Pretreatment of Oil Palm Frond (OPF) with Ionic Liquid

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Abstract. Pretreatment is the key to unlock the recalcitrance of lignocellulose for cellulosic biofuel production. Increasing attention has been drawn to ionic liquids (ILs) for pretreatment of lignocellulosic biomass because this approach was considered as a green engineering method over other conventional methods. In this work, Oil palm frond (OPF) was pretreated by using the ionic liquid 1-ethyl-3-methylimidazolium acetate \([\text{EMIM}]\text{Ac}\) at the temperature of 99˚C for 3 hours. The characterization of the untreated and pretreated OPF was conducted by using different techniques which are Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). The pretreatment of OPF with \([\text{EMIM}]\text{Ac}\) was demonstrated to be effective evidenced by the significant reduction of Lateral Order Index (LOI) from FTIR, reduction of Crystallinity Index (CI) based on XRD and the significant morphology changes indicated by SEM. The CI value for the pretreated OPF decreased from 0.47 (untreated sample) to 0.28 while the LOI value decreased from 1.10 to 0.24 after pretreatment with \([\text{EMIM}]\text{Ac}\) and the SEM morphology showed that the pretreated OPF becomes distorted and disordered.

1. Introduction
Lignocellulosic materials can be represented as softwood, hardwood, agricultural and forest residues, grasses, food industry wastes and also municipal solid wastes. Cellulose, hemicellulose and lignin are the major components present in the lignocellulosic biomaterials [1]. In Malaysia, one of the most abundant resources available would be the cellulose content from all parts from palm tree (Elaeis guineensis). Palm residues (Elaeis guineensis) particularly comes from palm oil industry such as palm frond and palm trunk which contain high cellulose content of 50.78% and 41.88%, respectively [2]. In order to fully utilize the lignocellulosic biomass by making cellulose accessible for hydrolysis, there must be an efficient pretreatment method for deconstruction of biomass [3]. Nowadays, the most efficient and green engineering technology for pretreatment is by using ionic liquid. Among all of the ionic liquids, \([\text{EMIM}]\text{Ac}\) has shown to be the most efficient ionic liquid in dissolving cellulose [3, 4, 5]. Ionic liquids have low toxicity, low hydrophobicity, high viscosity, low vapor pressure, thermal stability, enhanced electrochemical stability, and non-flammable properties, their use have low energy inputs and potentially minimal environmental impact. Therefore, in the last years, the use of ILs to dissolve many types of lignocellulosic biomasses has received more attention. In this study, the effect of \([\text{EMIM}]\text{Ac}\) on OPF was discovered.

2. Material and Method
Oil Palm Frond (OPF) was provided by Forest Research Institute Malaysia (FRIM). It was first dried at 40˚C in an oven before being ground by a horizontal mill. Next it was separated by a siever (Endecotts, USA) where the particle size falling between 250 and 75 microns. The moisture content of the sample was measured to be below than 10% before it was proceeded with pretreatment. 0.05 gram of OPF were mixed with 1 ml of \([\text{EMIM}]\text{Ac}\)-water mixture in Eppendorf tube and shaken by using Bioshake iQ (Quantifoil Instrument GmbH, Germany) at 1800 rpm shaking frequency, temperature of...
99°C for 3 hours. After pretreatment, 1 ml of deionized water was added as an anti-solvent for cellulose precipitation. The pretreated mixture was centrifuged and the supernatant (liquid) was removed until the clear liquid was observed. Finally, the IL-pretreated biomass was collected and oven-dried at 60°C for 24 hours for next analysis.

2.1 Fourier Transform Infrared (FTIR) Analysis
The structural analysis by using Fourier Transform Infrared (FTIR) (Perkin Elmer, USA) where the sample is pressed into a disc and the scanning is in the range of 4000-515 cm⁻¹ with a resolution of 4 cm⁻¹. The Lateral Order Index (LOI) for FTIR sample was calculated by using the absorbance (A) ratio \( A_{1430} / A_{898} \). From the raw data of FTIR, the transmittance (T) value was converted to absorbance by using Equation 1 from Beer’s Law.

\[
A = 2 - \log_{10}(\%T)
\] (1)

2.2 X-Ray Diffraction (XRD) Analysis
The cellulose crystalline structure is analysed by using X-Ray Diffraction (XRD) (Multi Purpose X-Ray Diffractometer, Ultima IV, Rigaku, USA). The scanning range, step size and step time was 10-30° (2θ), 0.02° and 1 second, respectively. The scans were conducted at 40 kV and 40 mA under ambient temperature. Crystallinity index (CI) was calculated by using peak height method developed by Segal and co-workers as in Equation 2.

\[
CI(\%) = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100
\] (2)

\( I_{002} \) = intensity of crystalline 002 peak at 2θ around 22.5°

\( I_{am} \) = intensity of diffraction of the non-crystalline material which is taken at 2θ around 18.5°

2.3 Scanning Electron Microscopy (SEM) Analysis
The morphology of the biomass before and after pretreatment was observed by using Scanning Electron Microscope (SEM) (Hitachi TM3000, USA). The acceleration voltage was 5.0kV. The sample was mounted on aluminium sample stubs and before analysis, the sputter coated the sample with a thin layer of gold.

3. Result and Discussion

3.1 FTIR Analysis
FTIR spectroscopy was frequently used to investigate the structure of constituents and its chemical changes occurring during the pretreatment of lignocellulosic biomass [10, 11]. Table 1 show the characteristic and variations of wavenumber in FT-IR spectra possible for cellulose. The FTIR spectra of cellulose samples irradiated with different doses are shown in Figure 1. In general, the patterns of the spectrum of the pretreated OPF were similar with the untreated OPF but the transmittance percentages of some bands were different. From Figure 1 at peaks 10, the obvious changes of the peaks are at the wavelength of 3100-3600 cm⁻¹ which correspond to alcohols (O-H) stretching vibration which give considerable information concerning the hydrogen bonds [6]. The untreated OPF has a broad band at this wavelength and the transmittance percentage increases causing flattening curve to be observed after pretreatment with [EMIM]Ac. The flattening peaks may represent the possible breakage of hydrogen bonds between O-H links and also possible disruption of crystalline cellulose. This result was supported by [7] which found that the peaks around 3100-3600 has a lower intensity compared to untreated samples and concluded that this was the effect of the scission of the intramolecular and intermolecular hydrogen bonds.

| Samples     | LOI  |
|-------------|------|
| Raw OPF     | 1.10 |
| Pretreated OPF | 0.24 |
Figure 1. FTIR Spectra of Untreated OPF and Pretreated OPF.

The next wavelength is the peaks around 2900 cm\(^{-1}\) at peak 9 in Figure 1 which indicates an alkane functional group which is C-H stretching vibration. After pretreatment with [EMIM]Ac, the peak of the FTIR spectrum again flatten and the transmittance percentage value increases. This result shows that the methyl (CH\(_3\)) group in OPF was disrupted. The decrease of the band at wavelength 2900 cm\(^{-1}\) was also found by [8] and [9] which concluded that the sample was more amorphous. The FTIR peak at the wavelength of 898 cm\(^{-1}\) as shown as in Figure 1 at peak 1 which characterizes the C-O-C stretching at \(\beta\)-1,4-glycosidic bond and also known as “amorphous absorption band” becomes more intense and strong with increasing of [EMIM]Ac concentration. It can be concluded that the cellulose becomes more amorphous after pretreatment with [EMIM]Ac. The finding of current study is consistent with [10] who found that the peaks at 897 cm\(^{-1}\) is becoming strong and sharp after pretreatment with various ionic liquids and concluded that the sample becomes more amorphous. The peak at 1430 cm\(^{-1}\) in Figure 1 as indicated by peak 5 which is assigned to CH\(_2\) bending vibration and also known as a “crystallinity band” becomes decrease after OPF was pretreated with [EMIM]Ac. This band is strong in crystalline cellulose and weak in amorphous cellulose [11]. The decrease of the peak indicates reduction in the degree of crystallinity in OPF. A low cellulose crystallinity index also indicates that a part of crystalline structure of cellulose was transformed into amorphous form. With the amorphous structure presence, higher possibilities of enzymatic hydrolysis could occur in comparison to the previous ordered arrangement of crystalline structures. The wavelength of ~1732 cm\(^{-1}\) as shown as a very sharp descending peak indicated by peak 8 in Figure 1 is assigned to the C=O stretching of carboxylic acids [10] and also corresponds to aldehydes in hemicellulose [12]. This particular peak was still retained in the pretreated OPF as shown as in Figure 1. However, it is very significant that the peak nearly disappeared after the pretreatment which may indicate a significant removal of hemicellulose.

Lignin main functional group is an aromatic compound (phenolic hydroxyl group) which has a structure of benzene ring. In FTIR spectroscopy, the wavelength which corresponds to the aromatic compound is \(~1500 – 1600\) cm\(^{-1}\) [7, 13]. From Figure 1, it can be observed that the peaks of \(~1550\) and \(~1600\) cm\(^{-1}\) at peaks 6 and 7, respectively, were almost disappeared after the pretreatment step which may indicate some removal of lignin. Other significant sharp peaks observed from the FTIR spectrum as shown as in Figure 1 are at the wavelength of \(~1031\) and \(~1230\) cm\(^{-1}\) as indicated at the peaks 2 and 3, respectively, corresponds to C-O-C stretching. While the observed peak of wavelength of \(~1365\) at peak 4 shown is assigned to C-H and C-O stretching in polysaccharides. All of the peaks was decreased after pretreatment with [EMIM]Ac. This also signifies the destruction of the ordered structure and dissolution of polysaccharides which are cellulose, hemicellulose and lignin.
3.2 XRD Analysis

XRD analysis was conducted in order to further examine the crystallinity of cellulose since the determination of the crystallinity index by FTIR spectroscopy only gives the relative values from both crystalline and amorphous regions. The XRD spectra and CI values for the effect of [EMIM]Ac pretreatment on OPF is as shown in Figure 2 and Table 2, respectively. In this study, two typical diffraction peaks were observed at 2θ = 15° and 22.5° which correspond to (101) and (002) peaks, respectively. The CI value for the pretreated OPF decreased from 0.47 (untreated sample) to 0.28. It might be due to the swelling of the cellulose matrix by [EMIM]Ac. From Figure 2, it can be observed that the XRD spectra for the pretreated OPF become decrease and flattened compared to the sharp peaks of raw OPF. Therefore, the CI result from XRD supported the FTIR result by showing a significance decrease of CI. The lower CI may thus provide a larger surface area accessible by cellulose enzyme which may then enhanced the reducing sugar yield after enzymatic hydrolysis of the IL-pretreated biomass. These results may suggest that [EMIM]Ac pretreatment can reduce the cellulose crystallinity in OPF.

![XRD Spectra of Raw OPF and Pretreated OPF.](image)

**Figure 2.** XRD Spectra of Raw OPF and Pretreated OPF.

| Samples         | CI  |
|-----------------|-----|
| Untreated OPF   | 0.47|
| Pretreated OPF  | 0.28|

3.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) which was further used to investigate the morphological structures of OPF after pretreatment with [EMIM]Ac can be seen as shown as in Figure 3(a) and Figure 3 (b).

![Scanning Electron Microscopy (SEM) images](image)

**Figure 3:** (a) Untreated OPF and (b) Pretreated OPF

It can be clearly seen that the raw OPF has a rigid, compact and ordered structure. After the pretreatment with [EMIM]Ac, the structure becomes disordered, loose and had some cracks or distorted surfaces as compared to the earlier image representing the untreated OPF. These changes of morphology probably happened because of the swelling of cellulose crystalline structure and
disruption of hydrogen bonds in cellulose after pretreatment with [EMIM]Ac. SEM analysis supported the results from FTIR and XRD where [EMIM]Ac pretreatment is effective in reducing the crystallinity of cellulose.

4. Conclusion
In summary, from the findings found from the investigations of the pretreatment of OPF with [EMIM]Ac can be said effective because of the significant decrease in Lateral Order Index (LOI) and the Crystallinity Index (CI) as well as the observed significant changes on the OPF morphology. The decrease of cellulose crystallinity will enhance the digestibility during enzymatic hydrolysis which may increase sugar yield for bioethanol production. Thus, it can be concluded that [EMIM]Ac is a promising green solvent to reduce cellulose crystallinity during pretreatment step.

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