Extraction and Comparative Analysis of Lignin Extract from Alkali and Ionic Liquid Pretreatment

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Abstract. Lignin, a heterogenous polymer which presents about 20-30% in lignocellulose emerged as promising feedstock for the production of specialty chemicals. However, due to lignocellulose recalcitrance structure, the pretreatment is necessary to obtain efficient utilization of biomass. In this work, the preliminary study was carried out focusing on the properties lignin extracted from bamboo (Gigantochloa scortcheninii) using alkali and ionic liquid pretreatment. It was discovered that about 42% and 11% of acid-insoluble lignin has been extracted from alkali and IL pretreatment respectively. The degradation onset temperature of alkali and ionic liquid extracted lignin were found to be 262.20 and 172.41ºC. FTIR spectra analysis showed the characteristic peaks of lignin for both samples. Desirable products such as aldehydes, phenol, hydrocarbons and aromatics were identified from pyrolysis GC-MS

1. Introduction
Malaysia is one of the key players in international agricultural industry and generated million tons of wastes contributed by oil palm cultivation, cocoa cultivation and forestry annually [1]. Less than 20% of waste has been used for finished products while remaining is disposed by burning. Global demand for energy resources has opened a new dimension in the production of biofuel from lignocellulosic biomass. Lignocellulosic biomass such as bamboo, palm kernel and rubberwood provide massive and extensive source to fulfill energy production industry requiring. Biomass provides unlimited sources for various chemical precursors which is currently produced from fossil fuels [2].

Lignin emerged as promising feedstock for chemical industry as starting material for valuable products such as cyclohexane, benzene, xylene and toluene [3]. Regardless, the complex amorphous structure in lignin cause lignin become resistance to biological and chemical attack thus prevents the maximum utilization of biomass [4]. Therefore, pretreatment is the key step to overcome the recalcitrance of lignocellulose. Several pretreatments such as Kraft process, pyrolysis and alkali have been widely reported [5-7]. Kraft process is commonly used to extract lignin from biomass [8]. However it possesses some drawbacks. This method requires high temperature and pressure [9] and
generates pollution which is against the Green Energy policy. Meanwhile, direct pyrolysis of biomass leads to degradation of cellulose and hemicellulose thus significantly diminished production of specialty chemicals. Several alternatives has been proposed such as soda pulping, oxidative delignification, organosolve process, acidic pretreatment [10, 22], and ionic liquid (IL) pretreatment in order to efficiently extract lignin.

The extraction process of lignin from biomass by dissolution in alkali medium such as sodium hydroxide, ammonium hydroxide, hydrogen peroxide is one of common methods that being used. During alkali pretreatment, the black liquor containing carbohydrate and lignin [11] was produced resulting from the disruptions of glycosidic bond in polysaccharides and hydrolysable linkages in lignin [12]. Due to removal of acetyl group and various uronic acid substitutions, the accessibility of cellulose and hemicellulose during hydrolytic enzymes process was increased [13].

IL, termed as designated solvents have attracted greater attention due to unique properties such as low vapour pressure, low melting point and high thermal stability [14]. Wide variety combinations of cations and anions enable IL to possess numerous physical and chemical properties [15] thus make them as promising alternatives for various applications. IL pretreated biomass has recently been practiced to replace existing methods. Wu et al., used recyled 1-ethyl-3-methylimidazolium acetate (EmimOAc) for corn stover pretreatment [16] and showed the ability of ILs to interrupt the crystallinity of cellulose remain unaffected. Hui and co-workers demonstrated the pretreatment of oil palm frond (OPF) using 1-butyl-3-methylimidazolium chloride (BmimCl). It was observed that there is improvement in cellulose hydrolysis into reducing sugar [17]. In addition to IL excellent performance for dissolution, ILs reportedly gives good recyclability [16, 18]. Pinkert et al., used ILs containing food additives (acesulfamate) to extract wood lignin. They found the IL extracted has large average molar mass and more uniform compared to lignin from Kraft process [19].

In the present work, the preliminary study related to the lignin extraction from bamboo (Gigantochloa scortcheninii) comparing two different processes i.e alkali and IL pretreatments are presented. The objectives to this study are to compare the properties of lignin extracted from both processes and possible products obtained from lignin. The alkali pretreatment was carried out using fixed concentration of sodium hydroxide (NaOH) solution whereas the ionic liquid lignin extraction was performed using (BmimCl). The same concentration of acid was added into supernatants obtained from both pretreatment to lower the pH with the aim to increase the precipitation of lignin. In this study, only the yield of insoluble acid lignin was calculated the yield of acid soluble lignin will not be included in this study. Both of extracted lignin samples were analyzed by developed methods, including Fourier transform infrared spectroscopy (FTIR) and pyrolysis gas chromatography and mass spectrometer (PyGC-MS). The morphology of lignin after each pretreatment was studied by field-emission scanning electron microscope (FESEM). Meanwhile thermal stability test of the samples was carried out by thermogravimetric analysis (TGA).

2. Materials and Methods

2.1 Materials

Chemicals of analytical grade were used for the pretreatment of biomass. The CAS number, source and grades of the chemicals used are as follows: sodium hydroxide (R&M Chemicals, 97 %), acetone (Merck, 99.8 %), hydrochloric acid (Merck, 37 %), acetonitrile (Merck, 99.5 %) and 1-butyl-3-methylimidazolium chloride (BmimCl) (Merck, 99 %). All chemicals were used without further purification. The bamboo (Gigantochloa scortcheninii, a native plant of Malaysia known as “buluh semantan”) was obtained from bamboo-processing industry located at Seri Iskandar, Perak, Malaysia. The bamboo was ground and sieved to obtain fractions of particle size of less than 125 µm using Retsch Test Sieve model AS 200.
2.2 Biomass pretreatment and precipitation of acid-insoluble lignin

Alkali pretreatment of bamboo biomass was conducted using the method proposed by Mousavioun et al., with slight modification [20]. 5.00 g of biomass was loaded into 40 mL of 10 g/g NaOH solution. The mixture was then heated at 125°C. The resulting black slurry of biomass/NaOH mixture was poured into ultrapure water to recover solid known as cellulose rich materials (CRM). The solid fraction was filtered and then washed with ultrapure water a few times before drying in oven at 80°C for 24 hours. The IL pretreatment was conducted according to method suggested by Muhammad et al with slight modification [21]. 5.00 g of biomass was added into 250 mL round bottom flask contained 100 g of BmimCl. The mixture was then heated in oil bath at temperature of 110°C with stirring at 250 rpm for 24 hours. The resulting viscous bamboo/IL mixture was poured into 500 mL acetone and water (7:3) L/L under rapid agitation thus formed precipitates. The resultant precipitates were filtered. The filtrates from both pretreatments were collected. Acetone in the filtrate from IL pretreatment was removed under reduced pressure causing lignin to precipitate out. Both of the filtrates were acidified to pH 2.0 with 0.5 M of HCl to enhance lignin precipitation. The precipitates were centrifuged at 4000 rpm 30 minutes. The precipitates obtained from both pretreatments known as lignin rich materials (LRM). The LRMs were filtered and washed with acidified water (pH 2.0) and dried in the oven at 80°C for 24 hours before undergone further analysis. The LRM extracted from both pretreatments were defined as follows:

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\text{mass of acid-insoluble lignin extracted} \times \frac{100}{\text{mass of initial biomass loading}}
\]

The recovery process of ionic liquid was carried out according to Lan et al., with slight modifications [18]. The filtrate containing IL, acidified water, degraded carbohydrates was collected and 0.5 mol of NaOH solution was added until the value of pH was 9.0. Water was removed under reduced pressure. Acetonitrile was added into the solution in order to dissolve IL and leave sodium chloride as precipitates. The acetonitrile was then removed under reduced pressure to recover back IL BmimCl.

2.3 Characterization of acid-insoluble lignins

2.3.1 Field Emmission Scanning Electron Microscope (FESEM). The morphology of both extracted LRMs was observed by FESEM.

2.3.2 Fourier Transform InfraRed (FTIR). The functional groups for both extracted lignin-rich materials were determined using FTIR-ATR diamond crystal. All spectra were recorded with FTIR spectrometer Nicolet iS10 by Thermo Fisher Scientific (Waltham, Massachusetts, USA) in the range of 4000 and 400 cm\(^{-1}\) under 32 scanning.

2.3.3 Thermogravimetric Analysis (TGA). The thermal analysis for both extracted LRMs were performed using a thermogravimetric analyzer (TGA) model STA 6000 from Perkin Elmer. The analysis was done at temperature range from 50 to 850°C with heating rate 10°C•min.

2.3.4 Pyrolysis Gas Chromatography and Mass Spectrometer (Py-GC/MS). The Py-GC/MS experiments were carried out in pyrolysis cell interfaced to a gas chromatograph and mass spectrometer with direct insertion probe and pyrolysis cell (GCMS-DIPPC) from Agilent model 7890A.

2.3.5 Nuclear Magnetic Resonance (NMR). The purity of the IL before and after pretreatment were characterized using Nuclear Magnetic Resonance spectroscopy.
3. Results and Discussions

3.1 Yield of acid-insoluble lignin from both pretreatments
The yield of acid-insoluble lignin was presented in Figure 1. Both alkali and IL processes able to extract about 11% and 42% of acid-insoluble lignin respectively. The percentage of lignin extracted from alkali pretreatment was excess the percentage of lignin present in *Gigantochloa scortcheninii* as reported by various literatures [33, 34]. Analysis on gas chromatography and mass spectrometry technique revealed the production furfural during pyrolysis process which indicates the presence of hemicellulose in lignin obtained through alkaline pretreatment. The remaining of hemicellulose left during fractionation process may contribute to the cause of excess recovery of lignin obtained in alkali preteatement. On the other hand, low yield IL extracted lignin could be contributed by high degradation rate of biomass in IL which occurred during dissolution process.

![Figure 1](image1.png)

*Figure 1.* The yield of acid-insoluble lignin obtained after undergo both pretreatments.

3.2 Morphology study of acid-insoluble lignins
The morphology analysis performed by FESEM showed that both of pretreatment processes altered the fibular structure of bamboo [27]. Figure 2 shows the morphology of both alkali and IL extracted lignin. Both of pretreatment processes had removed the fibrous structure which usually present in untreated bamboo. There were formations of pores on the surface of both extracted lignin which may cause by excessive pretreatment condition. SEM images in Figure 2 showed lignin extracted from alkali pretreatment Figure 2 (a and b) consist many pores compare to ionic liquid extracted lignin Figure 2 (c and d). This may occur due to the usage of strong alkali during pretreatment process resulting in high number of pores formed.
3.3 Functional groups analysis

The functional group analysis for commercial and both of extracted lignins were carried out and shown in Figure 3. The figure showed that lignin extracted from IL pretreatment possess almost similar spectra with commercial lignin and in agreement with reported literature [11, 19, 23-24]. The peak appeared shown in area 3300 cm$^{-1}$, 2900-2800 cm$^{-1}$ and 860-830 cm$^{-1}$ for all spectra indicated the presence of hydroxyl, methoxy and syringly respectively which represented the main functional groups present in lignin. However, spectrum obtained for extracted lignin from showed high intensity of OH band. This might occur due to the introduction OH group towards the lignin during alkali treatment. The OH high intensity also can be contributed by the hemicellulose that not completely removed during fractionation process. The linkage of C-O-C in 1037 cm$^{-1}$ represents the skeletal vibrations of pentose and hexose units which commonly present in hemicellulose [24].

3.4 Thermal stability of acid-insoluble lignins

Figure 4 and 5 show the thermal analysis of two extracted lignin samples. The $T_{onset}$ temperature of IL extracted lignin is 262.20°C while alkali extracted lignin started decompose at 172.41°C. The first stage of the weight loss at both samples which occur at $\approx$120°C is associated with water evaporation [25]. The appearance of a few peaks at range 350°C to 400°C were due to slow degradation process as lignin have a very complex structure [36]. There is absence of hemicellulose degradation peak in lignin IL but the peak appears in DTG of alkali lignin. This showed that the alkali pretreatment might not change the hemicellulose content significantly which then contribute to the peak appears in region corresponding to hemicellulose degradation. It is also due to the removal of acetyl group during pretreatment that cause the thermal stability of hemicellulose [5]. The chemical degradation of hemicellulose and lignin during alkali pretreatment could be due to low thermal stability to alkali extracted lignin [26].
Figure 4. The thermal decomposition of alkali and IL extracted lignin at heating rate of 10ºC •min⁻¹.

Figure 5. DTG curve for alkali and IL extracted lignin.

3.5 Pyrolysis products of acid-insoluble lignins
Py-GC/MS was employed to determine the effect of two different pretreatments in distribution of bamboo biomass pyrolysis products. From the chromatograms, the detected products generally can be classified into phenols, acid, aromatics (benzene, toluene, xylene and styrene) and furans as shown in Figure 6 which in agreement with Lou et al., and Lin et al [29-30].

Figure 6. Peak area represented pyrolysis products obtained from alkali and IL extracted lignin.
The pyrolysis of both extracted lignin revealed high percentage of peak area represented phenols such as 2-methoxyphenol, 2-methylphenol and 4-ethyl-2-methoxyphenol. This result of proven that lignin has been successfully extracted from both treatment process as 2-methoxyphenol (guaiacol) is the main intermediate product for depolymerization of lignin [35]. This result is supported by Lou et al., which stated that phenols are the main products being produced at higher temperature (40-800ºC) of pyrolysis For alkali lignin, there is high furfural production which represented by 37 % of peak area as shown in Figure 6. However, furfural is a common product obtained during hydrolysis of hemicellulose [31]. The presence of furfural proved that hemicellulose is not fully removed during alkali pretreatment.

Similar to phenol, aromatic hydrocarbons are commonly produced at high temperature (>800ºC) or in catalytic pyrolysis as high energy is needed in order to crack methoxyl and phenolic hydroxyl groups to aromatic monomers [32] The pyrolysis of both alkali and IL extracted lignin generated several aromatics such as benzene, toluene, xylene and styrene. However, the pyrolysis aromatic product of alkali extracted lignin was limited to toluene only may be due to contamination present. Besides, there was small amount of acid, aldehydes, vanillin and vanillic acid were detected on both samples.

3.6 Recovery of Ionic Liquids
About 80% of IL had been successfully recovered after undergo dissolution. However, due to the high pretreatment temperature, degradation of some components such as wax and addition of many recovery solvents had alter the physical appearance of recovered IL as shown in Figure 7 (b) [18]. Dark color of IL might be also caused by the degradation of carbohydrate and lignin materials during dissolution process. However, this not affecting the chemical structure of IL as proved by the $^1$H NMR. The unchanged in chemical structure of IL also suggest that IL did not degraded during treatment of biomass. This result proved that IL BMIMCl can be reused for the same process.

Fresh BMIMCl
$^1$H NMR (DMSO-d$_6$): 0.902 (m, 1H, CH$_3$), 1.249-1.264 (m, 2H, CH$_2$), 1.752-1.781 (m, 2H, CH$_2$), 4.167-4.195 (t, 2H, CH$_2$), 7.746 (d, 1H, CH), 7.817 (d, 1H, CH), 9.292 (s, 1H, imi).

Recycle BMIMCl
$^1$H NMR (DMSO-d$_6$): 0.899 (m, 1H, CH$_3$), 1.242-1.302 (m, 2H, CH$_2$), 1.792 (m, 2H, CH$_2$), 4.238-4.267 (t, 2H, CH$_2$), 7.872 (d, 1H, imi), 7.953 (d, 1H, imi), 9.625 (s, 1H, imi).

4. Conclusion
Different pretreatment processes did affect the properties of extracted lignin. The yield of lignin extracted through alkali pretreatment was higher compared to IL pretreatment due to the uncompleted removal of hemicellulose during alkali pretreatment. Based on the study carried out, IL BMIMCl is suitable in treated and extracted lignin compared to alkali pretreatment. The analysis showed that it is possible to obtained pure lignin by applied the IL pretreatment without further purification process. It also appears that IL extracted lignin has higher thermal stability compare to alkali extracted lignin. The pyrolysis GC-MS of both extracted lignin generates high content of lignin intermediate products and
aromatics along with the present of acid and aldehydes. The result of pyrolysis GC-MS also suggests that IL Based on 1H NMR of recycle IL showed there is no changes in IL structure which showed that IL is thermally stable for pretreatment of biomass.

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