Pretreatment of Oil Palm Frond (OPF) by Electron Beam Irradiation and Ionic Liquid Method

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Abstract— **In this study, the studied lignocellulosic biomass (LCB), oil palm frond (OPF) is pretreated with an ionic liquid (IL), 1-Ethyl-3-methylimidazolium acetate [EMIM]Ac. The pretreatment combines physical and physicochemical pretreatment for dissolving the biomass cellulose. The sample is first pretreated by electron beam irradiation (EBI) at Agensi Nuklear Malaysia which the irradiation dose of 100, 200, 400, 600, 800 and 1000 kGy with 2 MeV of voltage accelerator and 5 mA current flow. Ionic liquid pretreatment is then carried out using Bioshake at temperature 70 - 99°C for 4 hours with the speed of 800 rpm. Until the supernatant is achieved, the sample is being dried in the oven and analyzed by using Fourier Transformation Infrared (FTIR) spectroscopy. FTIR spectroscopy allows the composition of the biomass to be examined, qualitatively and quantitatively based on the absorbance of infrared radiation. TAPPI method is used to determine cellulose, hemicellulose and lignin degradation. The resulting FTIR spectra of the EBI and IL pretreated oil palm frond and cellulose show that the lignocellulosic biomass components degradation increases as the EBI doses increases, yet the spectra reading trend got a fluctuation due to error that possibly caused by contamination and particle size of LCB. The resulting TAPPI method shows that the cellulose composition and lignin degradation increase as the EBI doses increases, nevertheless the reading instability present in the middle of the doses range which probably due to excessive water presence in the ionic liquid and inhibitory factor that prevents the cellulose to be soluble and lignin to be degraded, respectively.**

Keywords— Ionic liquid, lignocellulosic biomass, nuclear irradiation, oil palm frond, pretreatment

I. INTRODUCTION

At the present time, the world are facing energy resources complications due to the documented issues of fossil fuels, i.e rising cost and unpredicted instabilities, releases of greenhouse gases, global warming and limited source. Thus, various alternatives have been strategized to overcome these difficulties. Fortunately, the awareness of these issue has been increasing, as can be seen from the developed field of renewable resources, sustainable energy which economically practical, for instance, bioethanol.

The bioethanol production from lignocellulosic material has been widely used all around the world because of the abundant renewable resources and the major constituent of biomass such as bagasse, wheat straw and wood chips. Lignocellulose which consists of hemicellulose, cellulose and lignin can be extracted into sugar for energy conversion.

However, the production of bioethanol from lignocellulosic material causes the process to be high in cost and not environmentally friendly and usually, the structure of the lignocellulose is hard to be broken and thus causing it hard to be analyzed. Furthermore, degradation of lignocellulosic facing few difficulties, where the peak is related to complex and occupied density of the lignin and carbohydrates (Zhu *et al.*, 2008; Lee *et al.*, 2009). Plus, whenever lignin structure is broken, only then the crystalline structure of cellulose could be unsettled.

Pretreatment is one of the way to ensure the biomass structure includes lignocellulose to be hydrolyzed. Pretreatment has many types, for example, physical, biological, chemical and physiochemical (Poornejad, Karimi and Behzad, 2014). This method allowing hydrogen bond to be disturbed and broken down besides increasing the area of the surface and the permeability. Nevertheless, pretreatment methods are facing several circumstances which give challenges in the pretreatment process. These include high cost and energy usage, environmental effluence and long residence time (Zhao *et al.*, 2009; Shill *et al.*, 2011).

Hence, in this research, the main purpose it is being conducted is to apply physicochemical pretreatment method which combines electron beam irradiation (EBI) and ionic liquid (IL) pretreatment using 1-ethyl- 3-methylimidazolium acetate, [EMIM]Ac on the lignocellulosic biomass (LCB) sample used, oil palm frond (OPF).

According to (Kristiani *et al.*, 2016), a broad practice of irradiation technologies especially electron beam irradiation is to transform the materials properties. EBI pretreatment produces gives out inhibitory or repressive byproducts fewer than the previous techniques which are thermochemical. The EBI pretreatment chain scission mechanism which is attacks of electron, emphasizes the alteration or degeneration of substrates crystallinity structure. Besides that, ionic liquid pretreatment is used due to its efficient ability as compared to other various methods. Furthermore, ionic liquid could be recycled back and reused repeatedly along the pretreatment process in spite of its high cost.

In addition, another purpose of the research is so that the analysis of the untreated and pretreated LCB sample were analyzed which obtained from X-ray diffraction (XRD) and Fourier Transformation Infrared (FTIR) spectroscopy to determine the biomass crystallinity and allow the composition of the biomass to be examined, qualitatively and quantitatively, respectively, based on the absorbance of infrared radiation.

II. METHODOLOGY

A. Preparation of lignocellulosic biomass (LCB) raw material and experimental parameters

At first, the lignocellulosic biomass underwent drying process. It was dried in the oven (Memmert, Germany) for 20 minutes with the temperature of 80°C. After that, the LCB underwent milling process which was being done at Manufacturing Workshop, Mechanical Engineering Faculty of UiTM Shah Alam. The LCB was then being sieved by siever so that the LCB's particle size was less than 250 µm. Next, the moisture content of the sample was being confirmed and calculated by putting in the oven at temperature of 105ºC and 24 hours. To get the moisture content, M_x which is below 10%, the formula (Equation 3.1 and 3.2) below was being used to justify its condition.

$$\text{Moisture content} = \frac{W_w - W_d}{W_w} \qquad \textit{(Equation 3.1)}$$

W_w = Weight of wet sample
W_d = Weight of dry sample

$$\text{Standard deviation of moisture content, } W_x = \left| \frac{W_f - W_i}{W_i} \right| \times 100\% \qquad \textit{(Equation 3.2)}$$

W_f = Weight of sample at time t_n
W_i = Weight of sample at time t_{n-1}

The optimized parameter is required to conduct LCB pretreatment, such as, dose amount for irradiation method. LCB were then undergoes pretreatment by ionic liquid after irradiation method is completed.

B. Electron Beam Irradiation Pretreatment

Electron beam irradiation pretreatment process was being performed at Agensi Nuklear (MINT), Bangi as the equipment required available there. The process was observed by engineers in MINT. As to begin the pretreatment process, the LCB samples were vacuumed by vacuum sealer. After that, the samples were irradiated at different dose; 100, 200, 400, 600, 800 and 1000 kGy. An electron beam accelerator was being utilized to complete the irradiation process with electron voltage of 2 MeV (Nissin EPS3000, Japan).

To prevent any occasion inside the irradiation room, the samples were put on the conveyor trolley and taped. Then, the conveyor trolley entered the irradiation room which moved by the conveyor tracks. The irradiation dose is set to 50 kGy for each round of irradiation. Since the dose has varies values, so, the conveyor trolley will pass across the irradiation room for certain round to fulfill the required dose for each sample, for instance, 20 rounds across the irradiation room to complete the dose of 1000 kGy. To prevent any dripping due to high temperature and blower presence in the process, the samples container for every 200 kGy, are changed and vacuum sealed. The parameter details are tabulated as below.

Table 3. 1 Parameters of conditions involved in pretreatment of lignocellulosic biomass by electron beam irradiation method

Parameter	**Range of parameter**
Irradiation Dose	100, 200, 400, 600, 800 and 1000 kGy
Irradiation Dose per Pass inside the accelerator	50 kGy
Voltage Accelerator	2 MeV
Current Flow	5 mA

C. Preparation of 0.5M of [EMIM]Ac solution

[EMIM]Ac is mixed with deionized water by using 500 mL volumetric flask. Measuring cylinder was used to measure the volume of [EMIM]Ac to be poured into a volumetric flask of 500 mL. Deionized water then was added slowly into the similar volumetric flask until the volume needed is achieved. Next, to ensure that the [EMIM]Ac solution and deionized water were mixed well, the volumetric flask was shaken. To complete the preparation of 0.5M [EMIM]Ac solution, the calculation by using Equation 3.3 as shown below, is used to obtain the volume needed of 95% [EMIM]Ac to produce 50% wt solution.

$$C_1V_1 = C_2V_2 \qquad \textit{(Equation 3.3)}$$

C_1 = Initial concentration of 95% [EMIM]Ac solution
V_1 = Volume of [EMIM]Ac needed to make 50% wt solution
C_2 = Final Concentration [EMIM]Ac solution
V_2 = Volume of final solution
Based on the calculation made, volume needed to prepare 500mL of 50% [EMIM]Ac solution is by using 263 mL of 95% [EMIM]Ac stock solution.

D. Pretreatment of LCB with ionic liquid

At first, LCB with mass of 0.25 g were mixed with 5 mL of diluted [EMIM]Ac solution. It was then being heated and shaken continuously at 50°C for 4 hours and 1800 rpm respectively. Next, the mixture was inserted into centrifuge machine and 5 mL of distilled water were added into the tube so the ionic liquid will be washed thoroughly. The pretreated mixture tube was then centrifuged at 14000 rpm for 4 minutes in 3˚C. The supernatant (liquid) should also be removed.

After that, using anti-solvent (water), the pretreated biomass was washed carefully and being centrifuged afterwards. As long as no colourless supernatant was gained, the washing and centrifuging steps were repeated more than 4 times. A colourless supernatant presence showed that the pretreated biomass has no more [EMIM][Ac]. Next, as for the afterward analysis preparation, the pretreated biomass was gathered and dried using oven at 60˚C for 24 hours. A table of pretreated LCB by ionic liquid parameter information is given in Table 3.2

Table 3. 2 Parameters of conditions involved in pretreatment of lignocellulosic biomass by ionic liquid method

Parameter	**Range of parameter**
Concentration of [EMIM]Ac	50%
Temperature	70 - 99°C
Mass loading of LCB	0.25 g
Speed for Bio-shake IQ	800 rpm
Time of pretreatment	4 hours

E. Determination of cellulose, hemicellulose and lignin

1) Alpha-cellulose determination

25.0 mL of mL of the filtrate and 10.0 mL of 0.5N potassium dichromate solution were pipetted into a 250-mL flask while swirling it. Then, 50 mL of concentrated H_2SO_4 was added cautiously into the flask. The solution was then allowed to stay hot for 15 min, then 50 mL of water was added and it was cooled to room temperature. After that, 2 to 4 drops of Ferroin indicator were added and 0.1N ferrous ammonium sulfate (R&M, USA) solution was titrated to a purple color. A blank titration was made afterwards to substitute the pulp filtrate with 12.5 mL of 17.5% NaOH and 12.5 mL of water.

a) Alpha-cellulose content calculation

$$\text{Alpha-cellulose, \%} = 100 - \frac{6.85\,(V_2 - V_1)\times N \times 20}{A \times W} \quad \textit{(Equation 3.4)}$$

Where:
V_1 = Pulp filtrate titration, mL
V_2 = Blank titration, mL
N = Exact normality of the ferrous ammonium sulfate solution
W = Oven-dry weight of pulp specimen, g
A = Volume of the pulp filtrate used in the oxidation, mL

2) Beta- and gamma-cellulose determination

50.0 mL of the pulp filtrate was pipetted into a 100-mL graduated cylinder with a ground glass stopper. Then, 50.0 mL of 3N H_2SO_4 was added and mixed meticulously by upturning it. After that, the cylinder was heated by submerging it in a hot water bath at 70°-90°C for several minutes to coalesce the beta-cellulose. The precipitate was allowed to resolve for few hours, if possible overnight, then if necessary, it was being filtered to gain a clear solution. Next, 50.0 mL of the clear solution and 10.0 mL of 0.5N $K_2Cr_2O_7$ are pipetted into a 300-mL flask and 90 mL of concentrated H_2SO_4 is added cautiously. The solution was allowed to stay warm for 15 min, then continued with titration. A blank titration was made afterwards to substitute the solution with 12.5 mL of 17.5% NaOH, 12.5 mL of water and 25 mL of 3N H_2SO_4.

a) Beta-cellulose content calculation

Beta-cellulose, % = 100 - (alpha-cellulose % + gamma-cellulose %) *(Equation 3.5)*

b) Gamma-cellulose content calculation

$$\text{Gamma cellulose, \%} = \frac{6.85\,(V_4 - V_3)\times N \times 20}{25 \times W} \quad \textit{(Equation 3.6)}$$

Where:
V_3 = titration of the solution after precipitation of beta-cellulose, mL
V_4 = blank titration, mL

3) Lignin degradation

Kappa number (K) method (Chai and Zhu, 2002) was used to determine lignin degradation which calibrated with Klason lignin method (Rowell, 2005). 0.05 g oven- dried treated OPF sample (ODT) was mixed with 2 M 20 mL of H_2SO_4 and 5 mL of 0.02 M $KMnO_4$ (R&M, USA). The mixture was agitated at 200 rpm for 5 min and it was then filtered. The filtrate was analyzed by using UV–vis at 546 nm and the reading of the absorbent was stated as A_c which was measured in nm. The experiment controller used was a blank sample containing $KMnO_4$ and H_2SO_4 mixtures. The blank sample absorbent was stated as A_o which was measured in nm. The OPF lignin degradation is calculated based on equation below.

$$\text{Kappa number, } K = 100\left[\frac{A_O - A_{CT}}{A_O}\right] \quad \textit{(Equation 3.7)}$$

$$\text{Content of lignin} = 0.15K \quad \textit{(Equation 3.8)}$$

$$\text{Lignin degradation (wt.\%)} = \frac{A_O - \frac{A_{CT}}{A_{VT}}}{A_O} \times 100 \quad \textit{(Equation 3.9)}$$

A_O = Absorbance of blank sample
A_{CT} = Absorbance of treated sample
A_{VT} = Absorbance of untreated sample

F. Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectrometry (Equinox 55, Bruker Germany) is used to characterize the samples chemical structure, whether it was treated or untreated. FTIR spectroscopy determines the samples changes in term of functional groups (Lai *et al.*, 2016). The samples were mixed with potassium Bromide (KBr) after being dried. Then, the samples were pressed into discs (Mood *et al.*, 2013) and were positioned on the tin-holder. The four scans were documented with range of the spectra directed are 4000–700 cm^{-1} at 4 cm^{-1} of resolution (Uju *et al.*, 2012).

$$\text{Lateral Order Index } (LOI) = \frac{A_{1424}}{A_{896}} \quad \textit{(Equation 3.10)}$$

At 1424 and 896 cm^{-1} as the ratio of peak areas which is gained from the FTIR spectrum (Ninomiya *et al.*, 2011).

III. RESULTS AND DISCUSSION

A. Fourier Transform Infrared (FTIR) Spectroscopy

i) Lateral Order Index

Table 1 LOI values of EBI pretreated oil palm frond

Dose	A1424	A896	LOI
0	77.94169	69.83428	1.116143
100	90.27213	86.18649	1.047405
200	91.85107	88.55058	1.037272
400	94.20529	91.7194	1.027103
600	90.50398	86.65542	1.044412
800	92.04511	87.9667	1.046363
1000	91.60669	88.1512	1.0392

Table 2 LOI values of EBI and IL pretreated oil palm frond

Dose	A1424	A896	LOI
0	77.94169	69.83428	1.116143
100	94.09019	90.61001	1.038408
200	94.34493	90.58164	1.041546
400	93.9166	90.93508	1.032787
600	92.94222	89.92027	1.033607
800	95.99536	93.69523	1.024549
1000	89.46169	83.81021	1.067432

Table 3 LOI values of EBI pretreated cellulose

Dose	A_{1424}	A_{896}	LOI
0 (Raw)	97.47853	95.539	1.020387
100	95.73932	96.78963	0.989149
200	96.50416	93.72745	1.029625
400	95.03004	91.03086	1.043932
600	92.647	87.18158	1.06269
800	90.42074	83.23368	1.086348

1000	93.39522	88.73945	1.052466

Table 4 LOI values of EBI and IL pretreated oil palm frond

Dose	A_{1424}	A_{896}	LOI
0 (Raw)	97.47853	95.539	1.020387
100	94.33537	90.43611	1.043116
200	96.10998	93.77498	1.0249
400	93.2347	88.81446	1.049769
600	92.20112	86.732	1.063058
800	93.90904	89.53282	1.048878
1000	94.22203	90.05413	1.046282

Lateral order index of oil palm frond which had been pretreated by electron beam irradiation exhibit decreasing value. Similar to oil palm frond which had been pretreated with electron beam irradiant and ionic liquid, yet its value slightly increased for dose 1000 kgY. As for cellulose samples which had been pretreated with electron beam irradiation and both of electron beam irradiation and ionic liquid, the results displayed were contrast with oil palm frond trend. LOI values of cellulose samples increased gradually but decreased when approaching 800 and 1000 kgY.

LOI value was interconnected with the cellulose overall degree. Based on the results, the increasing value of LOI values show that the high degree of crystallinity and more ordered cellulose structure than the decreasing value. It also showed that fraction of crystalline in cellulose is major (Kruer-Zerhusen, Cantero-Tubilla and B. Wilson, 2017). The decreasing or low LOI values possibly represents that the cellulose content was composed of more amorphous or unstructured areas (Poletto, Ornaghi and Zattera, 2014).

ii) IR Spectra

Figure 1 FTIR spectra of EBI pretreated OPF

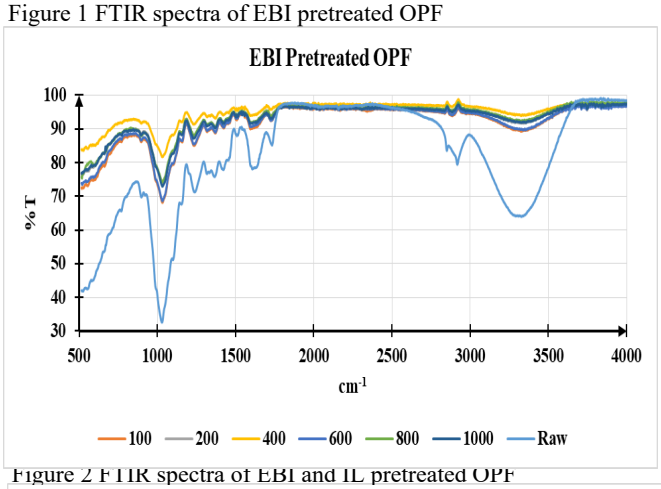


Figure 2 FTIR spectra of EBI and IL pretreated OPF

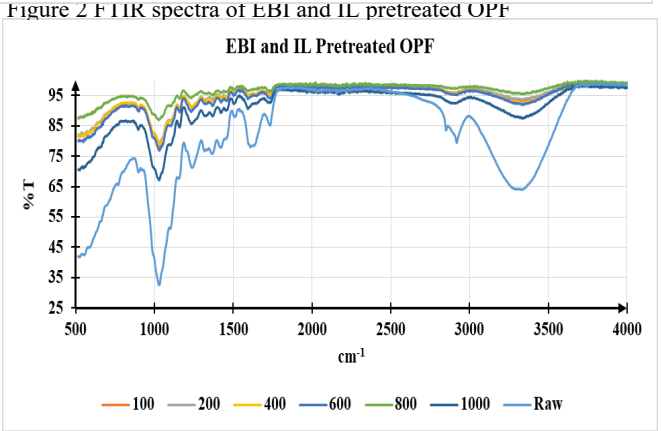


Figure 3 FTIR spectra of EBI pretreated cellulose

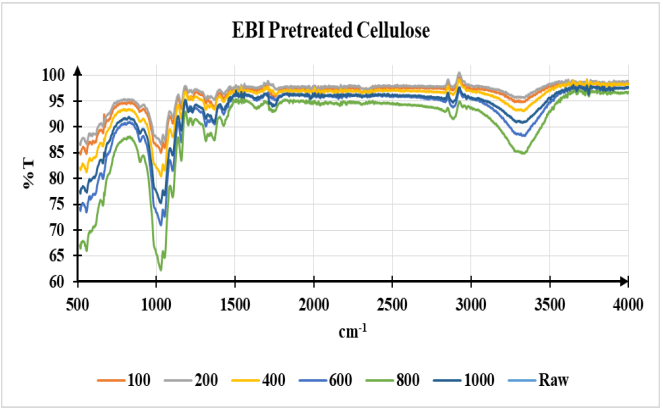
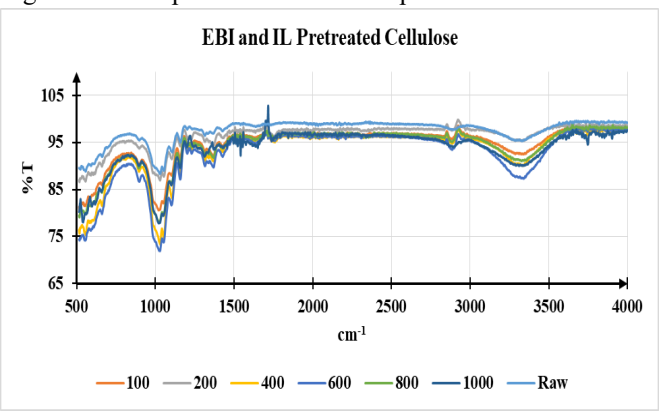


Figure 4 FTIR spectra of EBI and IL pretreated cellulose



Based on the Figure 4.4 and 4.5, the spectra shows that the functional group degraded as EBI dose increases from 100 to 1000 kgY. Cellulose and hemicellulose degradation were observed at 1159 cm^{-1} where C-O-C functional group stretched asymmetrically. At 1752 cm^{-1} shows hemicellulose degradation where there were unconjugated ketones, carbonyl and ester. At 1029 cm^{-1}, the degradation of cellulose, hemicellulose and lignin was observed, the peak also presents aromatic ring and primary alcohol functional group. Other chemical structure degradation detected from the resulting spectra were associated –OH (3311 cm^{-1}) and $-CH_2$ and $–CH_3$ (1370 cm^{-1}).

The inconsistency spectra trend reading may cause any error to happen. According to M. Chalmers, 2006, there are various causes which may contribute to the common errors. In this scope of research, the error may be due to the particle sizes of the lignocellulosic biomass sample. As to gain a good spectral contrast, good quality with low overlay of artifact/ anomalous effects, therefore, the particle's mean size should be below of the wavelengths used to analysis the sample. The approvable size range should be lower than 10 µm and if possible under 2 µm. Besides, the inconsistency may occur due to the contamination. The contamination here is meant by the materials that may cause the absorption bands to be misread rather than its predictable form. The contamination may present from the residual solvent in a sample container from causing it to be impurities.

B. The Composition of Cellulose

Table 5 Cellulose composition of EBI pretreated OPF

Dose (kgY)	Alpha (%)	Beta (%)	Gamma (%)

0	80.74693	22.32186667	3.0688
100	80.78347	29.044	9.827467
200	79.57787	29.1536	8.731467
400	81.40453	23.85626667	5.2608
600	81.95253	12.1656	-5.88187
800	85.05787	19.91066667	4.968533
1000	87.0672	10.99653333	-1.93627

Based on the results, it is shown that the undegraded or crystalline cellulose are the domain in the biomass sample, followed by the degraded cellulose and then hemicellulose. As the electron beam irradiation increases, percentage of beta- and gamma-cellulose or also known as degraded cellulose and hemicellulose, respectively, increases but then decreases at 600 kgY.
The cellulose content supposedly increases as the dose increases, indicating the higher force being applied for cellulose dissolution. The errors possibly occur due to the water presence in the ionic liquid. According to Mäki-Arvela *et al.*, 2010, the presence of water in the ionic liquid can cause the declination of cellulose solubility. This is due to inhibition of the solubilization of the cellulose microfibrils hydrogen-bonding. Addition of water to ionic liquid above 0.5 mole fraction of water, causing the solvent properties were expressively reduced, hence the cellulose was not soluble anymore.

C. Lignin degradation

Based on the results, it is shown that the lignin degradation increases by the increasing irradiation dose. Yet, its percentage decreases among the doses before the value get increased back, for example, 400 kgY for EBI pretreated OPF, 600 kgY for EBI and IL pretreated OPF and 200, 600 kgY for EBI pretreated cellulose. Lignin degradation supposedly increases gradually along with the increasing irradiation doses.

Table 6 Lignin degradation of EBI pretreated OPF

Dose	LD (%)
Raw (0)	N/A
100	2.144
200	11.941
400	7.909
600	2.230
800	4.551
1000	8.228

Table 7 Lignin degradation of EBI and IL pretreated OPF

Dose	LD (%)
Raw (0)	N/A
100	17.095
200	17.493
400	21.760
600	15.992
800	24.134
1000	32.075

Table 8 Lignin degradation of EBI pretreated cellulose

Dose	LD (%)
Raw (0)	N/A
100	46.382
200	-4.529
400	145.021
600	-2.728
800	65.305
1000	68.189

According to Jönsson and Martín, 2016, the degradation did not occur due to inhibitory formation. Although inhibitors formation is partial, the small ionic liquid amounts which remained in the pretreated biomass are possibly toxic to fermentative microorganisms and enzyme. Therefore, detoxification method is needed, such as usage of chemical additives including alkaline treatment or reducing agents and polymers, to overcome the inhibition problem of lignin degradation.

IV. CONCLUSION AND RECOMMENDATION

The objectives of the research study had been fulfilled. The biomass sample which is oil palm frond and cellulose as the reference, undergoes electron beam irradiation and ionic liquid pretreatment as to break down the chemical composition such as cellulose, hemicellulose and lignin. The resulting of the dissolution of the lignocellulosic biomass component were then analyzed by using Fourier Transform Infrared spectroscopy and TAPPI method which determines the cellulose composition.

The FTIR which allows the composition of the biomass to be examined, qualitatively and quantitatively based on the absorbance of infrared radiation, produced spectra of each of the oil palm frond sample and cellulose sample, with electron beam irradiation pretreated only and both of electron beam irradiation and ionic liquid pretreated. The transmission percentage of the lignocellulosic functional group decreases which represents degradation throughout the increasing doses but somehow increases among the doses due to some error, possibly caused by contamination and particle size. Therefore, it is recommended to be more alerted regarding the biomass particle sizes to be below than the limited range or at the preferable range. Moreover, the equipment used along the research should be cleaned in a proper way so that there is no contamination mixed with the samples which causes the error to happen.

As for the cellulose determination, the calculated results displayed positive feedback where the cellulose percentage increases as the doses increases, as well as the lignin degradation yet fluctuation happened between the lowest and the highest dose. This incident happened possibly due to excessive water content in the ionic liquid and inhibitory factor which cause the cellulose to be insoluble and the lignin to be undegraded, respectively. Therefore, it is compulsory for any chemical or material used to be complied with the standard volume or dosage to encourage the cellulose degradation, plus, use the chemical additives to detoxify the biomass and overcome the inhibition of lignin degradation.

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