Study of Crystallinity Index (CrI) of Oil Palm Frond Pretreatment using Aqueous [EMIM][OAc] in a Closed System

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Abstract. The objective of this preliminary study is to identify the Crystallinity Index (CrI) of Oil Palm Frond (OPF) pretreated with 40% concentration of 1-ethyl-3-methylimidazolium acetate ionic liquid ([EMIM][OAc]) in a closed system. The morphology and structural changes of the pretreated OPF were examined by using Fourier Transform Infrared Spectrometer (FTIR) and X-Ray Diffraction (XRD). The pretreatment process was carried out in triplicates by loading 40% of [EMIM][OAc] concentration with 10 wt% of OPF loading in the Bio-ionic liquid-reactor. The pretreatment process was conducted for 3 hours with working volume of 70 mL and temperature of 110°C. A Bio-ionic liquid reactor was purposely designed for the lignocellulosic pretreatment by using aqueous ionic liquid at high temperature (higher than boiling point of water). The CrI of OPF pretreated with 40% concentration of [EMIM][OAc] in a closed system observed was 9% lower from the untreated OPF and the result showed significant difference with 95% confidence level. Further examination of the untreated and pretreated OPF by using XRD showed that the diffraction pattern of the pretreated OPF samples was decreasing compared to the untreated OPF. The characteristic of the FTIR spectra of the pretreated OPF showed the presence of the cellulose I and occurrence of amorphous cellulosic in the samples. The findings from this study are expected to improve knowledge on pretreatment of OPF by using aqueous [EMIM][OAc] as a green economically viable process for future renewable energy.

1. Introduction
Bioethanol is one of the alternative renewable energy with low net Greenhouse Gases (GHG) emissions [1] produced from lignocellulosic biomass such as plant mass, grasses, wood and agricultural waste [2]. The cellulose obtained through lignocellulosic biomass pretreatment will be converted into sugars via enzymatic hydrolysis/saccharification process and sugars will be converted into bioethanol via fermentation process by bacteria/yeast. There are different kinds of chemical and physicochemical pretreatment methods, for instance alkaline delignification, diluted acid hydrolysis, steam explosion, alkaline peroxide, aqueous ammonia soaking, ultrasound irradiation, ionic liquid and ionic liquid-co-solvent were used for dissolution of lignocellulosic biomass [3, 4, 5, 6]. Deconstruction and fractionation of lignocellulosic biomass or called as pretreatment of lignocellulosic biomass [7] is an extremely important step in commercial biorefinery [8]. Towards low environmental impact on process, ionic liquid (IL) has provided a platform on the application of pretreatment of lignocellulosic materials in a green way [7]. Previous study indicated that IL pretreatment is a promising alternative in pretreatment of switch grass in terms of total process time to produce high yield of sugars from the recovered product, as compared to the dilute acid pretreatment [8]. Pretreatment of lignocellulosic biomass by using IL are resulted by a low inhibitor, low cost of equipment and energy and easier
chemical recovery as compared to other methods [9]. However, the cost of IL is expensive [9]. Therefore, by considering the cost of IL, ionic liquid-water mixture is considered to optimize the IL utilization. However, a study showed that the solubility of cellulose significantly decreased in the presence of water in the ionic liquid, presumably through competitive of hydrogen bonding to the cellulose microfibrils which inhibits the solubilisation [10]. Previous studies have demonstrated that 50–80% 1-ethyl-3-methylimidazolium acetate ionic liquid ([EMIM][OAc]) in water was efficient for cellulose dissolution which can match the performance of 100% [EMIM][OAc] in terms of glucose yields [11].

Interaction of 1-butyl-3-methylimidazolium acetate ionic liquid [BMIM][OAc] with native crystalline cellulose in plant cell wall shows that IL molecules progressively swell the biomass samples and the fraction of biomass samples swollen with IL transformed into a disordered structure [12]. [EMIM] [OAc] was indicated to be the most efficient for dissolving cellulose among ionic liquids tested [13]. Cellulose crystallinity, can be measured as Crystallinity Index (CrI) through X-Ray Diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR) [14, 15, 16, 17, 18, 19]. CrI were found to be significantly correlated with hydrolysis yield potential (HYP) after 24 hours of hydrolysis [20]. CrI were obtained through Lateral Order Index (LOI) in the FTIR spectra as a ratio of $A_{1430}$ to $A_{898}$ [16]. Absorption band, between 1420 and 1430 cm$^{-1}$ in the FTIR spectra is assigned to a symmetric CH$_2$ bending vibration, which is known as ‘crystallinity band’ and a band between 893 and 898 cm$^{-1}$ ($A_{898}$) is assigned to C-O-C stretching at β-(1→4)-glicosidic linkage, which is known as the amorphous band [17]. Malaysia produces about 47% of the world’s supply of palm oil. 12.9 metric tonne (mT) of Oil Palm Frond (OPF) and 15.8 mT of Empty Fruit Bunch (EFB) of biomass were produced in Malaysia [21, 22]. The OPF are available daily throughout the year when the palms are pruned during the harvesting of fresh fruit bunch for the production of oil [15]. OPF was identified with a higher cellulose content composition with 45.2% as compared to EFB of only 38.2% [23]. The objective of this preliminary study is to identify the Crystalinity Index (CrI) of OPF pretreated with 40% concentration of 1-ethyl-3-methylimidazolium ionic liquid in a closed system. The morphology and structural changes of the pretreated OPF were examined by using FTIR and XRD. The findings from this study are expected to improve knowledge on pretreatment of OPF by using aqueous 1-ethyl-3-methylimidazolium ionic liquid as a green and economically viable process for future renewable energy.

2. Materials and Methods

2.1. Materials and Preparation

Oil palm frond (OPF) stem pieces were obtained from Shah Alam were thoroughly washed with water, dried and stored in a closed tight container at room temperature. The OPF samples were then grounded by a grinder (A11, IKA, Malaysia) and sieved using a 120 mesh siever (OCTAGON 2000, Endecotts, United Kingdom) then stored in a sealed plastic bag before being used in the experiments. The ground OPF was named as ‘untreated OPF’. Ionic liquid, 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) was purchased from Sigma-Aldrich (Germany). The OPF was weighted to 10 wt% OPF loading in 70 mL of the pretreatment working volume. The 40% of ([EMIM][OAc]) was prepared by mixing the ([EMIM][OAc]) with deionized water.

2.2. Bio-ionic liquid reactor

A Bio-ionic liquid reactor was designed for lignocellulosic pretreatment by using aqueous ionic liquid at high temperature pretreatment process (higher than boiling point of water) and fabricated up to 200 mL of working volume, 0-130°C working temperature and 150 psi maximum pressure.

2.3. Aqueous ionic liquid pretreatment

The pretreatment process was carried out in triplicates by loading 40% of [EMIM][OAc] concentration with 10 wt% of OPF loading in the Bio-ionic liquid reactor. The pretreatment process was conducted for 3 hours with a working volume of 70 mL and temperature at 110°C. The heat-up
time was ~10-15 minutes. After 3 hours, warm water was added to the slurry by ratio 1:1. Cellulose could be precipitated from the IL solution by adding water [10]. The water was used as an anti-solvent for cellulose regeneration and for recovering any solubilized biomass [11]. The samples were centrifuged at 10,000g for 20 minutes in order to separate the solid and the liquid. The supernatant containing IL was removed, and the precipitate was washed 4 to 5 times [8, 11, 12] until there is no residue of ionic liquid.

2.4. Crystallinity and chemical changes measurements

X-Ray diffraction samples were collected on a RIGAKU RINT 2500 and was used to determine the CrI of both untreated and pretreated OPF. The OPF crystallinity, as expressed by CrI was determined from XRD data and calculated by using Segal formula in the equation (1) below. Based on the equation (1), the $I_{002}$ is the maximum intensity of the 002-lattice diffraction and $I_{am}$ is the intensity of diffraction at 2$\theta$. The $I_{002}$ and $I_{am}$ were in arbitrary units (a.u.) [14]. The XRD data were collected using CuK$\alpha$ radiation, which generated at 40 kV and 26 mA. The scans were collected from 5$^\circ$–35$^\circ$ 2$\theta$ in 2.5 minutes per 2$\theta$ intervals.

$$CrI = \left( \frac{I_{002} - I_{am}}{I_{002}} \right) \times 100$$

Fourier Transform Infrared spectra of approximately 2 mg of untreated and pretreated OPF samples were measured by using PerkinElmer Spectrum One Fourier Transform Infrared Spectrometer. The sample spectra were obtained with a resolution of 4 cm$^{-1}$ and the frequency range between 4000–500 cm$^{-1}$ in the transmission mode.

3. Result and Discussion

3.1. Bio-ionic liquid reactor

A Bio-ionic liquid reactor for aqueous ionic liquid pretreatment was fabricated as shown in figure 2(a) and 2(b).

3.2. Aqueous ionic liquid pretreatment

Normality test shown in table 1 demonstrates that the data was significantly normal (Shapiro-Wilk). The CrI of OPF pretreated with 40% concentration of [EMIM][OAc] in a closed system was compared to the untreated OPF. The result showed significant difference with 95% confidence level as shown in table 2 and 3.

| Table 1. Normality of CrI data of the pretreated OPF |
|-----------------------------------------------|
| **Statistic** | **Kolmogorov-Smirnov** | **Shapiro-Wilk** |
| **Kolmogorov-Smirnov** | **Degree of Freedom** | **Significant** | **Degree of freedom** | **Significant** |
| CrI | 0.204 | 6 | 0.200 | 0.883 | 6 | 0.281 |

| Table 2. CrI of untreated and pretreated OPF |
|-----------------------------------------------|
| **Oil Palm Frond (OPF)** | **N** | **Mean** | **Standard Deviation** | **Standard Error** | **95 % Confidence Interval for Mean** | **Minimum** | **Maximum** |
| Untreated | 3 | 62.5745 | 1.4378 | 0.8301 | 59.0028 | 66.1461 | 60.99 | 63.81 |
| Pretreated | 3 | 54.1319 | 2.2408 | 1.2937 | 48.5655 | 59.6982 | 52.62 | 56.71 |
| Total | 6 | 58.3532 | 4.9212 | 2.0091 | 53.1886 | 63.5177 | 52.62 | 63.81 |
### Table 3. Anova analysis on CrI of untreated and pretreated OPF between and within groups.

|                  | Sum of Squares | Degree of freedom | Mean Square | F       | Significant |
|------------------|---------------|------------------|-------------|---------|-------------|
| Between Groups   | 106.916       | 1                | 106.916     | 30.167  | 0.005       |
| Within Groups    | 14.177        | 4                | 3.544       |         |             |
| Total            | 121.093       | 5                |             |         |             |

#### 3.3. Crystallinity and chemical changes measurements

An X-ray diffractogram of untreated and pretreated OPF is shown in figure 3. The diffraction pattern of the pretreated OPF samples is lower as compared to the untreated OPF, showed by the two main peaks of the cellulose and amorphous background band. \( I_{002} \) at the Bragg angle \( (2\theta) \) in the x axis represents the maximum intensity at \( 2\theta = 22^\circ \). \( I_{am} \) shows the minimum intensity at \( 2\theta = 18^\circ \). The CrI of untreated OPF and pretreated OPF are 63% and 54%, respectively. Figure 4 shows the FTIR spectra in the fingerprint region between 4000 and 500 cm\(^{-1}\) for untreated and pretreated OPF. The characteristic of hydrogen bonds or O-H stretching vibration are shown in the 3600-3100 cm\(^{-1}\) region of the OPF pretreated peak (3320 cm\(^{-1}\)) where the peak became sharper as compared to the untreated OPF. This is correlated with the scission of the intra and intermolecular hydrogen bonds. Figure 4 shows the intensity of the adsorption band of 2908 cm\(^{-1}\) (C-H stretching vibration) which strongly decreased as correlated to the presence of amorphous or cellulose II. The adsorption band 1165 cm\(^{-1}\) assigned to C-O-C asymmetric (bridge oxygen stretching) of the cellulose. Adsorption band 1425-1430 cm\(^{-1}\) showed CH\(_2\) bending as correlated to the presence of cellulose (crystallized I and amorphous) or also known as “crystallinity band”. Decrease in the intensity of the band reflects a reduction in the degree of crystallinity of the samples (CrI).
4. Conclusion
The CrI of pretreated OPF with 40% concentration of 1-ethyl-3-methylimidazolium ionic liquid in a closed system obtained from XRD analysis was 14% lower from the untreated OPF and the result shows significant difference with 95% confidence level. The diffraction pattern of the pretreated OPF samples was lower compared to the untreated OPF. The characteristic of the FTIR spectra of the pretreated OPF shows a presence of cellulose I and occurrence of amorphous cellulosic in the samples. The findings from this study are expected to improve knowledge on pretreatment of OPF by using aqueous 1-ethyl-3-methylimidazolium acetate ionic liquid as a green and economically viable process for future renewable energy.

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