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Mild temperature fractionation of bagasse with ionic liquid for later conversion to sugars

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Abstract: In this work, the fractionation of sugarcane bagasse into cellulosic material and lignin was studied with various operating temperatures in 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) ionic liquid. It was observed that the dissolution temperature at 100°C for 22 h using [Bmim]Cl as solvent with bagasse loading of 5 wt.% gave the maximum regenerated cellulose 71%. Moreover, this temperature was sufficient to decrystallize the native structure of cellulose I to the low crystallinity of cellulose II, which was beneficial for subsequent hydrolysis step. The fractionation process at temperature lower than 100°C was not enough to dissolve bagasse whereas the process at higher temperature than 120°C generated higher amount of lignin in regenerated cellulosic material that led to the remaining structure of cellulose I in sample. Moreover, the ionic liquid was observed to be reusable. The reusability tests showed that lignin was a major impurity in recovered ionic liquid that affected on impurity of regenerated cellulosic material.

Keywords: Fractionation; Ionic liquid; 1-Butyl-3-methylimidazolium chloride

1. Introduction

Currently, the depletion of fossil fuels and global environmental problems drive the use of renewable resources as feedstocks for production of chemicals, fuels and energy. Lignocellulosic biomass offers a great promise to use as a new feedstock because it is abundantly available, renewable and inexpensive energy sources. However, it is not readily utilized because of net-structure constructed by cellulosic compounds and lignin. Therefore, the fractionation of lignocellulosic biomass into cellulosic fraction and lignin fraction are essential to further utilization.

Among the different routes that have been developed for the fractionation of lignocellulosic biomass, the use of ionic liquids (ILs) has received much attention. The structure of ionic liquids that are composed solely of ions with immeasurable combinations of anions and cations is helpfulness for fractionation process. The anions and the cations of ILs interact with hydrogen and oxygen atoms from the hydroxyl group of cellulose, respectively to dissolve lignocellulosic biomass in ILs [1]. Selective component is then recovered by addition of anti-solvent in solution. Therefore, the complete dissolution of lignocellulosic biomass is an important requirement for efficient biomass fractionation. The effects of types of ionic liquids and dissolution operating conditions for fractionation of biomass were widely studied at the present. For example, the biomass solubility improves when particle size of biomass decrease and the stirring speed and dissolution time increase [2]. The solubility of biomass reduces with the presence of water [3]. The chloride anion of ILs showed interesting potential to dissolve lignocellulosic biomass because of the small size and the strong electronegativity. In addition, the small size of the cation led to an easier dissolution of lignocellulosic biomass [4-5]. Herein, in this work, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) was used to fractionate of biomass to cellulosic fraction and lignin fraction. Moreover, the effect of dissolution temperature on fractionation of lignocellulosic biomass was examined to find the suitable conditions. In addition, the reusability of ionic liquid was evaluated.

2. Materials and Method

2.1 Chemical and raw materials

Analytical grade chemicals were purchased from Sigma-Aldrich. Milli-Q water (18 MΩ) obtained from a Milli-Q water purification system (Elix® Advantage system, Merck Millipore) was used to prepare various solutions. For enzyme hydrolysis, the commercial cellulose (Ctec 2, 161.43 FPU/ml) was purchased from Novozyme.

In this work, sugarcane bagasse was chosen as a biomass feedstock. Sugarcane bagasse obtained from Mitr Phol Sugar Corp., Ltd. According to the standard NREL analysis, the biomass contains cellulose 34.9 wt.%, hemicelluloses 25.0 wt.%, lignin 19.6 wt.%, and ash 2.4 wt.%.

Firstly, sugarcane bagasse was ground with a ball-milling to become find particle with the average particle size less than 75 μm. Then, sugarcane bagasse was fractionated by using ionic liquid.

2.2 Fractionation process

Sugarcane bagasse was fractionated into two fractions: cellulosic fraction and lignin fraction by using ionic liquid. In this work, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) was used as a solvent in biomass fractionation process. The bagasse sample and ionic liquid was vacuum dried at 70°C overnight prior to the fractionation step to eliminate the moisture.

Dried bagasse was added to the ionic liquid with 5%w/w biomass loading and the mixture was heated at temperature of 90-120°C for 22 h under continuous stirring for dissolution of bagasse. After dissolution step, the stirring was stopped and the mixture was maintained heated at high temperature for 1 h to sediment the suspended material. Then, the liquor containing ionic liquid and dissolved bagasse was collected by centrifugation. The 20 mL of acetone/water (1:1 v/v) was added to the 4 g of liquor

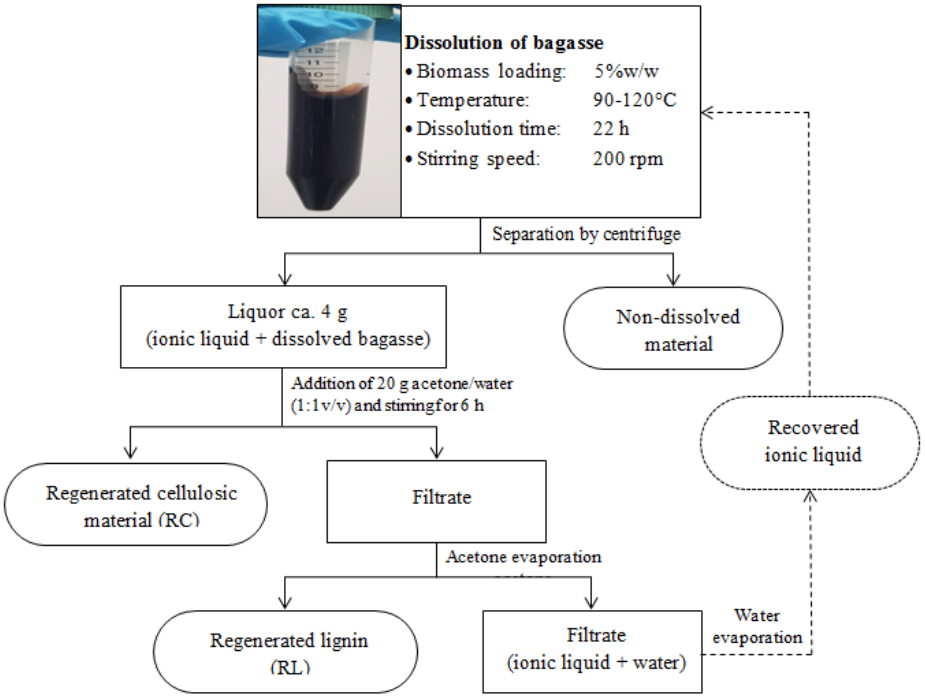

Figure 1. Schematic of biomass fractionation process.

$$\text{Mass percentage of regenerated cellulosic material (wt.\%)} = \frac{weight\ of\ regenerated\ cellulosic\ material\ (g)}{weight\ of\ cellulose\ in\ initial\ bagasse\ (g) + weight\ of\ hemicellulose\ in\ initial\ bagasse\ (g)} \times 100 \quad (1)$$

$$\text{Mass percentage of regenerated lignin (wt.\%)} = \frac{weight\ of\ regenerated\ lignin\ (g)}{weight\ of\ lignin\ in\ initial\ bagasse\ (g)} \times 100 \quad (2)$$

$$\text{Mass percentage of remain fraction in ionic liquid (wt.\%)} = \frac{weight\ of\ initial\ bagasse\ (g) - (weight\ of\ RC\ (g) + weight\ of\ RL\ (g))}{weight\ of\ initial\ bagasse\ (g)} \times 100 \quad (3)$$

Table 1 Composition of sugarcane bagasse in this work.

Cellulose	Hemicellulose	Lignin	Ash	Other	Total
34.94%	25.00%	19.62%	2.41%	18.03%	100%
0.07 mg	0.05 mg	0.039 mg	0.005 mg	0.036 mg	0.2 mg

and the mixture was stirred for 6 h to precipitate the cellulosic material. The regenerated cellulosic material (RC) was filtered by vacuum filtration, washed repeatedly with acetone/water and dried in an oven. These regenerated cellulosic materials were called as RC-120, RC-110, RC-100 and RC-90 corresponding to the dissolution temperature. Then, the filtrate was evaporated to precipitate lignin. After filtering lignin, the ionic liquid was recovered. The recovered ionic liquid was then reused for new bagasse fractionation to investigate the reusability of ionic liquid. A schematic of biomass fractionation process is presented in Figure 1.

The regenerated cellulosic material and the regenerated lignin were characterized by several techniques. Fourier transform infrared spectroscopy (FT-IR) analysis was performed using Perkin-Elmer System 2000 and the samples were prepared by using KBr pellet method. The measurement resolution was set at 4 cm^{-1} with 64 scans in the range from 4000 to 450 cm^{-1}. The microstructure of the regenerated cellulosic material was analyzed by X-ray diffraction (BRUKER, D8 Advance). The scans were collected from 5 to 60° of 2θ.

The mass percentages of each fraction were calculated using the equations (1)-(3).

2.3 Enzymatic hydrolysis

Enzymatic hydrolysis of the regenerated cellulosic materials was conducted at 50°C for 72 h and 1% (w/w) of the samples in a shaking incubator. The mixture containing biomass and 0.1 M sodium citrate buffer (pH 4.8) with 2% sodium azide was prepared with an enzyme load of 53.3 FPU/g cellulase. After hydrolysis, the mixture was heated in water bath for 5 min. Each sample was filtrated to remove insoluble solids and filtrates were used to measure reducing sugar concentrations by HPLC instrument coupled with a UV-Vis detector (Shimadzu, SPD-10AVP) with a Aminex HPX-87H (300 x 7.8 mm) column.

3. Results and Discussion

3.1 Effects of dissolution temperature

Sugarcane bagasse was used as a raw material in this work. Before fractionation testing, the characteristics of bagasse and each composition were examined. The composition of bagasse was presented in Table 1. In this work, 4 g of the solution containing ionic liquid and 0.2 g of bagasse was used as the initial solution for fractionation.

The effect of dissolution temperature on purity of regenerated cellulosic material was investigated in order to find the suitable condition. Dissolution experiments were carried out at temperature of 90-120°C for 22 h under continuous stirring. Table 2 presents the mass percentage of each fraction received by different dissolution temperatures. The bagasse could not dissolve in ionic liquid at 90°C attributed to the high melting

point of ionic liquid. Therefore, the all composition was regenerated in the first fraction. Bagasse could dissolve in ionic liquid by increasing the dissolution temperature. A mixture of acetone and water was then added in solution to precipitate the cellulosic material. The results present that 40-70% of cellulosic materials were regenerated in first fraction with difference conditions. After cellulosic material was regenerated, the acetone was evaporated from remaining solvent to precipitate lignin. The results present that only 1-7% of lignin was regenerated in this step. That might be due to the low lignin content in bagasse. The remaining solvent was then heated to evaporate water and the recovered ionic liquid was received for next reuse. The bagasse could not regenerate from this process and still remain in the recovered. Ionic liquid were presented in 'remain in ionic liquid' column in Table 2. These data indicate that the most of bagasse was still in the liquid fraction and could not be regenerated.

Table 2. The mass percentage of each fraction received by different dissolution temperatures.

Dissolution temperature (°C)	Regenerated cellulosic material (wt.%)	Regenerated lignin (wt.%)	Remain in ionic liquid (wt.%)
90	100	-	-
100	54.17	1.03	67.30
110	72.33	6.67	55.30
120	43.67	-	73.80

The composition and structure of regenerated cellulosic material (RC) were identified and compared to the commercial microcrystalline cellulose (MCC) by FT-IR and XRD. Figure 2 shows the FT-IR spectra of (a) commercial microcrystalline cellulose (MCC), (b) commercial lignin and (c) commercial xylan. The spectra of three samples present a broad band at 3500-3200 cm^{-1} that was assigned to O-H stretching vibration and a band at 2950-2850 cm^{-1} that was assigned to asymmetric and symmetric C-H stretching of CH, CH_2 and CH_3 groups. The main chemical bond vibrations of lignocellulosic materials were detected in the region of 1800-800 cm^{-1}. Therefore, this region was selected for the analysis of all samples considered in this work. The characteristic peaks of MCC appeared at 1380 cm^{-1} (a bending of C-H group in cellulose), 1166 cm^{-1} (C-O asymmetric bending vibration), 1118 cm^{-1} (secondary alcohol C-O stretching vibration), 1049 cm^{-1} (primary alcohol C-O stretching vibration) and 898 cm^{-1} (vibration of β-glycosidic C-H deformation with a ring vibration contribution (hexoses/pentoses) characteristic of glycosidic bonds). On the other hand, the characteristic peaks of lignin appeared at 1609, 1510, 1456 and 1422 cm^{-1} that are associated with aromatic skeleton vibrations. The peak at 1710 cm^{-1} assigned to C=O stretching in unconjugated ketone, carbonyl, and ester groups. The band at 1115 cm^{-1} assigned to the characteristic absorption of p-hydroxypheny (H) units. In addition, the out of plane C-H vibration in guaiacyl (G) units was observed at 840 cm^{-1}. For xylan, the band at 1631 cm^{-1} was associated with water present in the sample and 1044 cm^{-1} that was assigned to C-O stretching and C-O-C glycosidic linkage in xylan were detected.

Figure 3 shows the FT-IR spectra of the regenerated cellulosic material (RC) that received by different dissolution temperatures. The bands at 1380, 1166, 1118, 1053 and 898 cm^{-1} were characteristic peaks of cellulose, which could be mainly observed in the spectrum of RC samples. The results indicate that the most content of RC samples was cellulose. However, the characteristic peaks of lignin containing 1510, 1456 and 840 cm^{-1} were also observed in the spectrum of RC samples, especially RC-90 and RC-120. In addition, the appearance of new peaks at 1734 cm^{-1} that was assigned to ester-linked acetyl, feruloyl and p-coumaroyl groups between hemicellulose and lignin was detected. The regenerated cellulosic material was achieved through the dissolution of sugarcane bagasse in ionic liquid by their hydrophobic property and the precipitation of cellulosic material by addition of anti-solvent [1, 6]. The results revealed that [Bmim]Cl is an effective solvent to solubilize bagasse and the acetone/water mixture is an effective anti-solvent to regenerate cellulosic material with low lignin content. However, the limit dissolution of biomass at low temperature caused the high lignin content in RC-90 whereas the high lignin content in RC-120 might be due to the high interaction of ionic liquid with the aromatic lignin with high temperature [7]. In addition, with the dissolution temperature at 100 and 110 °C, a new peak appeared at 3447 cm^{-1} (O-H stretching intramolecular hydrogen bonds of cellulose II). The results indicate that cellulosic material regenerated at 100 and 110°C and the crystalline structure was transformed from cellulose I to II.

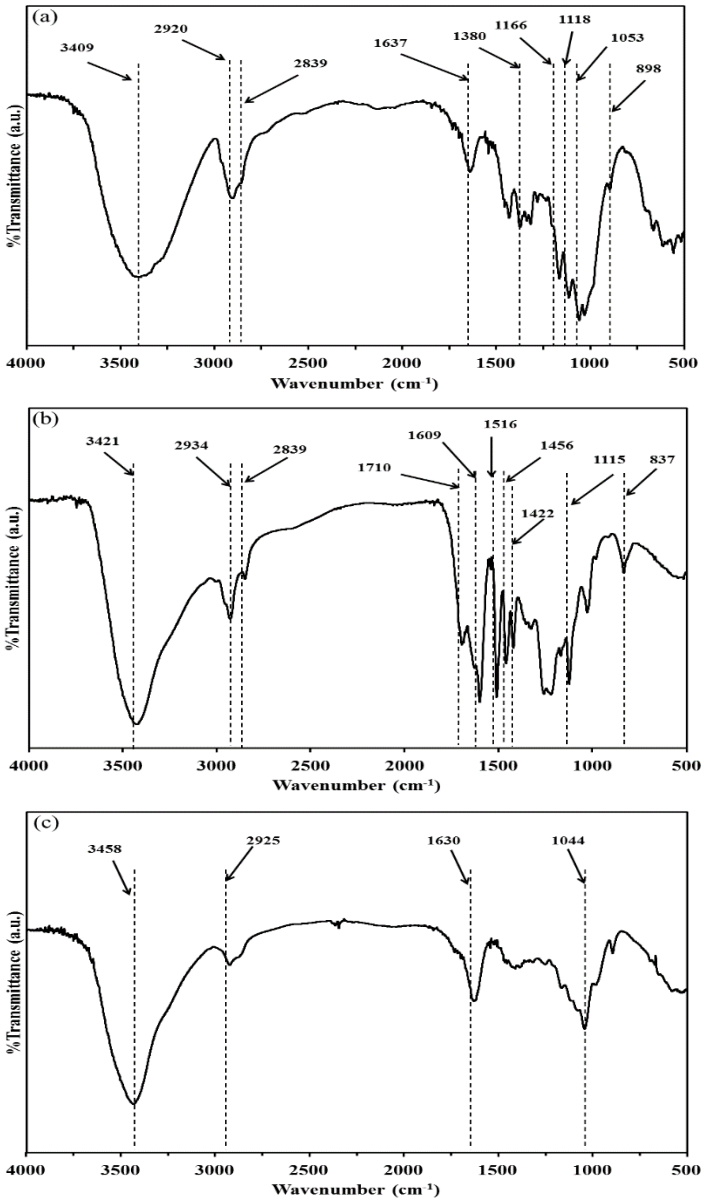

Figure 2. The FT-IR spectra of (a) commercial microcrystalline cellulose (MCC), (b) commercial lignin, and (c) commercial xylan.

Figure 4 shows the XRD patterns of the microcrystalline cellulose (MCC) and the regenerated cellulosic material (RC) that received by different dissolution temperatures. MCC presented the characteristic peaks of cellulose I containing the main peak at 22.5° and the broad peak at 16°. The peaks at 22.5° and 16° were assigned to the distance between hydrogen bonds in cellulose

I and the amorphous structure, respectively. For RC-100 and RC-110, the asymmetric doublet peak at 20.0° and 21.5° and the peak at 12° that were the characteristic peaks of cellulose II, were detected. This result indicates that the native form of cellulose I of bagasse was converted to cellulose II, which was in accordance with the FT-IR result. Compared with cellulose I, the van der Waals interaction between hydrogen-binding layers of the cellulose II was weaker [7]. Therefore, it is assumed that the cellulose II was more efficiently hydrolyzed than cellulose I. Moreover, with the increase of dissolution temperature from 100 to 110°C, a decrease in intensity of these peaks was observed. The results revealed that the high dissolution temperature disrupted the tissue network that led to the decrease in the crystallinity of regenerated material. However, the both characteristic peaks of cellulose I and cellulose II were detected in the XRD pattern of RC-120. This result indicates that RC-120 were recrystallized to both cellulose I and cellulose II or remained as an amorphous structure. The recent report noted that the lignin interrupted the recrystallization of cellulose because strong combination of cellulose, lignin and hemicellulose reduced cellulose mobility [8]. Then, the cellulose I structure in RC-120 was the result of the higher of lignin content in the sample, which was in accordance with the FT-IR result.

3.2 Enzymatic hydrolysis

The efficiency of fractionation was evaluated by enzyme hydrolysis. As shown in Figure 5, RC-90 presented the lowest sugar yield that was result from dissolution limit. The highest glucose yield after hydrolysis was obtained with RC-100. This result reveals that the low crystallinity of cellulose II was useful for enzymatic hydrolysis [7]. For RC-110, the glucose yield after hydrolysis was lower than RC-100 due to the presence of hemicellulose. On the other hand, the low glucose yield after hydrolysis in RC-120 was contributed by the contamination of lignin in structure. The presence of lignin, hemicellulose and other compounds were known to hinder the access of cellulosic material to sugar [9]. These results indicated that the increase of dissolution of bagasse with increasing dissolution temperature could directly affect the purity of regenerate cellulosic material, which were in accordance with the FT-IR and XRD results.

For comparison, Trinh et al. studied the fractionated mixed softwood (Pinus rigida and Pinus densiflora) with [Bmim]Cl [7]. This work used water as an anti-solvent to precipitate cellulosic material, which was difference with our system. They reported that most of softwood was recovered (83.6%). The high mass recovery might be a result of performance of anti-solvent. However, RC-100 presented the higher purity of cellulosic material than the regenerated cellulosic material of previous work [7], as shown in Table 3. This result indicates that the mixtures of acetone/water presented the higher selectivity to recovered cellulosic material than water.

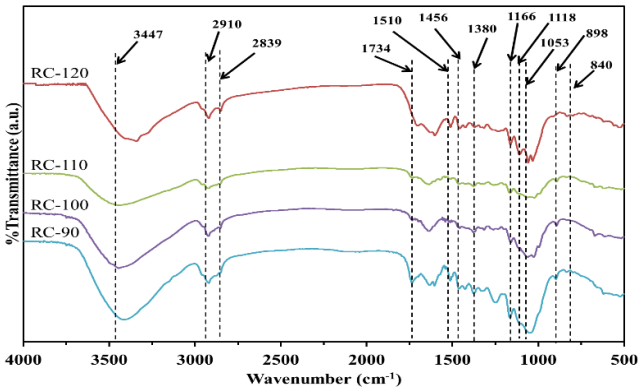

Figure 3. FT-IR spectra of the regenerated cellulosic material (RC).

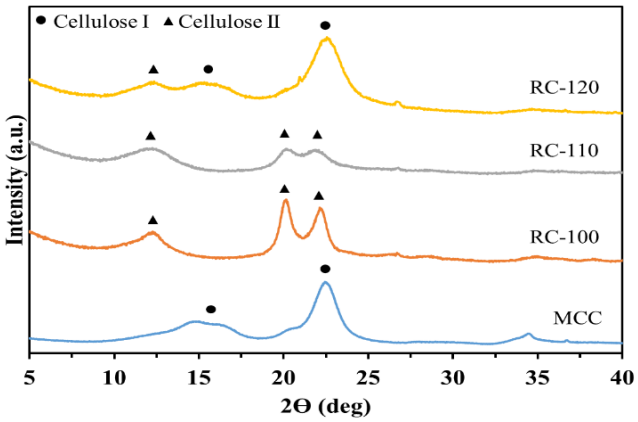

Figure 4. The XRD patterns of the microcrystalline cellulose (MCC) and the regenerated cellulosic material (RC) received by different dissolution temperatures.

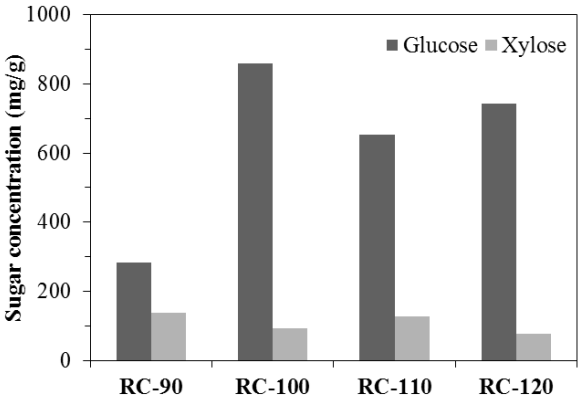

Figure 5. Sugar yield from enzymatic hydrolysis of the regenerated cellulosic material (RC) received by different dissolution temperatures.

Table 3. Fractionation of biomass in this work compared with Trinh et al. [7].

Biomass	Operating condition	Composition of regenerated cellulosic material			Reference
		Cellulose	Hemicellulose	Lignin	
Mixed softwood (Pinus rigida and Pinus densiflora)	130°C, 15 h	40.9%	8.5%	37.3%	[7]
Sugarcane bagasse	100°C, 22 h	71.2%	3.2%	12.4%	This work

Table 4 The mass percentage of each fraction received by fresh and recovered ionic liquid.

Sample	Regenerated cellulosic material (wt.%)	Regenerated lignin (wt.%)	Remain in ionic liquid (wt.%)
Fresh ionic liquid	43.67	-	73.80
Recovered ionic liquid	49.08	195.90	32.35

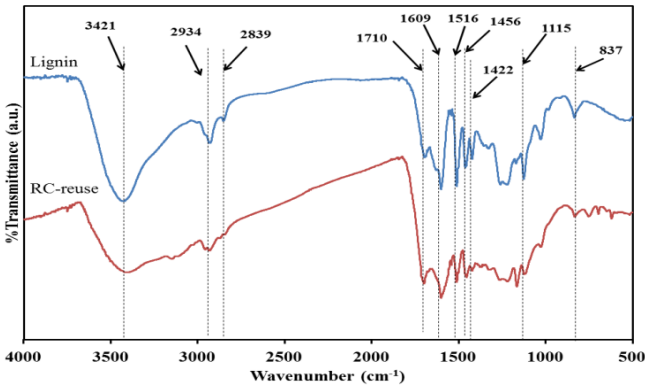

Figure 6. FT-IR spectra of commercial lignin and the regenerated cellulosic material received by using recovered ionic liquid (RC-reuse).

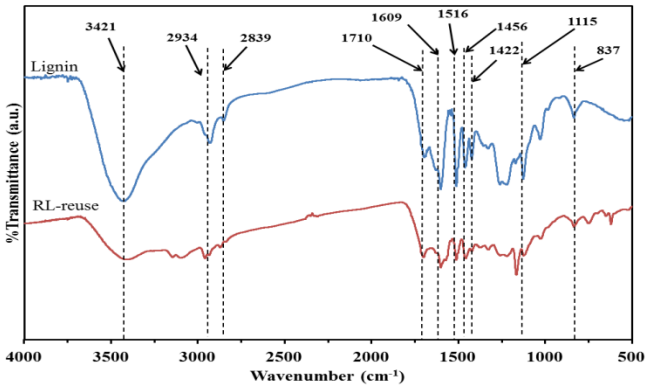

Figure 7. FT-IR spectra of commercial lignin and the regenerated lignin received by using recovered ionic liquid (RL-reuse).

3.3 Recycled ionic liquid performance

The recovered ionic liquid containing ionic liquid and residual bagasse was reused for fractionation new bagasse fractionation at 120°C. As shown in Table 4, the recovered ionic liquid presented the high mass percentage of both regenerated cellulosic material and regenerated lignin compared with fresh ionic liquid. It might be a result of high remaining biomass in ionic liquid that referred to high solid loading in recycles cycle. The mass percentage of each fraction received by recovered ionic liquid presented in Table 4 was calculated based on mass of new added bagasse. The mass of residual bagasse contaminated in recovered ionic liquid was ignored to calculate. Therefore, the more than 100 wt.% of mass percentage of regenerated lignin that received by using recovered ionic liquid was a result of the regenerated of residual bagasse in this fraction. These results suggest that high solid loading could enhance the fractionation performance in terms of quantity.

The regenerated cellulosic material (RC-reuse) and regenerated lignin (RL-reuse) that received by using recovered ionic liquid were characterized by FT-IR , as shown in Figure 6 and Figure 7, respectively. However, the FT-IR spectra of RC-reuse mainly presented the characteristic peaks of lignin. This result indicates that lignin was the main composition of RC-reuse instead of cellulosic material. It might be due to the high residual water content or residual bagasse that referred to high lignin content. The recent report noted that the water content in recovered ionic liquid was increase during recycled ionic liquid [10-11]. This residual water acted as competitor with cellulose to bond with the anion of ionic liquid. Moreover, the residual lignin affected the rheological properties and purity of recovered ionic liquid that led to the decrease of the solubility of cellulose and the low cellulose content in regenerated cellulosic material. These results present that although the quantity of regenerated material was increase by increase the high solid loading, the quality of regenerated material was decrease. Therefore, it suggests that recovered ionic liquid should be purified before reuse.

As presented in Figure 6, FT-IR spectra of RL-reuse completely accorded with that of commercial lignin. This result indicates that the most content of RL-reuse is lignin. According to a previous reason, the formation of hydrogen bonds between lignin and ILs led to the highly lignin content in regenerated material.

4. Conclusions

The dissolution of sugarcane bagasse was carried out by 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) ionic liquid. Regeneration of cellulosic material was carried out by precipitation with acetone/water mixture. The process was efficient to regenerate the high purity cellulosic compounds. The dissolution at 100°C helps the transition from the native structure of cellulose I to the low crystallinity of cellulose II. In addition, the study of reusability of ionic liquid presented that the impurity in recovered ionic liquid caused the high lignin content in regenerated cellulosic material.

Acknowledgements

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References

[1] Li, W., Ju, M., Wang, Y., Liu, L. and Jiang, Y. 2013. Separation and recovery of cellulose from Zoysia japonica by 1-allyl-3-methylimidazolium chloride, *Carbohydrate Polymers*, 92, 228-235.

[2] Carneiro, A.P., Rodríguez, O. and Macedo, E.A. 2017. Dissolution and fractionation of nut shells in ionic liquids, *Bioresource Technology*, 227, 188-196.

[3] Hou, Q., Ju, M., Li, W., Liu, L., Chen, Y. and Yang, Q. 2017. Pretreatment of lignocellulosic biomass with ionic liquids and ionic liquid-based solvent systems, *Molecules*, 22, 490-513.

[4] Badgujar, K.C. and Bhanage, B.M. 2015. Factors governing dissolution process of lignocellulosic biomass in ionic liquid: Current status, overview and challenges, *Bioresource Technology*, 178, 2-18.

[5] Muhammad, N., Man, Z., Mutalib, M.I.A., Bustam, M.A., Wilfred, C.D., Khan, A.S., Ullah, Z., Gonfa, G. and Nasrullah, A. 2015. Dissolution and separation of wood biopolymers using ionic liquids, *Chem. Bio. Eng. Rev.*, 2, 1-23.

[6] Olivier-Bourbigou, H., Magna, L. and Morvan, D. 2010. Ionic liquids and catalysis: Recent progress from knowledge to applications, *Applied Catalysis A: General*, 373, 1-56.

[7] Trinh, L.T.P., Lee, Y.J., Lee, J.W. and Lee, H.J. 2015. Characterization of ionic liquid pretreatment and the bioconversion of pretreated mixed softwood biomass, *Biomass and Bioenergy*, 81, 1-8.

[8] Kim, H., Anh, Y. and Kwak, S.Y. 2016. Comparing the influence of acetate and chloride anions on the structure of ionic liquid pretreated lignocellulosic biomass, *Biomass and Bioenergy*, 93, 243-253.

[9] da Costa Lopes, A.M., João, K.G., Rubik, D.F., Bogel-Łukasik, E., Duarte, L.C., Andreaus, J. and Bogel-Łukasik, R. 2013. Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation, *Bioresource Technology*, 142, 198-208.

[10] Xu, J., Liu, B., Hou, H. and Hu, J. 2017. Pretreatment of eucalyptus with recycled ionic liquids for low-cost biorefinery, *Bioresource Technology*, 234, 406-414.
[11] Ding, J.C., Xu, G.C., Han, R.Z. and Ni, Y. 2016. Biobutanol production from corn stover hydrolysate pretreated with recycled ionic liquid by Clostridium saccharobutylicum DSM 13864, *Bioresource Technology*, 199, 228-234.