

## Characterization Of Solid Products From Ozonolysis Pretreatment Of Oil Palm Fronds

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### ABSTRACT

This present work focused on the characterization of treated oil palm frond (OPF) from ozonolysis pretreatment. The ozonolysis pretreatment was performed in a conventional semi-batch reactor at ambient condition. The effectiveness of ozonolysis pretreatment was validated by investigating the degradation of lignin and composition of solid product from pretreatment (treated OPF). In addition, crystallinity, surface area, pore structure, and morphology of untreated and treated OPF, as well as functional groups present were examined accordingly to study the effect of ozonolysis pretreatment on physical properties of OPF. The ozonolysis pretreatment on OPF was found to degrade the lignin structure, and exerted only a slight effect on the hemicellulose structure. Meanwhile, the cellulose yield was increased. As comparison to the untreated OPF, the crystallinity index OPF was increased while the surface area, pore volume, and mean pore diameter for the treated was decreased. This observation explained that the ozonolysis reaction has degraded the C=C bond of acid insoluble lignin into acid soluble lignin. Thus, the lignin could be removed easily by washing with water. Removing the amorphous component would lead to higher crystallinity index. Meanwhile, the pore properties of treated OPF would collapse during washing and drying process. Results of the present study indicated ozonolysis pretreatment as an effective method for OPF pretreatment to produce a washed clean substrate rich in cellulose and xylan. In addition, the physical properties of OPF were improved and ready for subsequent downstream bioconversion process such as acid hydrolysis for glucose production.

**Keywords:** *Lignocellulosic biomass; pretreatment; ozonolysis; oil palm fronds; FTIR, XRD.*

### 1. INTRODUCTION

Recently, ozonolysis pretreatment (OP) is claimed as an effective method to remove the lignin from biomass by slightly degrading the hemicellulose but

almost not affecting the cellulose (Binder et al., 1980; García-Cubero et al., 2009; Kumar et al., 2009; Lee et al., 2010; Li et al., 2015; Neely, 1984). Ozone has been recognized as a powerful and green oxidant since it can be decomposed to form oxygen ( $O_2$ ). For that reason, no toxic is produced from the OP. In addition, the process was performed under ambient temperature and pressure (Kumar et al., 2009); making the process becomes energy efficiency. Hence, the possibility to employ the ozonolysis method for pre-treatment of the biomass is higher. However, the method is scarcely explored especially on Malaysia's biomass such as oil palm frond (OPF). The OPF is the largest abundant biomass produced from palm oil industry and very suitable for the process since it contains high cellulose and low lignin content (Tan and Lee, 2012). To date, reports on the effect of ozonolysis pretreatment of biomass on physical properties of biomass resulting from ozonolysis treatment is still lacking. The objective of this work is to investigate the transformations of OPF characteristic after undergoing ozonolysis pretreatment and relationships of the transformation with lignin degradation.

## 2. EXPERIMENT

### 2.1. Materials

OPF, supplied by KESEDAR Renok Baru Plantation, Kelantan, Malaysia, was grounded and meshed at the Polymer Laboratory, N29, Faculty of Chemical and Energy Engineering (FKT), Universiti Teknologi Malaysia (UTM), Malaysia. The OPF was then stored in a sealed container at room temperature for further characterization and subsequent reaction. Chemicals such as sulphuric acid,  $H_2SO_4$  (95-98%)(Qrec, NZ), potassium iodide (Qrec, NZ), and potassium permanganate (Fisher Brand, UK) were in analytical grade reagent. Oxygen gas (170 bar,  $8.4\text{ m}^3$ ) was supplied by Mega Mount Gases Sdn Bhd, Malaysia.

### 2.2. Design of Experiment (DOE)

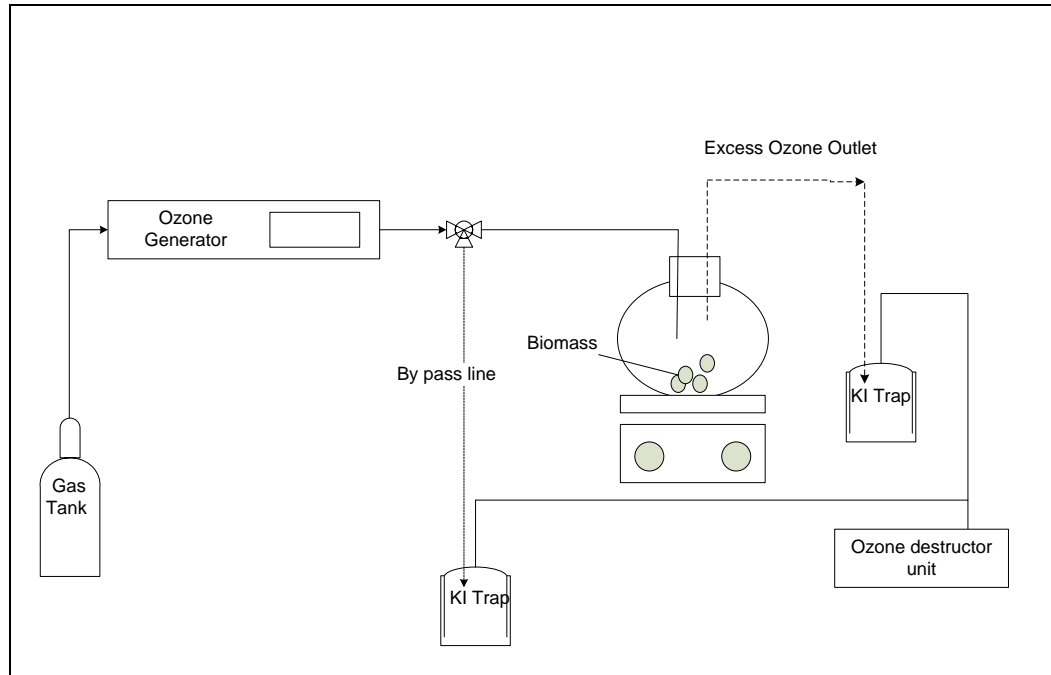
The experiment was designed from 2-level fractional design. The moisture content ( $X_1$ ), particle size ( $X_2$ ), reaction time ( $X_3$ ), ozone flowrate ( $X_4$ ), and ozone concentration ( $X_5$ ) were selected as process variables while lignin degradation as response. The untreated is coded as UTP, while the treated were coded as R3, R4, R5 and R6, respectively. The experimental condition set for each run was tabulated in Table 1.

Table 1: Experimental condition and results.

Sample Coded	$X_1$ (wt.%)	$X_2$ (mm)	$X_3$ (hr)	$X_4$ (mL/min)	$X_5$ (wt.%)	Lignin Degradation (%)
UTP	10	0.8	-	-	-	-
R3	30	0.8	1	30	40	89.73
R4	70	0.8	1	60	20	90.15
R5	30	0.3	2	60	20	39.02
R6	70	0.3	2	30	40	1.83
ANOVA; Response: Lignin Degradation; R-sqr=.99954; Adj:.9968 DOE; 5 factors at two levels; MS Residual=4.242836 DV: Var7						

### 2.3. Ozonolysis Procedure

A total of 10 g oven-dried OPF was moisturized with distilled water until the desired moisture content attained. The moist OPF was then placed in the glass reactor, and the reactor was connected to the ozone generator (model LAB 2A, Triogen, Scotland), and ozone destructor. The ozonolysis experimental system was set up as Figure 1. The ozone concentration was monitored using ozone monitor (Honeywell EC-P2) by controlling the oxygen feed flowrate and voltage supply. Initially, ozone flowed through a bypass to regulate the rate prior to continuous flow through the reactor at a specified flow rate and reaction time. When the desired reaction time was achieved, the ozone generator was switched off and the system was flushed with oxygen for 10 min. The ozone-treated substrate (known as treated OPF after this) was immediately washed and rinsed with 100 mL of distilled water to suspend the lignin and other impurities for 1 h. The mixture was then separated via vacuum filtration. After filtration, the wet treated OPF was dried in the oven at 105 °C for 50 min. The dried treated OPF was weighed and stored in a crucible for further analysis.



**Figure 1:** Ozonolysis Treatment of Biomass System

## 2.4. Analytical Method.

### 2.4.1. Chemical Analysis

The composition of cellulose and hemicellulose was determined by cellulose isolation method and modification of chlorination method, respectively. The method was followed from Tan and Lee (2012) with a little modification. The cellulose and hemicellulose content was calculated using Eqs. 1 and 2, respectively. The lignin content was determined by Kappa number method (Chai and Zhu, 2002). The lignin degradation of OPF was calculated based on Eq 3. The concentration of total reducing sugar (TRS) in treated OPF was determined from 3,5 Di-nitrosalicylic Acid (DNS) method. The glucose was selected as a standard for TRS calibration curve.

$$\text{Cellulose content (\%)} = \frac{\text{Weight of residue from isolation cellulose method (g)}}{\text{Initial weight of OPF sample (g)}} \times 100 \quad (1)$$

$$\text{Hemicellulose content (\%)} = \frac{\text{Weight of residue from chlorination method (g)}}{\text{Initial weight of OPF sample (g)}} \times 100 - \text{Cellulose content (\%)} \quad (2)$$

$$\text{Lignin degradation (wt.\%)} = \frac{\text{Untreated lignin content} - \text{treated lignin content}}{\text{untreated lignin content}} \times 100 \quad (3)$$

### 2.4.2. Physical Properties Characterization.

The functional group presence in untreated and treated sample was identified by Fourier Transform Infrared (FTIR) spectrometer (Pelkin Elmer). The instrument is located at Analysis Laboratory, N18, FKT, UTM, Malaysia. About 2 mg samples were prepared by mixing with 120 mg of spectroscopic grade KBr powder then pressed in a standard device using a pressure to produce 13 mm diameter pellets. The background spectrum of pure KBr was subtracted from that of the sample spectrum. The spectra ( $4000\text{--}600\text{ cm}^{-1}$ ) were recorded with a resolution of  $4\text{ cm}^{-1}$  and 16 scans per sample.

The crystallinity of OPF was determined by powder X-ray diffraction (XRD). The XRD patterns were taken with a Bruker D8 Advance diffractometer, which has a Dynamic Scintillation Detector with low background (0.4 cps) and high dynamic range (up to  $2 \times 10^6$  cps) allowing rapid data acquisition for most samples, which was located at P23, Faculty of Mechanical, UTM, Malaysia. The diffractometer uses Cu K  $\alpha$  radiation (40 kV 40 mA) with a wavelength ( $\lambda$ ) of 1.54 Å. The samples were recorded from  $15^\circ$  to  $50^\circ$  ( $2\theta$ ) with step scan of  $0.05^\circ$  every 1s. The phases were identified using the powder diffraction file (PDF) database (JCPDS, international Centre for Diffraction Data).

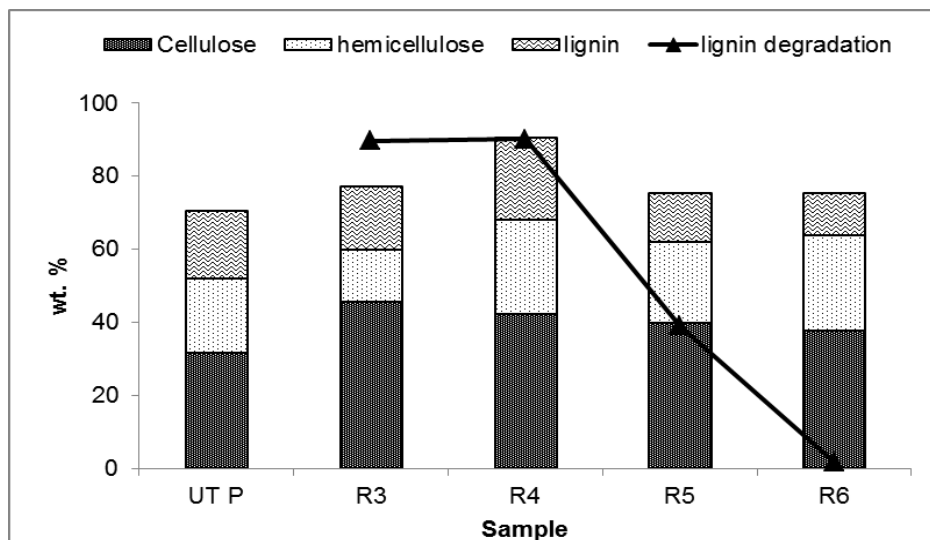
The total surface areas, pore volume, and diameter of pore, as well as the structure of the microporous material of catalyst were determined using isotherm nitrogen ( $\text{N}_2$ ) adsorption at 77 K (Micromeritics ASAP 2020 V4). Before adsorption measurements, all the samples were outgassed for 20 h at  $100^\circ\text{C}$ . Quantachrome Autosorb-1 instrument is designed for the surface area and pore size analysis of powdered and porous materials.

## 3. RESULTS AND DISCUSSION

### • Composition of Treated OPF

Figure 2 illustrates the histogram of the composition of untreated and treated OPF and the relationship of the composition with lignin degradation. Overall, the lignin yield decreased after pretreatment indicating that OP on OPF is effective to degrade lignin. In addition, the yield of cellulose component in treated OPF was increased, and slightly affecting the hemicellulose yield. The amount of cellulose recovery for treated OPF increased and correspondence with the lignin degradation of OPF. But, the amount of hemicellulose recovery was independent with lignin degradation as well as lignin recovery. The ozonation of OPF would break the acid insoluble lignin (AIL) into acid soluble lignin (ASL) at the surface of OPF as explained by Garcia-Cubero (2009). The hemicellulose and lignin losses could be attributed to amorphous nature of lignin and hemicellulose. The ASL and other soluble compound could be removed during the washing steps.

However, the cellulose structure is not affected in the process since the cellulose exhibits high hygroscopic nature with water (Wertz et al., 2010).



**Figure 2: The relationship of lignin degradation and chemical composition of OPF**

- **Physical Properties of Treated OPF**
- i. **Functional group identify by Fourier Transform Infrared Spectroscopy (FTIR).**

The graph of IR band of each sample is shown in Figure 3. As observed, the functional group presence in each treated OPF is slightly similar. Besides, the IR- spectra is weakling for treated sample indicating that the crystal structure of OPF is disrupted. The vibration strength of the IR-spectra for the treated sample is weaker compared to untreated OPF. The shaking vibration at  $3390\text{ cm}^{-1}$  (1) revealed that the hydrogen bonding between the OH groups of OPF is breaking. On the other hand, the functional group presence for cellulose, hemicellulose, and lignin which is hardly defined for untreated sample appeared after the pretreatment. After pretreatment, the clear shoulder at  $2900\text{ cm}^{-1}$  (2) representing the functional group of aliphatic saturation hemicelluloses were detected (Conley, 1972). Meanwhile, the phenolic compound at  $\sim 1600\text{ cm}^{-1}$  represent the lignin compound was not detected in untreated OPF and treated OPF sample. But, the presence of C=O vibration at  $\sim 1640\text{ cm}^{-1}$  (3) and/or  $\sim 1730\text{ cm}^{-1}$  (4) indicated that the ozone could have degraded the phenolic structure to ester with six ring i.e lactone or ketones, respectively (Conley, 1972). The other vibration presence at finger print region ( $\sim 1100\text{-}700\text{ cm}^{-1}$ ) explained the structure of cellulose in the sample (Conley, 1972). Moreover, the amorphous cellulose detected at  $897\text{ cm}^{-1}$  (12) for each treated product reveals the appearance of cellulose in OPF after pre-treatment (Conley, 1972; Zhang et al., 2012).

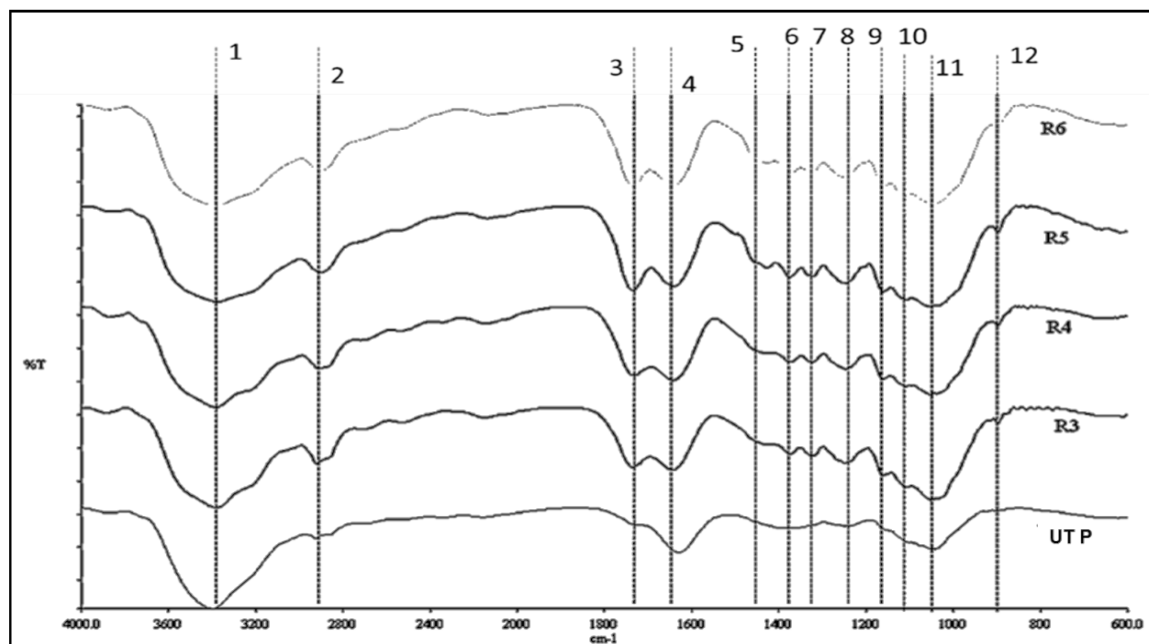


Figure 3: IR- Spectra of OPF (UT P) and treated OPF (R3, R4, R5 and R6) by FTIR

## ii. Crystallinity determination by XRD

XRD measured the crystallinity of the entire material (i.e. hemicellulose, lignin and cellulose) in OPF or treated OPF. Thus, the crystallinity of OPF is mostly attributed by amorphous region of hemicellulose and lignin and crystal region of cellulose. As depicted by **Error! Reference source not found.4**, the treated sample increased the intensity of crystal structure. On the other hand, higher lignin degradation (R4) showed the highest intensity but not for crystallinity index (CrI) which explained that the ozone treatment does not decreased the crystallinity of cellulose structure of OPF but, it affects most of hemicellulose and lignin part. Overall, the crystallinity index of treated OPF is increased for the entire treated sample (Table 2).



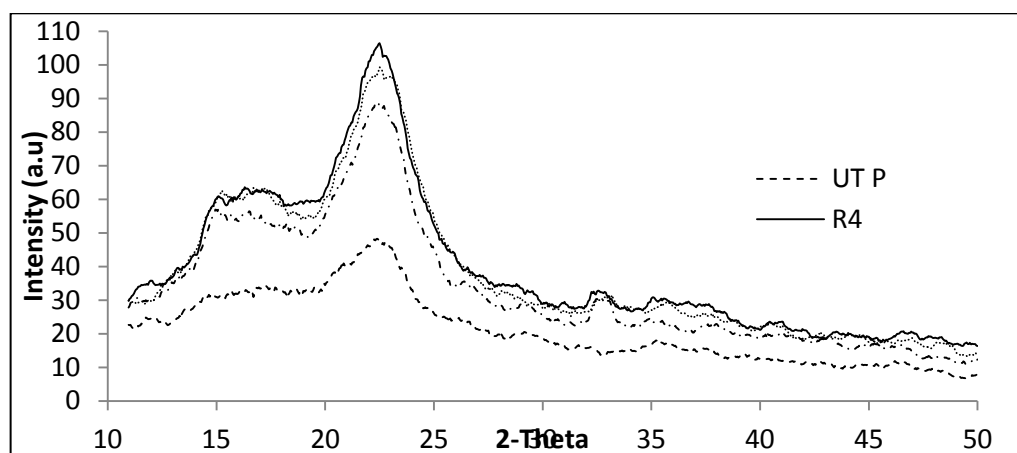


Figure 4: Diffractogram of XRD

### iii. Surface area and porosity by multipoint N<sub>2</sub>- absorption

The pore distribution of untreated OPF is a multi-pore structure; majoring the macropores by showing the Type IV isotherm adsorption with no knee hysteresis. However, the pore distribution of OPF is changed after the treatment. From multipoint BET analysis, the surface area of the treated sample was found to reduce slightly except for R5 (

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**Table 2: Crystallinity index, Surface area and pore properties of OPF and treated OPF**

Sample	Lignin Degradation (%)	TRS Yield (mg/mL)	Crytallinity Index (%)	BET Surface Area (m <sup>2</sup> /g)	Pore Diameter (nm)	Pore Volume ( $\times 10^{-3}$ cm <sup>3</sup> /g)
UT P	n/a	7.8	36.1	1.03	11.40	2.93
R4	90.15	9.1	51.9	0.74	11.10	2.05
R5	39.02	9.6	52.1	1.07	98.74	2.65
R6	1.83	9.0	50.1	1.00	11.01	2.76

#### iv. Morphology study by SEM

The morphology images of untreated and treated OPF sample at different lignin degradation were taken at 500x magnification presented in **Error! Reference source not found.**The untreated OPF images show that the OPF structure is highly fibrillar and intact morphology crystal structure (**Error! Reference source not found.3A**). The structure of OPF was evolved during the process for each part of OPF consistently with lignin degradation. The structure is totally disrupted at lignin degradation over than 90% **Error! Reference source not found.**(**Error! Reference source not found.3B**). The structures become curly, disordered and loose. Meanwhile, the OPF structure was slightly disrupted at medium lignin degradation (**Error! Reference source not found.3C**) and lower lignin degradation (**Error! Reference source not found.3D**). This indicates the polymer chain of lignin is breaking up with lignin degradation

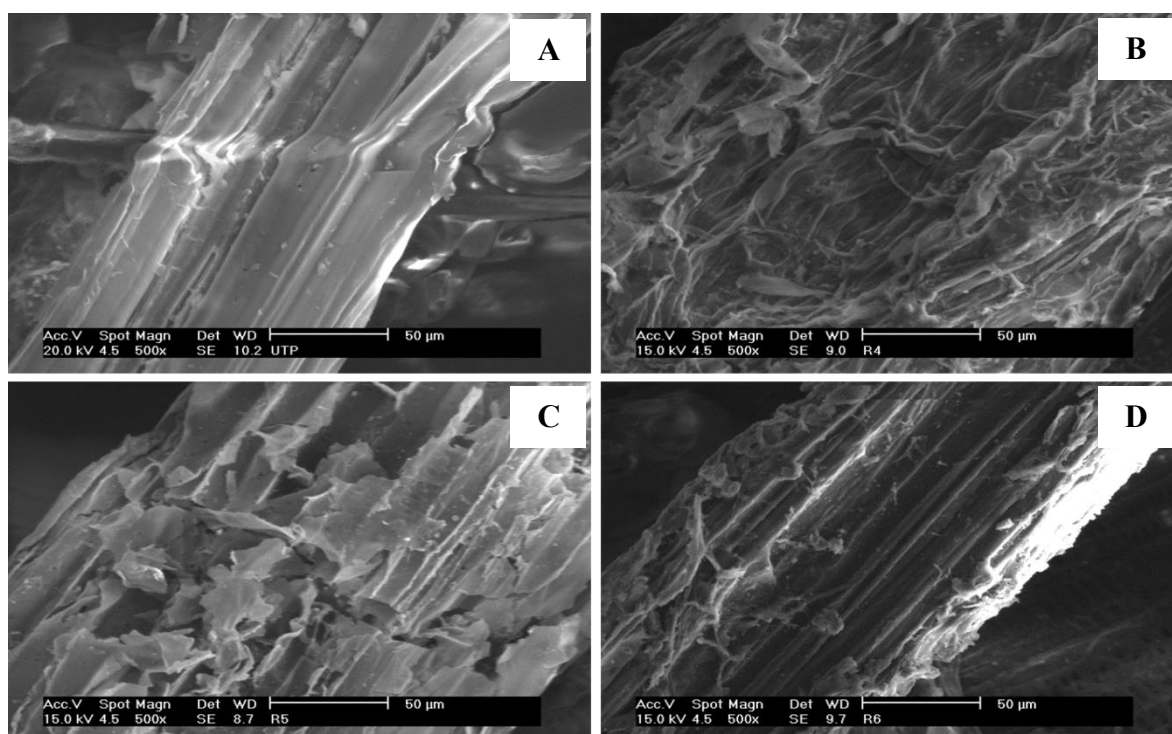


Figure 1: Electron micrograph of OPF (A) and treated OPF (B-D)

#### 4. CONCLUSION

The ozone was found to permeate at the external surface of OPF and degrade the lignin structure into soluble component (carboxylic compound) and exposed the structure of hemicellulose. The exposed hemicellulose layer was then removed as well during the washing step since it is tending to be soluble in water due to the presence of acetyl- and methyl-substituted groups. However, the cellulose layer is not affected during the treatment due to its high resistance to water swelling. By removing the lignin and hemicellulose, the recovery of cellulose structure is increased as well as the crystallinity of the OPF. Unfortunately, the surface area and pore properties of OPF were not associated with lignin degradation but have a connection with the process parameter which further study is needed. However, the ozonolysis pretreatment of OPF would increase the TRS recovery compared to untreated OPF due to the removal of lignin.

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