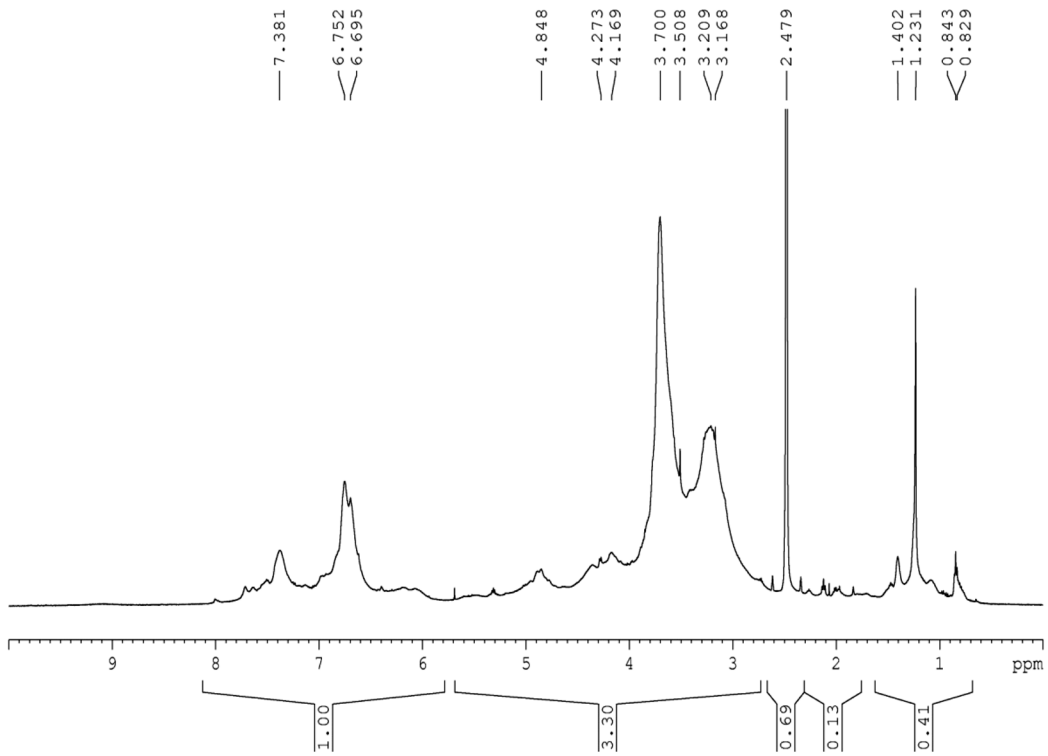
Pretreatment of sugarcane bagasse by [EMIM]oAc recovered 90% lignin. The recovered lignin was analysed by NMR.

## 1D and 2D NMR analysis of lignin

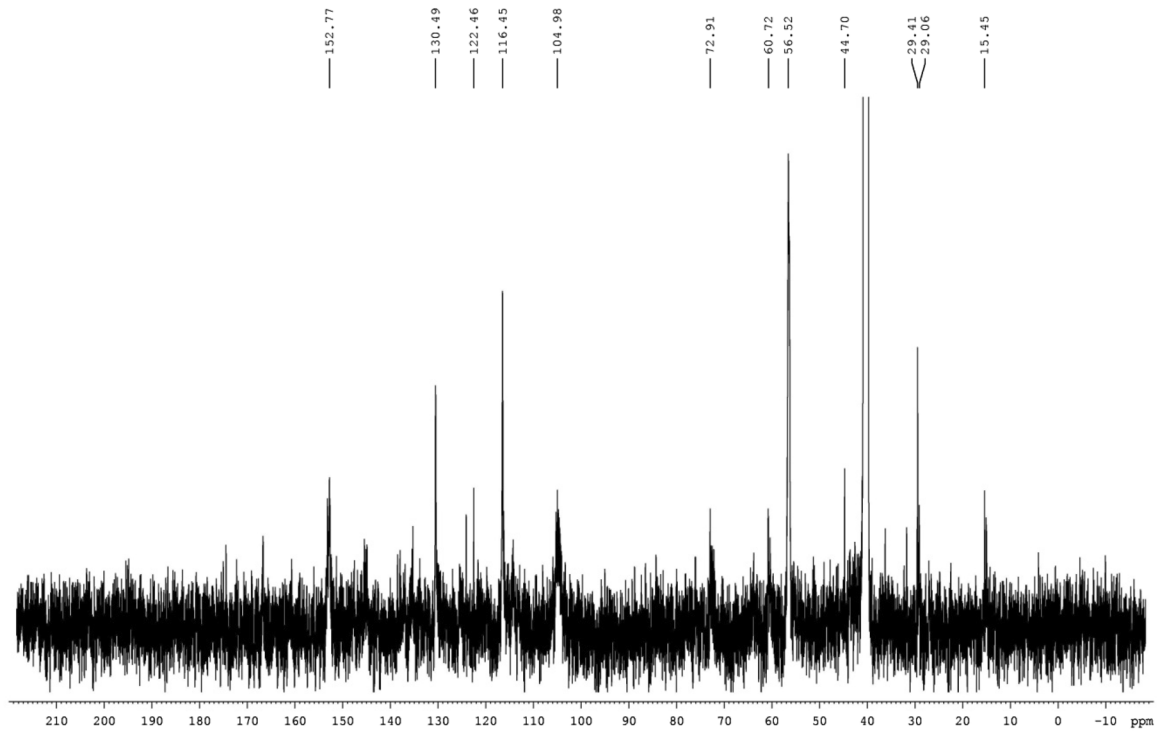
Nuclear magnetic resonance spectroscopy is the most reliable analysis of lignin as it presents both qualitative characteristics as well as some extent of quantitative characteristics. Proton NMR ($^1$H NMR) spectra quantify methoxyl groups proton, benzylic proton, aliphatic and aromatic acetate and hydrocarbon contaminant whereas carbon NMR ($^{13}$C NMR) spectra reveal more detailed information of lignin structure. $^{13}$C NMR quantifies aliphatic/

**Table 2** $^1$H NMR peak assignment of sugarcane bagasse lignin extracted with [EMIM]oAc

| Signal (ppm) | Functional group |
|---|---|
| 1–1.5 | Proton in aliphatic acetate |
| 0.7–0.9 | Aliphatic contaminants |
| 2.2–2.6 | Proton in aromatic acetate |
| 3.2–3.4 | $H_\beta$ in β–β structure |
| 3.4–4 | Methoxyl proton |
| 4.1–4.6 | Hγ in β-*O*-4 aryl ether |
| 4.8 | $H_\beta$ in β-*O*-4 aryl ether |
| 5.3–5.8 | $H_\beta$ in benzyl aryl ether |
| 6.6 | Aromatic H in syringyl unit |
| 6.7–6.9 | Aromatic H in guaiacyl unit |
| 7.3–7.6 | Ethylenic and aromatic protons in *p*-coumaric and ferulic acids |

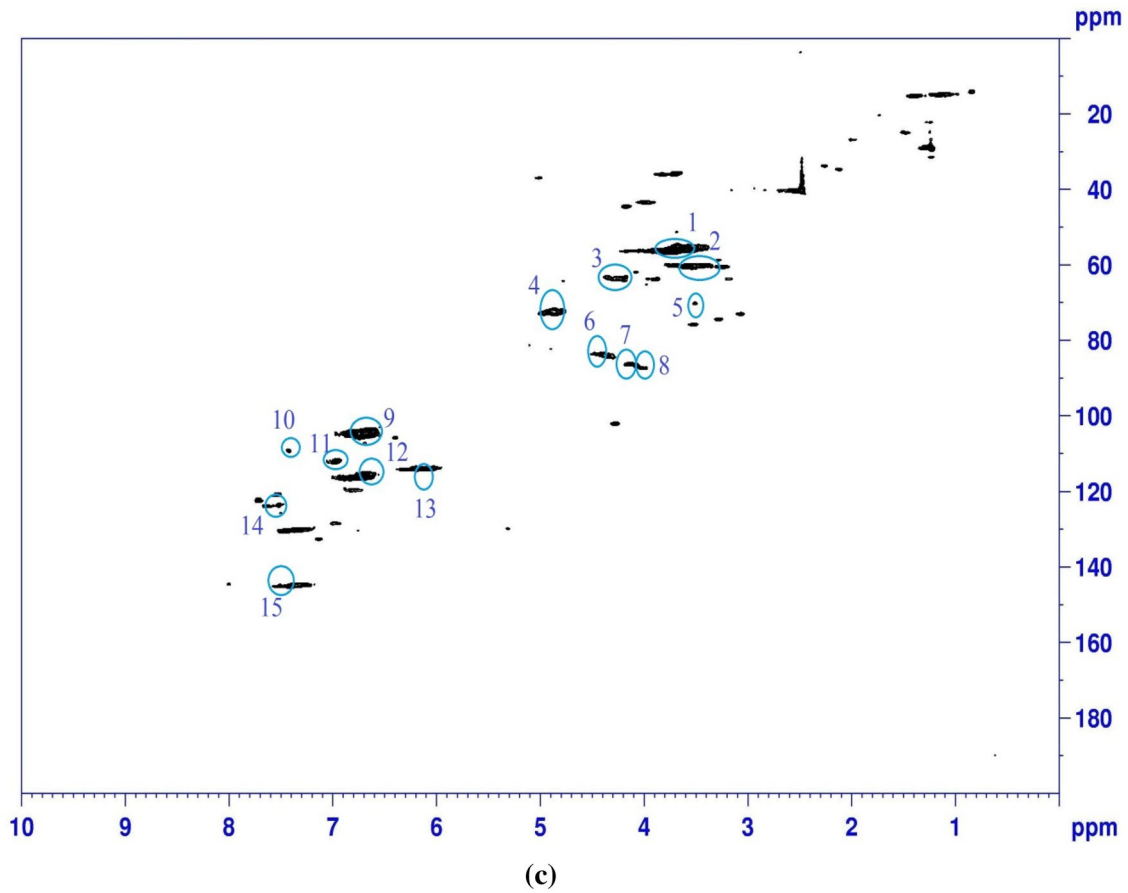



**(a)**




**(b)**

**Fig. 5** **a** $^{1}$H NMR, **b** $^{13}$C NMR and **c** 2D HSQC NMR spectra of lignin




(c)

**Fig. 5** (continued)

phenolic hydroxyl group and provides inter-unit bonding pattern. As the structural framework of lignin is very complex, thus resonance overlap occurs while quantifying lignin by 1D NMR. This hinders precise quantification of complex structure. Two dimensional NMR (2D NMR) which correlates both proton and carbon NMR provides enhanced resolution by separating overlapping proton and overlapping carbon by differing chemical shift. It provides both qualitative and quantitative information of inter-unit bonding pattern (Ghaffar and Fan 2013). Both hetero- and homo-nuclear 2D NMR was used to characterize lignin in the previous report (Ede and Brunow 1992). Hetero-nuclear-single-quantum-coherence (HSQC) approach of 2D NMR have been widely used for characterization of lignin isolated from different lignocellulosic biomass by ionic liquid aided pretreatment (Sun et al. 2013; Yuan et al. 2013; Moghaddam et al. 2014; Xu et al. 2015). $^{13}C$ NMR and $^{1}H$ NMR were also studied to characterize lignocellulosic lignin (Kim et al. 2011; Casas et al. 2012). For $^{1}H$ NMR peak at 7.38 ppm corresponds to *p*-coumaric and *p*-ferulic acid of lignin (Hoareau et al. 2004). The peak at 5.7 ppm signifies ether linkage (Cox and Ekerdt 2013). Detailed assignment of proton NMR spectrum was presented in Table 2 based on earlier researches (Hoareau et al. 2004; Garcia et al. 2009; Kim et al. 2011; Cox and Ekerdt 2013). Figure 5a shows $^{1}H$ NMR spectrum of lignin. The signals appeared from 100 to 155 ppm, 60–86 ppm and 55–57 ppm in $^{13}C$ NMR spectra detected

**Table 3** $^{13}C$ NMR peak assignment of sugarcane bagasse lignin extracted with [EMIM]oAc

| Signal (ppm) | Functional group |
|---|---|
| 15.45 | γ methyl in *n*-propyl side chain |
| 29–31 | α, β methylene groups |
| 56.52 | $OCH_3$ in guaiacyl and syringyl ring |
| 60–63 | $C_\alpha$ with C=O, $C_\gamma$–O in *p*-coumaryl ester |
| 70–76 | $C_\alpha$–OH in β-*O*-4 |
| 104–106 | $C_2$, $C_6$ of syringly ring |
| 116–117 | $C_5$ of guaiacyl ring |
| 130–137 | $C_1$, $C_4$ of Guaiacyl or syringly ring |
| 150–155 | $C_3$, $C_4$ of guaiacyl etherified or $C_3$, $C_5$ of syringly β-*O*-4 |

**Table 4** 2D HSQC signal assignment of sugarcane bagasse lignin extracted with [EMIM]oAc

| No of signals | Signal ($\delta_C/\delta_H$) | Assignment |
|---|---|---|
| 1 | 56.52/3.7 | C–H in methoxyl group |
| 2 | 60.12/3.5 | $C_\gamma$–$H_\gamma$ in β-*O*-4′ substructure |
| 3 | 63/4.35 | $C_\gamma$–$H_\gamma$ in γ-acetylated β-*O*-4′ substructure |
| 4 | 72/4.84 | $C_\alpha$–$H_\alpha$ in β-*O*-4′ substructure linked to syringyl unit |
| 5 | 71/3.6 | $C_\gamma$–$H_\gamma$ in β–β′substructure |
| 6 | 85.4/4.5 | $C_\alpha$–$H_\alpha$ in β–β′substructure |
| 7 | 85.8/4.22 | $C_\beta$–$H_\beta$ in β-*O*-4′ substructure linked to syringyl unit (erythro form) |
| 8 | 86/4.1 | $C_\beta$–$H_\beta$ in β-*O*-4′ substructure linked to syringyl unit (threo form) |
| 9 | 103/6.7 | $C_{2,6}$–$H_{2,6}$ in etherified syringyl unit |
| 10 | 106.7/7.28 | $C_{2,6}$–$H_{2,6}$ in $C_\alpha$ oxidized syringyl unit |
| 11 | 111/6.9 | $C_2$–$H_2$ in guaiacyl unit |
| 12 | 115/6.7 | $C_5$–$H_5$ in guaiacyl unit |
| 13 | 117/6.2 | $C_2$′–$H_2$′in spirodienone substructure |
| 14 | 130/7.5 | $C_{2,6}$–$H_{2,6}$ in *p*-hydroxycinnamyl alcohol or p-hydroxybenzoate |
| 15 | 146/7.5 | $C_\alpha$–$H_\alpha$ in *p*-hydroxycinnamyl alcohol or p-hydroxybenzoate |

the presence of aromatic carbon, aliphatic side chain and methoxy carbon, respectively (Kim et al. 2011). No peak occurred in the region of 102–90 ppm, which signified almost absence of residual sugar in ionic liquid extracted lignin sample (Hage et al. 2009). Detailed assignment of $^{13}C$ NMR spectra of bagasse lignin was represented in Table 3 based on previous research (Hage et al. 2009; Kim et al. 2011; Casas et al. 2012; Moubarik et al. 2013), and spectra are shown in Fig. 5b. Due to signal overlapping in $^{13}C$ NMR, 2D HSQC NMR was performed to resolve resonance overlap and explore detailed structure. 2D NMR spectra can be divided into two regions. Side chain region: $\delta_C/\delta_H$ 50.0–90.0/2.50–6.00 and aromatic region: $\delta_C/\delta_H$ 100.0–135.0/5.50–8.50 (Moghaddam et al. 2014). Cross signals of side chain region correspond to details regarding inter-unit linkage of lignin (Moghaddam et al. 2014) whereas the aromatic region provided information regarding syringyl (S), guaiacyl (g) and *p*-hydroxyphenyl (H) unit and also substructures such as spirodienone, *p*-hydroxycinnamyl alcohol and p-hydroxybenzoate substructure (Yuan et al. 2013). Figure 5c shows HSQC NMR spectra combining both the regions. Detailed assignment of 2D NMR cross signals was depicted in Table 4 relating to earlier publication (Sun et al. 2013; Yuan et al. 2013; Moghaddam et al. 2014).

**Table 5** Weight average molecular weight ($M_w$), number average molecular weight ($M_n$) and polydispersity index (PDI) ($M_w/M_n$) of lignin extracted by fresh and recycled IL

| Lignin | $M_w$ (g/mol) | $M_n$ (g/mol) | $M_w/M_n$ |
|---|---|---|---|
| Extracted with fresh IL (Saha et al. 2017a, b) | 1769 | 1098 | 1.611 |
| Extracted with recycled IL | 1765 | 1072 | 1.646 |

## Recovery and reuse of ionic liquid after bagasse pretreatment

Ionic liquid was recycled and reused after the first pretreatment process to verify the effectiveness of ionic liquid for lignin extraction from bagasse. The quality of extracted lignin by recycled ionic liquid was determined by GPC analysis. Gel permeation chromatography of lignin recovered by recycled ionic liquid showed the almost equal molecular weight of the lignin which was extracted with fresh ionic liquid in the previous study by Saha et al. (2017a, b). Table 5 strongly confirmed this result as it showed similar molecular weight ($M_n$ and $M_w$) and polydispersity index (PDI) as the lignin drawn out by pure ionic liquid.

## Conclusion

The preliminary study demonstrated the influence of imidazolium ionic liquid on sugarcane bagasse pretreatment. The introduced method recovered 90% lignin. The potentiality of this method was manifested by observing the change in bagasse structure as a result of pretreatment. In contrast to the untreated bagasse, yield of reducing sugar increased while pretreated bagasse was hydrolyzed. The purity of extracted low molecular weight lignin was determined by NMR analysis regarding the presence of essential functional groups. Recycling and reuse of [EMIM]oAc as verified by GPC analysis of lignin established the overall process as sustainable and eco-friendly. Based on this study, further investigation is necessary to study the fermentation of hydrolysate to ethanol which determines the overall efficiency of

1-ethyl-3-methylimidazolium acetate in the processing of sugarcane bagasse to bioethanol.

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