Physicochemical and Morphological Characterisation of the Native and Alkaline Pre-treated Fibre Pressed Oil Palm Frond for Fermentable Sugars Production

Fatin Syazwana Hashim[a], Hafizuddin Wan Yussof*,[a], Mior Ahmad Khushairi Mohd Zahari[a], Roshanida A Rahman[b], Rosli Md Illias[b]

[a]Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Kuantan, Pahang, Malaysia.
[b]Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.
hafizuddin@ump.edu.my

Lignocellulosic biomass (LCB) is the most abundant renewable biomass that gives high potential source for the production of value added products. LCB is mainly composed of cellulose, hemicellulose and lignin. During pretreatment process, cellulosic biomass structure will be altered and delignification occur which make cellulose more accessible to the subsequent process of converting it into simple sugars. In this study, fibre pressed oil palm frond (FPOPF) was introduced as a raw material in order to maximise the utilisation of oil palm waste. This study was conducted to analyse and compare the physical, chemical and morphological characteristics of FPOPF before (native) and after alkaline pretreatment (pre-treated). The FPOPF was subjected to an alkaline pretreatment at 4.42 % w/v of sodium hydroxide solution which operated at 100 °C for an hour (Sukri and Rahman, 2014). From the study, it was found that characterisation of native FPOPF produced 40.7 % glucan, 26.1 % xylan, 4.5 % extractives, 26.2 % lignin and 1.8 % ash. Pre-treated FPOPF gave 61.4 % glucan, 20.4 % xylan, 0.3 % extractives, 13.3 % lignin and 1.3 % ash. The FPOPF samples were characterised using X-ray Diffraction spectrometry (XRD), Fourier Transform Infrared spectroscopy (FTIR), and Scanning Electron Microscopy (SEM) to evaluate the properties and structural changes of FPOPF between native and pre-treated FPOPF. This information is useful in order to understand the properties and structural changes for native and pre-treated FPOPF.

1. Introduction

A significant amount of biomass is produced every year across a variety of crops especially in agricultural and forestry sector such as palm oil, switch grass, rubber, sugarcane and wood (forest residues) which produce large amount of wastes. Most of the plant fibres composed of cellulose, hemicellulose, lignin, waxes, pectin, and water soluble compounds. Lignocellulose refers to the plant dry matter known as lignocellulosic biomass (LCB). Recently LCB has gained increasing research interests and special importance because of their renewable nature. It is the most promising feedstock as natural and renewable sources to produce energy, platform chemicals and materials in modern industrial societies. Most of the agricultural LCB composed of 40 - 50 % cellulose, 25 - 35 % hemicellulose and 15 - 20 % lignin (van Dyk and Pletschke, 2012). Distribution of each component varies significantly between different types of plants (Jorgensen et al., 2007). Cellulose, hemicellulose, and lignin that form major constituents of the biomass might differ depending on plant age, environment, soil condition, weather effect, and others (Han and Rowell, 1997).

Oil palm frond (OPF) is currently the most LCB studied because OPF is daily generated in oil palm mills and oil palm plantations. Utilisation of OPF into more valuable product has been reported recently as a livestock feed (Ebrahimi et al., 2013), biofuel production (Ofori-Boateng and Lee, 2014), renewable sugar (Zahari et al., 2012) and composite board (Rasat et al., 2011). Utilisation of FPOPF can create extra income for the oil palm settler, and new job opportunities as well as generating income to the country as a whole. The aim of this

study is to carry out systematic studies on physicochemical properties and morphological structure of FPOPF lignocellulosic biomass, before (native) and after the alkaline pretreatment (pre-treated). NREL procedures have been employed to characterise the composition of both samples in terms of xylan, glucan, lignin, ashes and extractives. Microscopy (SEM) and spectroscopy (NMR, FTIR, and XRD) techniques also been applied to figure out the structural properties of biomass.

2. Materials and methods

Oil palm frond (OPF) was obtained from a local palm oil plantation at Kuantan, Pahang. The OPF was pressed by using sugarcane machine to remove its juice. Then the FPOPF itself was sun dried for 2 - 3 days. Dried FPOPF was shredded into pieces and sieved into particle size less than 2 mm. Dried FPOPF was stored in sealed plastic bag at room temperature.

2.1 Characterisation of FPOPF

Characterisation of FPOPF was performed based on the National Renewable Energy Laboratory (NREL) methods to determine the composition of xylan, glucan, lignin (Sluiter et al., 2008a), ashes (Sluiter et al., 2008b) and extractives (Sluiter et al., 2008c). The compositional characterisation analysis was performed on both the native FPOPF and pre-treated FPOPF. The removal of extractives was carried out using DIONEX ASE 350 (Termo Scientifc, US). Ethanol and water were used as solvents. Water extraction sample was used to determine the soluble sugar content in the FPOPF such as glucose, fructose and sucrose. This sample was further hydrolysed using sulfuric acid (H_2SO_4) to obtain the oligomers. Ethanol extraction sample was used to determine the ethanol extractive that includes chlorophyll, proteins fats, and oils. Total extractive in the FPOPF was determined from the mass difference between the native and extracted FPOPF. Next, analysis on acid insoluble lignin and acid soluble lignin was performed to determine the lignin content inside the FPOPF. Ash content was determined by using muffle furnace at 575 ± 25 °C for 24 ± 6 h.

2.2 Scanning electron microscopy (SEM)

The morphology of the FPOPF was analysed by EVO 50 SEM (Carl Zeiss, Germany) for native FPOPF and pre-treated FPOPF. Prior to analysis, the samples were fixed onto aluminium stubs using carbon tape and then coated with a carbon layer. Sample imaging operated at acceleration voltage of 5 kV and a working distance of 11 mm for 400x magnification and 11.5 mm for 1,000x magnification.

2.3 X-ray diffraction spectrometry (XRD)

X-ray diffraction approach gives more detailed data on features of crystalline and less on the non-crystalline fraction of cellulose. The inherent crystalline nature of the FPOPF lignocellulosic biomass for native and alkaline pre-treated were analysed using a MiniFlexII XRD (Rigaku, Japan) which generated at a voltage of 30 kV and a current of 15 mA. The 2θ scan range was from 5° to 80°, with a step size of 0.02° and a step time of a second. The crystallinity index (CrI) is defined by means of the empirical method described by Segal et al. (1959) as shown in Eq. (1):

$$\text{CrI} = \frac{(I_{002} - I_{am})}{I_{002}} \times 100 \text{ \%} \tag{1}$$

where CrI represents the relative degree of crystallinity (%), I_{002} is the peak intensity of the 002 crystal plane at 2θ = 22°, and I_{am} is the peak intensity of the amorphous phase at 2θ = 18°.

2.4 Fourier transform infrared spectroscopy (FTIR)

FTIR of native and pre-treated FPOPF samples were recorded on a Spectrum 100 FTIR spectrometer (Perkin Elmer, USA). Changes in the functional groups due to the alkaline pre-treatment were evaluated by FTIR analysis. The scanning range was from 4,000 to 400 cm^{-1} with an accumulation of 4 scans per samples.

3. Results and discussion

3.1 Composition analysis of FPOPF

Proximate chemical analyses of native and alkaline pre-treated FPOPF were carried out in accordance to the NREL's methods. The compositional analysis of both FPOPF were analysed for glucan, xylan, lignin, ashes and extractives as shown in Table 1. The total structural carbohydrate content for native FPOPF was found to be 66.8 % with 40.7 % of glucan as the major structural carbohydrate followed 26.1 % of xylan. Pre-treated FPOPF shown 81.8 % of total carbohydrates which comprise of 61.4 % glucan and 20.4 % xylan. Lower yield

of lignin was observed after alkaline pretreatment process which indicates that alkaline pretreatment promotes the removal of lignin from FPOPF biomass.

Table 1: Composition of native and pre-treated FPOPF

Composition	Native FPOPF (%)	Pre-treated FPOPF (%)
Total carbohydrates	66.8	81.8
1. Glucan	40.7	61.4
2. Xylan	26.1	20.4
Lignin	26.2	13.3
Ashes	1.8	1.3
Extractives	4.5	0.3

The C6 sugar content (glucan or cellulose) was found to be in line with Zahari et al. (2012) and Tan et al. (2016) which are 41.7 % and 42.8 %. The differences recorded between this present work and two reports were less than 5.16 %. Report on C5 sugars (xylan and arabinan or hemicellulose) and lignin contents obtained by Zahari et al. (2012) and Tan et al. (2016) were lower with differences about 43.30 % and 33.21 % compared in this study due to the different types of pre-treatment methods been applied. Different geographic locations, ages, climate, and soil conditions also can be the reasons chemical composition varies between others (Han and Rowell, 1997).

3.2 Scanning electron microscopy (SEM)

The SEM micrographs were taken for native and alkaline pre-treated FPOPF. The images were captured at 400x magnification in Figure 1 and 1,000x magnification in Figure 2. From the SEM image in Figure 1(a) and Figure 2(a), it has been seen clearly that the native FPOPF show a smooth and rigid surface structure. For Figure 1(b) and Figure 2(b), the outer layer was disrupted which suggests caused by a partial removal of hemicellulose and lignin. The cell wall was distorted during alkaline pretreatment resulting the cellulose fibre was exposed. The morphological features of the LCB from FPOPF observed in this study were comparable to those studies of cellulose fibres obtained by Pereira et al. (2016) and Tan et al. (2011) after the removal of hemicellulose and lignin from sugarcane bagasse, sweet sorghum bagasse and oil palm frond.

3.3 X-ray diffraction spectrometry (XRD)

The X-ray diffraction profile of the native and pre-treated FPOPF showed a semi-crystalline pattern as in Figure 3. It can be seen that all samples had diffraction peaks in the vicinity of $2\theta = 16°$, $2\theta = 22°$ and $2\theta = 35°$ that resembled a typical amorphous cellulose pattern. Both samples showed the typical XRD peaks of cellulose.
Based on Figure 3, it was found that crystallinity index (CrI) in native FPOPF (44.3 %) was lower than the pre-treated one (63.3 %). The increasing of CrI from native to preteated sample might be due to the increasing in glucan composition of native (40.7 %) and alkaline pre-treated FPOPF (61.4 %) as shown in Table 1. Delignification that occurred during alkaline pretreatment resulted in increasing trend of CrI due to the decrease in the amorphous region and alteration of cellulose microfibrils in the lignocellulosic biomass structure. Similar observation was found by Pereira et al. (2016) and Subhedar and Gogate (2014). This suggests that alkaline pre-treatment can reduce the amorphous substance (i.e. lignin) hence increase the accessibility of enzymes in the hydrolysis due to greater porosity of the pre-treated FPOPF.
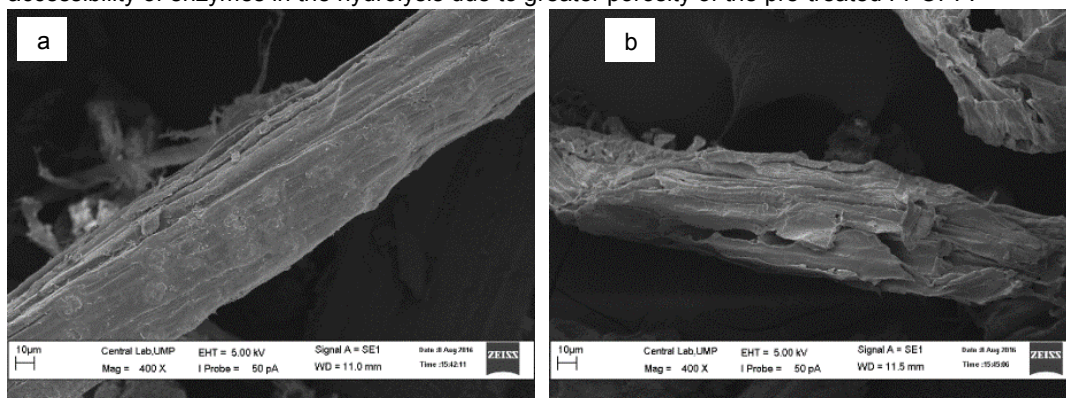

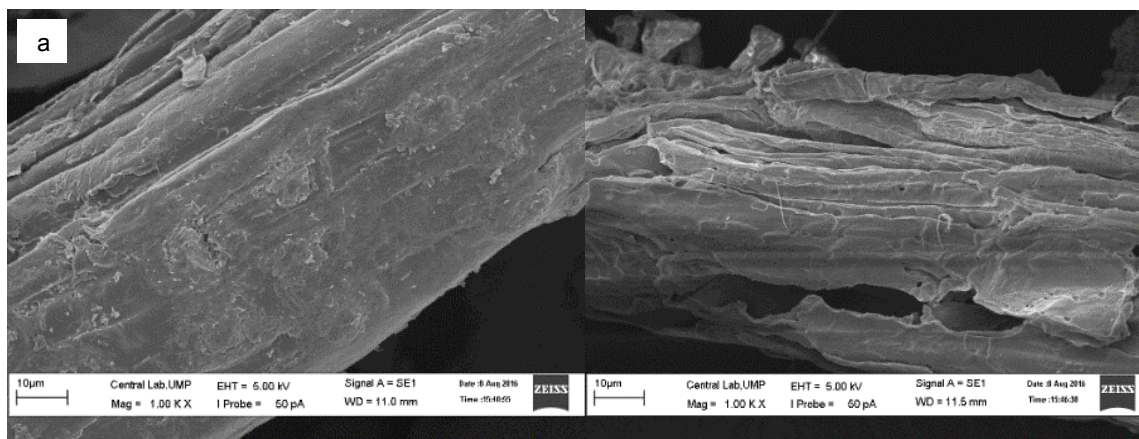*Figure 1: SEM image under magnification of 400x: a) Native FPOPF, and b) Alkaline pre-treated FPOPF*

Figure 2: SEM image under magnification of 1000x: a) Native FPOPF, and b) Alkaline pre-treated FPOPF
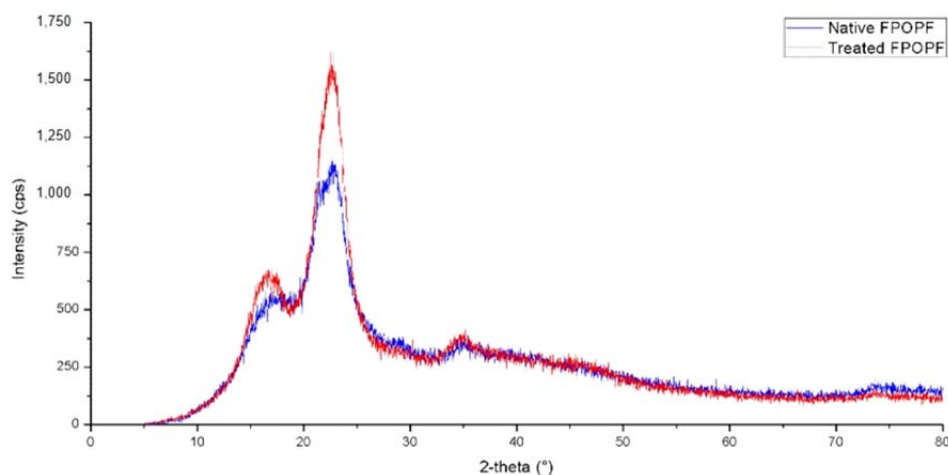

Figure 3: X-ray diffraction pattern of FPOPF for native and alkaline treated FPOPF

3.4 Fourier transform infrared spectroscopy (FTIR)

FTIR was used as a tool to evaluate the changes in chemical composition due to the alkaline pre-treatment, by analysing the bands that referred to the main components of LCB in terms of cellulose, hemicellulose, and lignin. The FTIR spectra of native and pre-treated FPOPF are illustrated in Figure 4. It is noteworthy that the FTIR spectrum of the pre-treated sample was similar to those of the untreated samples (native), suggesting that the alkaline pretreatment did not cause any serious changes to the biomass. Same result also obtained by Pereira et al. (2016). However, there were some differences in the spectral bands of the three residue fractions, as can be seen in Figure 4.

Arellano et al. (2016) stated that broad band in the region of $3,600 – 3,200$ cm^{-1} is attributed to the OH (e.g., water, alcohol, and phenol) and N-H (amines) stretching vibrations. The broad band at $3,446$ cm^{-1} was attributed to the phenolic and aliphatic OH group presence in FPOPF. This strong band can be clarified due to present in lignin structure that linked by carbon–carbon and ether bonds, including three major phenylpronanoid monomers consist of syringyl alcohol, guaiacyl alcohol and p-coumaric alcohol similar to Ngadi and Rusli (2014). Three bands at $1,637$ cm^{-1}, $1,505$ cm^{-1}, and $1,468$ cm^{-1} were referred to aromatic ring vibration of the lignin skeleton which also obtained by Pereira et al. (2016) and Ngadi and Rusli (2014). The small band at $1,420$ cm^{-1} relates to C–H vibration of methyl group. The small signal showed the conjugate C–H content in lignin of OPF was relatively low. The band at $1,263$ cm^{-1} happened because of the bending vibration of the syringyl ring breathing with C–O stretching (Ngadi and Rusli, 2014). The guaiacyl ring was attributed at the bands $1,218$ cm^{-1}. Removal of outer layer of biomass such as lignin and other depositions during delignification was in agreement with weaker FTIR bands at $1,263$ cm^{-1} and $1,637$ cm^{-1} after alkaline

pretreatment. The disruption of FPOPF structure after pretreatment caused the total exposure of internal structure and fibrils to the enzymes for efficient hydrolysis to happen.
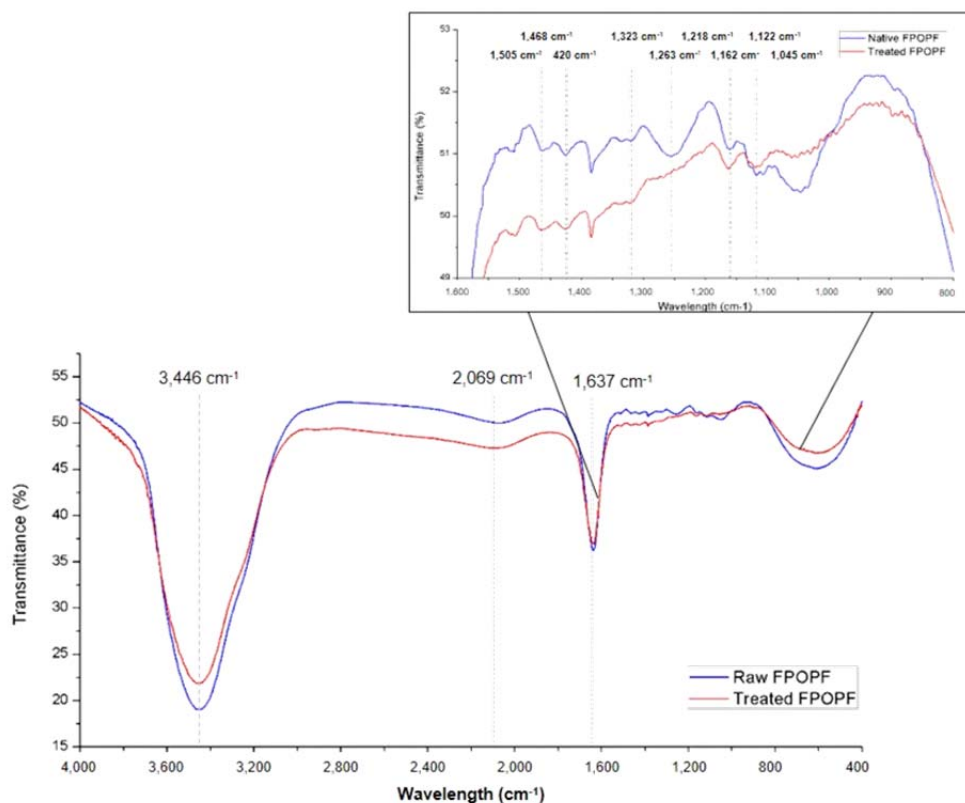

Figure 4: RID pattern of FPOPF for native and alkaline treated FPOPF

4. Conclusion

An amount of 40.7 % glucan, 26.1 % xylan, 4.5 % extractives, 26.2 % lignin and 1.8 % ash could be obtained from native sample. Pre-treated FPOPF gave 61.4 % glucan, 20.4 % xylan, 0.3 % extractives, 13.3 % lignin and 1.3 % ash. An increase in glucan after the alkali pretreatment was due to the cell wall distorted resulting the cellulose fibre to expose. The decrease in xylan after alkaline pretreatment was due to the removal of the amorphous hemicellulose, which revealed by CrI values determined from the XRD analysis. The results of the SEM analysis showed that alkaline pretreatment cause changes in the structure of the FPOPF where the outer layer was disrupted resulting the exposure of cellulose fibre. These disrupted outer layers partially removed hemicellulose and lignin. FTIR analysis of the pretreated sample was similar to those of the untreated samples (native), suggesting that the alkaline pretreatment did not cause any serious changes to the FPOPF biomass. Reduction amount of lignin was observed after alkaline pretreatment process which indicates that alkaline pretreatment promotes the removal of lignin from FPOPF biomass. Successful removal of outer layer of biomass such as lignin and other depositions during delignification was confirmed with weaker FTIR bands at 1,263 cm^{-1} and 1,637 cm^{-1} after alkaline pretreatment. The disruption of FPOPF structure after alkaline pretreatment has resulted in the total exposure of internal structure and fibrils for efficient enzymatic hydrolysis to occur. Information on physicochemical properties and morphological structure of FPOPF lignocellulosic biomass, for native and pre-treated FPOPF are very useful to understand the changes before (native) and after the alkaline pretreatment (pre-treated).

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