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Research Article

Chemical and Structural Changes of Ozonated Empty Fruit Bunch (EFB) in a Ribbon-Mixer Reactor

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Abstract

Agricultural wastes especially empty fruit bunch (EFB) are abundantly available to be utilized as a feedstock for biochemical synthesis or biofuel production. The components of the waste include lignin, hemicellulose and cellulose. Cellulose, the polymer of glucose, is the active component for producing bio-based chemicals. However, it is difficult to isolate cellulose since lignin, the most outer layer in the waste is recalcitrant. Therefore, the agricultural wastes need to be pre-treated prior to downstream processing. The aim of this study was to investigate the effect of ozone pretreatment on lignin degradation and total reducing sugar (TRS) yield. EFB was pre-treated using ozone gas in a ribbon-mixer reactor. The chemical and structural changes of ozonated EFB were analysed. The highest delignification obtained were 95.7 wt.% and TRS yield was enhanced to 84.9% at a moisture content of 40 wt.% with 60 g/m^3 ozone concentration within one hour of reaction time. Both NMR and FTIR spectra conferred major peaks inferring higher lignin degradation could be achieved using ozonolysis.

Keywords: Ozone; Oil Palm Biomass; Pre-treatment; Delignification; Empty Fruit Bunch

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1. Introduction

Biomass is one of the renewable energies that has huge potential to be utilize as natural source of fuel and energy. It is widely available, sustainable, and also cheap. Around five million tons of biomass comprises of organic materials from grasses, trees, flowers and agricultural wastes, such as: residues from sawmills, corncob, sugarcane bagasse, oil palm waste, wheat straw, and rapeseed oil pressing, were produced annually [1]. It consists of high molecular weight compounds, such as: lignin, cellulose, hemicellulose, and other nutrients, such as: lipids, phenolic compounds, pectin, and proteins. These natural compounds have a huge potential to be utilized for various industries including food and beverages, coatings, paints, cosmetic and pharmaceutical.

Besides, lignocellulosic biomass has huge potential to be utilized as raw material for second-generation biofuels, such as: methane [2], biodiesel [3], biohydrogen [4], and bioethanol, thus creating sustainable biomass supply chains by adopting "waste-to-wealth". Unfortunately, the

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utilization of biomass for bio-chemical and biofuel production has not been well-established yet.

In Malaysia, about 420 oil palm mills process fresh fruit bunches (FFB) to produce crude palm oil, and almost 70% of FFB are turned into wastes in the form of empty fruit bunch (EFB), fibers and shells, as well as liquid effluent [5]. These by-products can be converted to higher value-added products or energy to generate additional profit for the palm oil industry [6]. Among the wastes, the EFB is underutilized and some of it is currently used either as mulching material or sometimes fed into the boiler as a low energy-efficiency combustion fuel [7]. Currently, most EFB is left to decompose near the mill due to its high moisture content. This could cause detrimental effect to the environment.

One of the main constraints to utilize EFB for industrial application is the needs for pretreatment steps to break down the recalcitrant structure of lignocellulose into its fibrous components of holocellulose and lignin. It is due to low efficiency of enzymatic hydrolysis of polysaccharide which can be influenced by several factors including particle size, lignin content and cellulose degree of polymerization [8]. Among these factors, lignin content has the most significant impact on biomass degradability since the lignin removal promotes binding of enzymes and increases the total reducing sugar yield [9], hence the best pretreatment should maximize lignin removal.

Various pre-treatment methods have been developed, including biological, chemical and physical methods, as well as combinations of these methods, with the goal to find the most suitable and efficient pre-treatment steps to degrade biomass. Among those pretreatments, ozonolysis is one of the most promising pretreatment methods since it can effectively delignify biomass (up to 85%) with minimal impact on cellulose. The ozone acts as a strong oxidative agent that attacks the electron rich lignin component of the biomass and exposed the fibrous components of hemicellulose and cellulose. Many types of agroindustry waste such as cereal straw [10], wood pulp and wood chips [11], cotton stalk [12], grass [13], among others, have been investigated for their behaviors and characteristics using ozone pretreatment and showed promising results.

There are many research findings reporting on the effectiveness of ozonated biomass in enhancing product efficiency and yield. The ozonated samples have been studied for biofuel production via fermentation process for bioethanol production [14,15], biohydrogen production [16], and anaerobic digestion process for methane production [17]. In addition, the ozonated biomass has also been studied in the waste water treatment plant [18]. Therefore, the ozone pretreatment is a suitable candidate to be used as pretreatment method of biomass for industrial applications.

The effectiveness of ozonolysis pretreatment depends on the reactor design, which affects ozone consumption, sugar release, and reaction kinetics by the contact between ozone with substrate. In the previous study on ozonolysis of wheat straw using fixed bed reactor [19], 50% delignification was achieved at operating parameter of 40 % (w/w) moisture for 2 h reaction time. The reaction consumed 100% ozone in the first 60 min and gradually decreased once breakthrough time was attained. Previous studies by Vidal and Molinier [19] concluded that the stirred semi batch reactor provided higher ozone concentration compared to the fixed bed reactor, while Cesaro and Belgiorno [20] investigated the ozonolysis of municipal solid waste using Drechsel trap reactor and bubble column reactor. The bubble column reactor was more effective as higher amount of ozone reacted with the substrate while Drechsel trap obtained poor ozone contact with substrate and yielded high residual ozone [21]. Hence, in this study, the ribbon-mixer reactor was designed and used to improve the ozone distribution to enhance ozone contact with the substrate in order to favor the oxidation reaction.

In this work, lignin degradation and total reducing sugar of untreated sample (raw) and ozone treated EFB (OTE) were studied using the novel ribbon-mixer reactor. The major components were analyzed using NMR, FTIR, SEM-EDX to monitor the impact of ozone oxidation on samples. The insights of lignin modification through ozone pre-treatment could provide useful information for the development of ozonolysis pre-treatment process and its product characteristics.

2. Materials and Methods

2.1 Materials

The EFB sample was collected at palm oil mill located at Penggeli, Kulai, Johor, Malaysia in shredded form. The particle size of EFB was reduced to 0.3 mm and 0.5 mm. The EFB was then dried in the oven at 105 °C for 24 h to eliminate excess moisture and stored in sealed container to prevent microorganism growth and further characterization.

All chemicals used in the experiments were of analytical reagent grade. Sulphuric acid (H_2SO_4, 95–98%, Qrec, NZ), sodium carbonate (Na_2CO_3, Qrec, NZ), potassium iodide (KI, Qrec, NZ), and potassium permanganate ($KMnO_4$, Fisher Brand, UK) were used in the chemical analysis. Oxygen gas (170 bar, 8.4 m^3) was utilized for the ozonolysis pretreatment. Acetone (CH_3COCH_3, Merck Brand, Germany) was used to wash the ozonated samples. Distilled water was used for moisturizing the EFB and preparation of solutions. D-Glucose ($C_6H_{12}O_6$, Qrec, NZ) and D-Cellulose $(C_6H_{10}O_5)n$, Sigma–Aldrich, USA) were adopted as the model compounds for the identification of chemical compositions.

2.2 Chemical Composition of Empty Fruit Bunch (EFB)

The cellulose isolation method was conducted to quantify the compositions of cellulose and hemicellulose [22]. Meanwhile, the acid insoluble lignin, acid soluble lignin and ash contents were measured by following the standard laboratory analytical procedure of National Renewable Energy Laboratory (NREL/TP-510-42618).

2.3 Measurement of Bulk Density and Void Fraction

The physical properties of untreated EFB in terms of bulk density and void fraction were determined according to our previous work [6]. The effect of various moisture contents (20%, 30%, and 40%) and different particle sizes (0.3 mm and 0.5 mm) on the physical properties were evaluated. The moisture content was measured using moisture analyzer (SASTECTM, ST-LSC60 model, Malaysia) [6].

2.5 Ozonolysis Pretreatment

EFB ozonolysis pretreatment experiments were conducted using OzBiONY® pretreatment prototype as shown in Figure 1(a). The piping and instrumentation diagram (P&ID) of the OzBiONY® prototype consists of ozone generator (model LAB 2A, Triogen, Scotland), ozone reactor (ribbon mixer semi-batch reactor, 2.47 L), ozone monitor (Eco Sensors, UV-106M), and ozone destructor (Figure 1(b)).The ozonolysis pretreatment method was thoroughly described in our previous work [22]. Several steps were slightly adjusted in the current study. 20 g of moistened EFB was placed inside the ozone reactor with the rotation speed of 60 rpm. After completing the ozonolysis pretreatment, the ozonated EFB was immediately swelled in 300 mL water-acetone (270/30 mL v/v) mixture for 2 h to suspend the lignin and other impurities. The ozonolysis experiments were repeated for the EFB samples at moisture contents of 20%, 30%, and 40%. with different particles sizes (0.3 mm and 0.5 mm). The values of ozone inlet concentrations were also var-

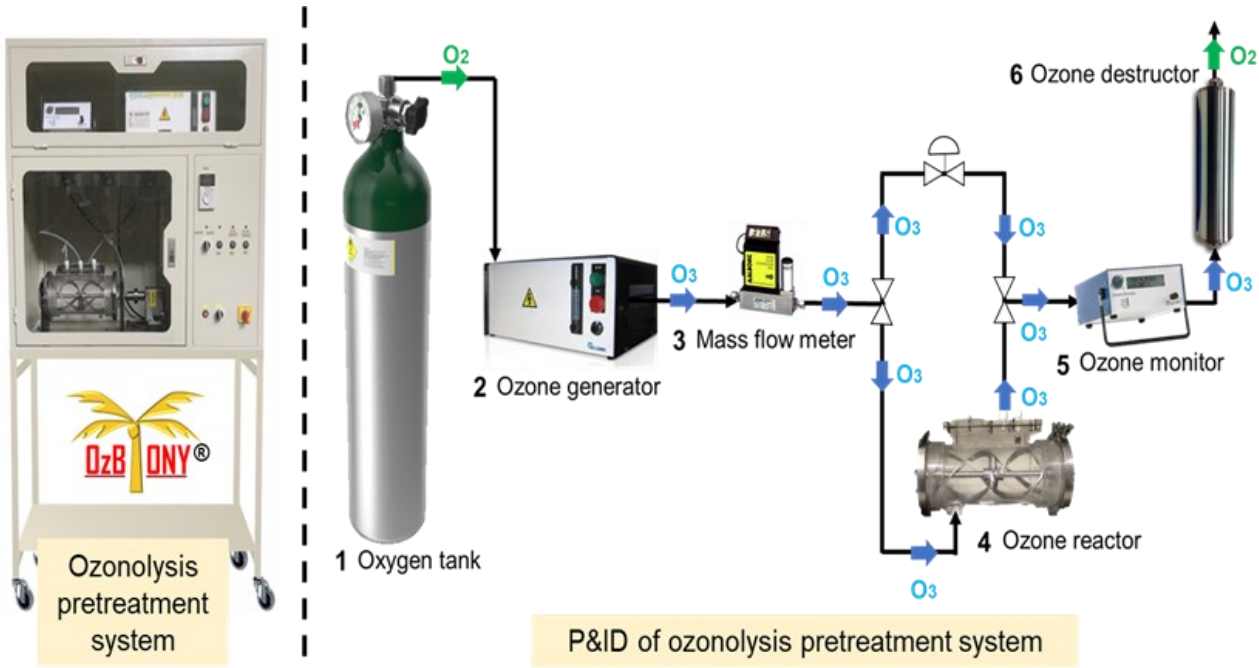

Figure 1. OzBiONY® pretreatment prototype system (a) and the piping and instrumentation diagram (P&ID) consists of (1) oxygen tank, (2) ozone generator, (3) mass flow meter, (4) ribbon mixer ozone reactor, (5) ozone monitor, and (6) ozone destructor (b).

ied at 40, 60 and 80 g/m^3 and at different reaction times (60, 90, and 120 min) to investigate the effectiveness of ozone consumption on lignin degradation.

2.6 Two-step Acid Hydrolysis

The detail of two-step acid hydrolysis step was reported elsewhere [22] to determine the amount of TRS yield.

2.7 Analytical Methods

2.7.1 Lignin degradation

The lignin degradation was determined using Kappa number (K) method calibrated with Klason lignin method [22]. 0.05 g OTE was mixed with 20 mL of H_2SO_4 (2 M) and 5 mL of 0.02 M $KMnO_4$. The mixture was stirred at 200 rpm for 5 min and then filtered. The filtrate was analyzed using UV–vis at 546 nm and the absorbent reading was recorded as A_c. A blank sample consists mixtures of $KMnO_4$ and H_2SO_4 was used as the controller. The absorbent of the blank sample was recorded as A_0. The lignin degradation of the EFB was calculated based on Eqs. (1)–(3):

$$Kappa\ number, K = 100\left[\frac{A_0 - A_c}{A_0}\right] \quad (1)$$

$$Lignin\ content = 0.15K \quad (2)$$

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

2.7.2 Determination of total reducing sugar (TRS)

The amount of TRS in the hydrolysis product was determined by biochemistry analyzer (2950D YSI biochemistry analyzer, US). The YSI biochemistry analyzer was calibrated using glucose standard before starting the analysis. About 1.0 mL of hydrolysate from acid hydrolysis process is added into sample tray and the TRS concentration results was recorded in (g/L). The TRS yield in the EFB hydrolysate was computed by Eq. (4) [22]. The TRS yield was multiplied with the total weight of OTE sample to determine the total weight of TRS produced after the pre-treatment. The TRS recovery was then calculated by Eq. (5):

$$TRS\ yield\left(\frac{g}{g\ OTE}\right) = \frac{TRS\ concentration\left(mg/mL\right) \times V \times df \times 0.9}{300\ mg\ OTE} \quad (4)$$

$$TRS\ recovery(\%) = \frac{Total\ Weight\ of\ TRS\ from\ OTE\ (g)}{Weight\ of\ initial\ EFB\ (g)} \times 100 \quad (5)$$

where total volume (V) is equal to 0.087 L, dilution factor (df) is equal to 1, and 0.9 is the anhydrous correction for glucose.

Table 1. Chemical composition of EFB.

Chemical composition (dry basis)	(wt.%)
Cellulose	53.00
Hemicellulose	15.44
Acid insoluble lignin (AIL)	26.67
Acid soluble lignin (ASL)	0.89
Ash	4.00

2.8 Characterization of EFB and OTE Samples

The untreated EFB and OTE samples were dissolved in DMSO and characterized by proton (1H-NMR) in the range of 1–6 ppm. The 1H-NMR spectra of the products were acquired at 25 °C using Bruker AV400 high resolution multinuclear NMR spectrometer. The FT-IR spectroscopy (Perkin-Elmer spectrum GX FT-IR system, USA) was used to detect major functional group in the extracted cellulose and the absorption mode was recorded in the range of 4000–600 cm^{-1} using ATR type. The morphology study of untreated EFB and OTE samples was performed using scanning electron microscope, SEM-5410LV with 500X magnification while the elemental composition of the products was detected by using energy disperse X-ray spectrometry (EDX).

3. Results and Discussion

The chemical compositions of the EFB are summarized in Table 1. The results have proved that the EFB is significantly dominated by cellulose components, which are 53 wt.% is cellulose and 15.44 wt.% is hemicellulose. The highly cellulose content has made the EFB as an ideal source for cellulose-based natural products. The cellulose component should be exposed to efficiently convert into higher value-added materials by removing the complex lignin structure which occupies about 27.56 wt.% of EFB.

3.1 Effect of EFB Moisture Content on the Physical Properties of EFB

The physical properties of EFB particles in terms of bulk density and void fraction were studied for particle sizes of 0.3 mm and 0.5 mm at different moisture contents; 20%, 30%, and 40%. For operational control and design of a re-

actor containing gas-particle flow, the void fraction and bulk density of solid particles are vital parameters [23]. Void fraction (porosity) is a measure of the void spaces in a solid material which is usually defined as the fraction of the volume of void space available for the flow of fluid over the total volume [24]. In ozonolysis process, the void space is occupied by ozone gas, which flows through the EFB particles. Bulk density is an indicator of particle compaction, which is measured by the mass weight of particles in a unit volume.

The void fraction shown in Figure 2 (a) is decreased and bulk density in Figure 2 (b) is increased as moisture content increased. This proved that the water particles play significant effect towards the change in physical characteristics of EFB. The increase of void volume in the EFB decreases the EFB bulk density. The difference in density, size and shape among EFB particles often leads to particle segregation during flow with large particles rising to the upper surface and small particles percolating down to the base [25].

3.2 Ozonolysis of EFB

In ozonolysis of EFB, ozone acts as oxidizing agent and reacts with the surface of EFB to degrade lignin without affecting the cellulose component [26]. Based on Criegee mechanism [27], the ozone preferably reacted with aromatic compounds, alkenes, and ketones and break the olefinic double bonds and cycloaddition complexes. Another reaction mode is the ozone insertion into carbon-hydrogen bonds in alcohol-, aldehyde- and ether- type structures. In the case of aryl and alkyl ethers, the reaction results in the cleavage of the ether bond [28].

The subsequent hydrolysis reaction could convert OTE to attain glucose yield as high as 45.09% [29] which can be used as feedstock for production of 5-hydroxymethylfurfural (5-HMF) [30] bioethanol [31], and levulinic acid (LA) [32]. The ozone pretreatment is proven to efficiently reduce the recalcitrance in EFB for the conversion of cellulose and enhancement of sugar release. In hydrolysis reaction, β-1,4-glycosidic bonds of cellulose chains are broken into glucose molecules by cellulose enzymes via enzymatic hydrolysis [33] or acids via acid hydrolysis [34]. On the other hand, the OTE can also be purified by using acid hydrolysis [35] or sodium hydroxide [36] to be employed as cellulose fiber for box packaging, and raw material for green chemicals production, such as: car-

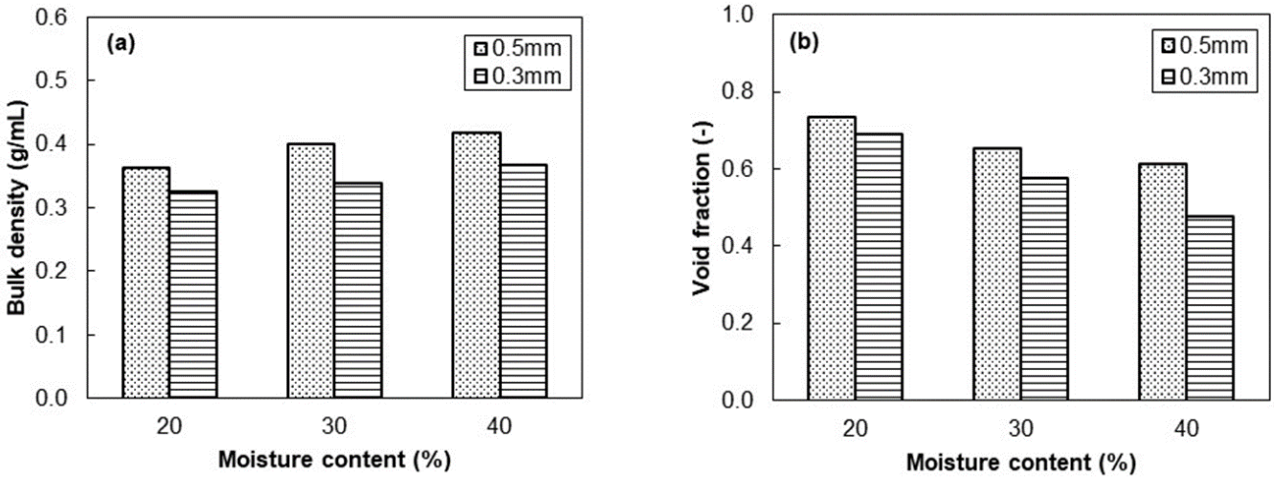

Figure 2. (a) Void fraction and (b) bulk density of EFB over moisture content at 0.3 mm and 0.5 mm particle sizes.

Table 2. Process parameters of ozonolysis pretreatment in different types of reactor and biomass.

Raw material	Reactor design	Moisture content (%)	Ozone Concentration (g/m^3)	Flow (L/h)	Time (h)	Delignification (%)	Ref.
EFB	Ribbon mixer	40	60	2	1	95.7	This study
Oil Palm Fronds	Semi batch	30	n.a	3.6	0.5	98.7	[49]
Wheat straw	Batch	95	9.5	60	5	50	[50]
Poplar sawdust	Fixed-bed	30	0.5	60	3	2	[19]
Coastal Bermuda grass	Rotatory	30	n.a	60	1	31	[13]
Red oak	Cylindrical	50	n.a	30	2	-	[51]

boxymethyl cellulose (CMC), microcrystalline cellulose (MCC), and cellulose nanocrystal (CNC), which are widely used as hydrogels, pickering emulsifier, binders, membranes and polymer coatings [35]. The effect of process parameters and reactor design is discussed in this section.

3.2.1 Effect of reactor design

Various factors affect the effectiveness of ozonolysis process as describe in previous literature including the interaction between ozone with substrate, reactor design and reaction parameters. The optimum process parameters for ozonolysis process such as moisture content, reaction time and ozone flowrate are compared with the previous studies as shown in Table 2. The results show the highest delignification of biomass was obtained using ribbon mixer reactor design. The radial mixing generated by the ribbon mixer helps to increase reaction rate and interfacial contact of the biomass with ozone in order to favor the oxidation reaction. The pre-treatment of biomass in general and EFB in particular becomes more effective with higher lignin degradation rate and increment of the TRS yield.

3.2.2 Effect of process parameter; moisture content, reaction time and ozone concentration

Moisture content is arguably the most important process parameter for ozonolysis reactor to occur, since its form mass transport medium and influence the generation of radicals. As mentioned in previous studies, the optimal moisture content is in the range of 10 to 50 wt.% for ozonolysis of oil palm biomass [37,22], hence the ribbon mixer reactor used in this study helps to homogenous ozone concentration with moisture content in the biomass. From Figure 3 (a), it shows that the lignin degradation and TRS yield increase with the rise of moisture content. At low moisture content, ozone mass transfer was limited since the ozone was beginning to react with bounded water. At 40 wt.%, the EFB 0.3 mm and EFB 0.5 mm obtained high lignin degradation of 86.8% and 95.6% with highest TRS yield of 0.76 wt.% and 0.54 wt.%, respectively. However, if the water content is excessive, the water particles will block the biomass pore with the formation of thick film which favors larger residence time of ozone, thus enhances the decomposition of hydroxyl radical. Hence, moisture content more than 40 wt.% is not favorable since the lignin will not properly degraded [38].

The effect of EFB ozonolysis with reaction time is shown in Figure 3 (b), where the results shown concur with previous study involving other types of biomass. The delignification of cereal straws [21] was rapidly increased in 60 min reaction time, and reduced within 90 min due to acid insoluble lignin (AIL) degradation. Wan *et al.* [22] also discovered the oil palm

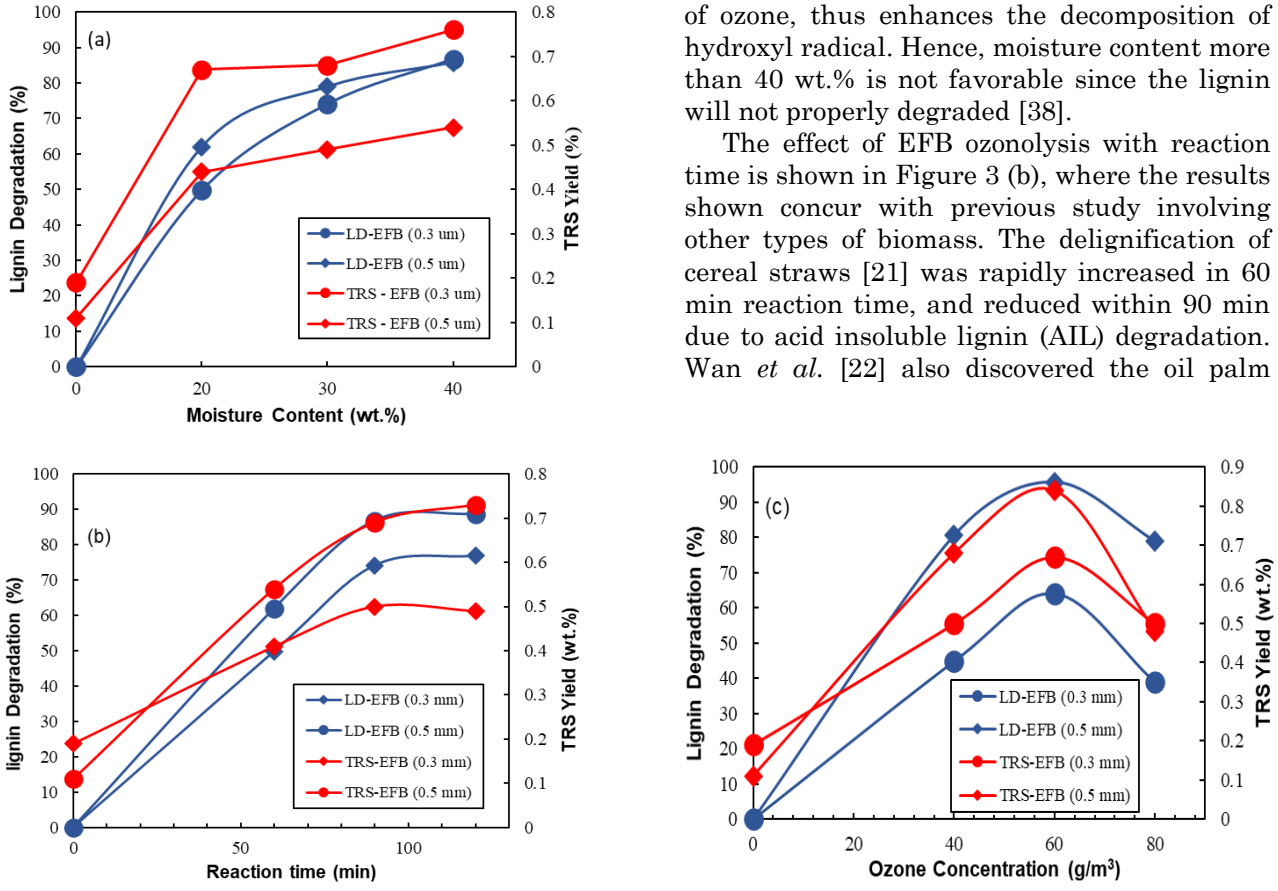

Figure 3. Effect of ozonolysis of EFB over (a) moisture content, (b) reaction time, (c) ozone concentration, (d) ozone consumption.

fronds reaction with ozone decrease the lignin degradation after reaction time more than 60 min since the ozone could react with hemicellulose on the exposed surface after the lignin layer was degraded.

It is clearly observed that the delignification has three visible stages [39]: (1) Initial, (2) Bulk, and (3) Residual. At the beginning of the reaction time, the delignification occurred very fast. In the initial phase, around 20% of lignin degraded mainly due to the reaction of phenolic and carbonyl structures. The bulk phase is up to 90 min, where the highest delignification obtained for EFB 0.3 mm and EFB 0.5 mm were 74.1% and 86.8%, respectively. The TRS yield also increased in relation of more surface area of cellulose is exposed while lignin bonds break down. After 90 min, the retarded delignification is established in the residual phase due to very small amount of lignin is dissolved. The residual lignin remains intact even after a prolonged ozone exposure because of three main reasons: (1) The presence of alkaline-stable native lignin structure, (2) The presence of alkaline-stable covalent linkages between lignin and carbohydrates and, (3) The occurrence of condensation reactions in lignin.

Figure 3 (c) shows the effect of ozonolysis of EFB with ozone concentration. The lignin degradation and TRS yield are increased until 60 g/m^3 and started to decrease afterwards. High lignin degradation of 95.7% and 64.1% was obtained for EFB 0.3 mm and EFB 0.5 mm, respectively, while high TRS yield of 0.84 and 0.67 was obtained for EFB 0.3 mm and EFB 0.5 mm, respectively. This is due to high inhibitory compounds were generated by degraded sugar with low molecular lignin compounds at higher ozone concentration [40]. The increase in TRS yield was attributed to the disrupted lignin structures, which allowed cellulose to be more accessible for subsequent hydrolysis steps.

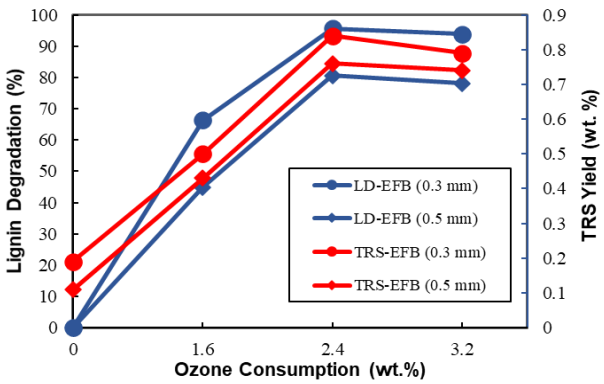

Figure 4. Effect of ozone consumption on TRS yield and lignin degradation.

3.2.5 Effect of ozone consumption

Previous studies by Wan *et al.* [22] and Schultz-Jensen *et al.* [26] have discussed the increase in cellulose component and decrease in lignin in ozonated biomass. However, although the TRS yield in hydrolysate was increased, the TRS yield in OTE might not be large due to excessive ozonation which could lead to sugar losses and low OTE weight after the pretreatment.

The ozonolysis pre-treatment is not economically feasible for full scale industrial implementation due to high cost of ozone production and maintenance, hence minimal ozone consumption is required to increase the feasibility of ozone pretreatment. As stated by Travaini *et al.* [41], ozone consumption can be defined as the amount of ozone consumed per gram biomass. The relationship between ozone consumption with lignin degradation of EFB are shown in Figure 4. The ozone consumption is directly dependent on reaction time, ozone concentration and inlet gas flow [42]. The glucose yield in hydrolysate increased rapidly with ozone consumption, which attributes to the sugar release at higher ozone concentration and ozonation time. However, lignin degradation and glucose yield slightly decreased and became constant after 3 wt.% ozone consumption as shown in Figure 4. This could be attributed to the delignification process being suppressed since the pores at the outermost layer gradually became impermeable during ozone-lignin reactions thus, preventing the ozone from diffusing into the lignin interior [43]. The formation of impermeable barrier limited the ozone mass transfer to complete the degradation of lignin. The loss of moisture due to ozone-lignin reaction could also be another reason for reducing delignification and the presence of residual lignin. Similar trends have been found in previous reports on the delignification of sweet sorghum bagasse obtained by alkali pre-treatment [44] and sugarcane bagasse obtained by chemical pre-treatment [45]. Excessive ozone consumption could lead the formation of inhibitory compounds by sugar degradation and expensive cost operation, hence operation above 3 wt.% ozone consumption is not favorable.

3.3 Characteristic and Structural Changes on Ozone Pre-treated Lignocellulosic Biomass

3.3.1 NMR Characterization

The chemical characterization of ozonated products are shown in Figure 5. It distin-

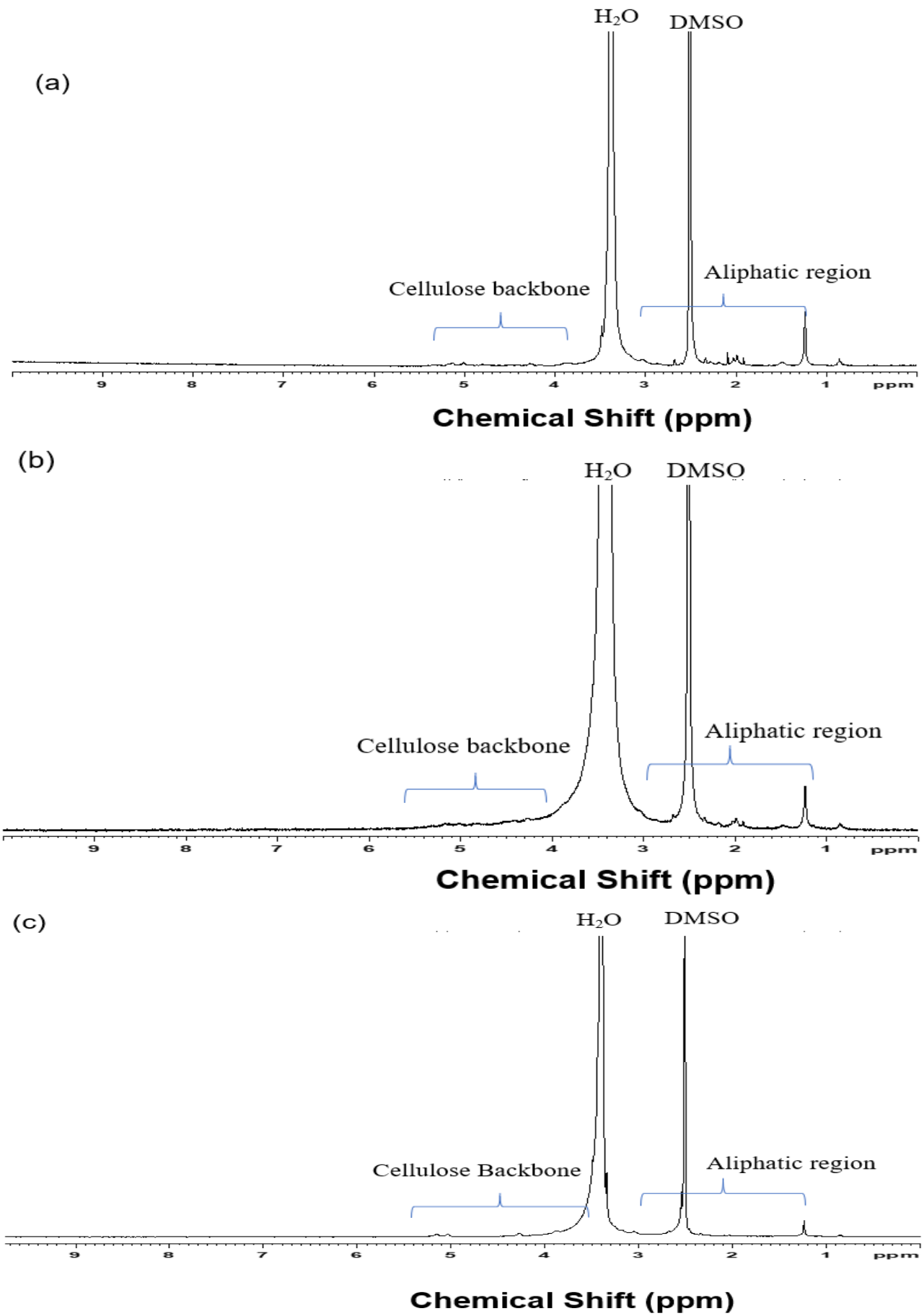

Figure 5. NMR spectra of ozone treated EFB at (a) 40 g/m^3, (b) OTE 60 g/m^3 and (c) 80 g/m^3.

guishes the distribution of the proton aliphatic region shift values at 1 to 3 ppm, indicating ozone has a prominent effect on the biomass component. These results are also supported by other researchers [46,41], stating that aromatic component of lignin was very susceptible to ozone reactions. The signal corresponding to cellulose backbone occurred at 4 to 5.5 ppm in the ozonated samples as ozone concentration increased.

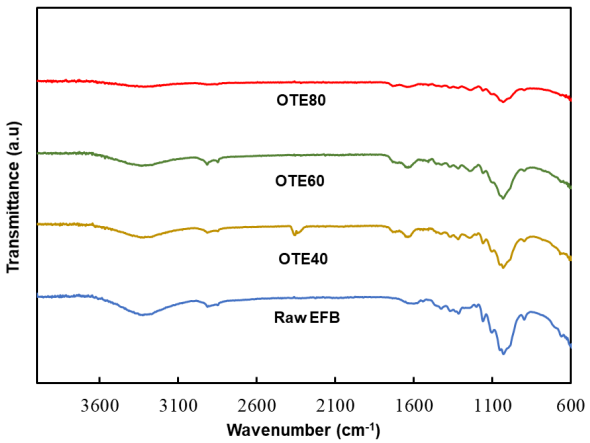

Figure 6. ATR-FTIR spectra of EFB raw and ozonated sample.

3.3.2 ATR FT-IR Characterization

Based on the Fourier transform infrared (FT-IR) analysis in Figure 6, the destruction of aromatic rings and ketones and aldehydes formation as ozone concentration increases up to 60 g/m^3 are evident in the OTE samples. Higher ozone concentrations promote the production of inhibitory compounds by sugar degradation and by reaction with low molecular lignin compounds. This is due to the partial oxidation reaction between hemicellulose and lignin. According to Criegee's mechanism, ozone attacks lignin double bonds and leads to formation of carbonyl compounds and aliphatic carbon acids [24]. Besides, the peak intensity was significantly reduced between 1458 cm^{-1} which is attributed to the aromatic stretching vibration of lignin degradation [47]. The decrease in the peak intensity at the absorption peak of 1750 cm^{-1} and 1025 cm^{-1} could be attributed to the disappearance of ester C=O stretching and ether symmetric stretching.

3.3.3 SEM Characterization

The SEM images of raw EFB and ozonized EFB samples are exhibited in Figure 7. The raw EFB tends to appear smoother than the

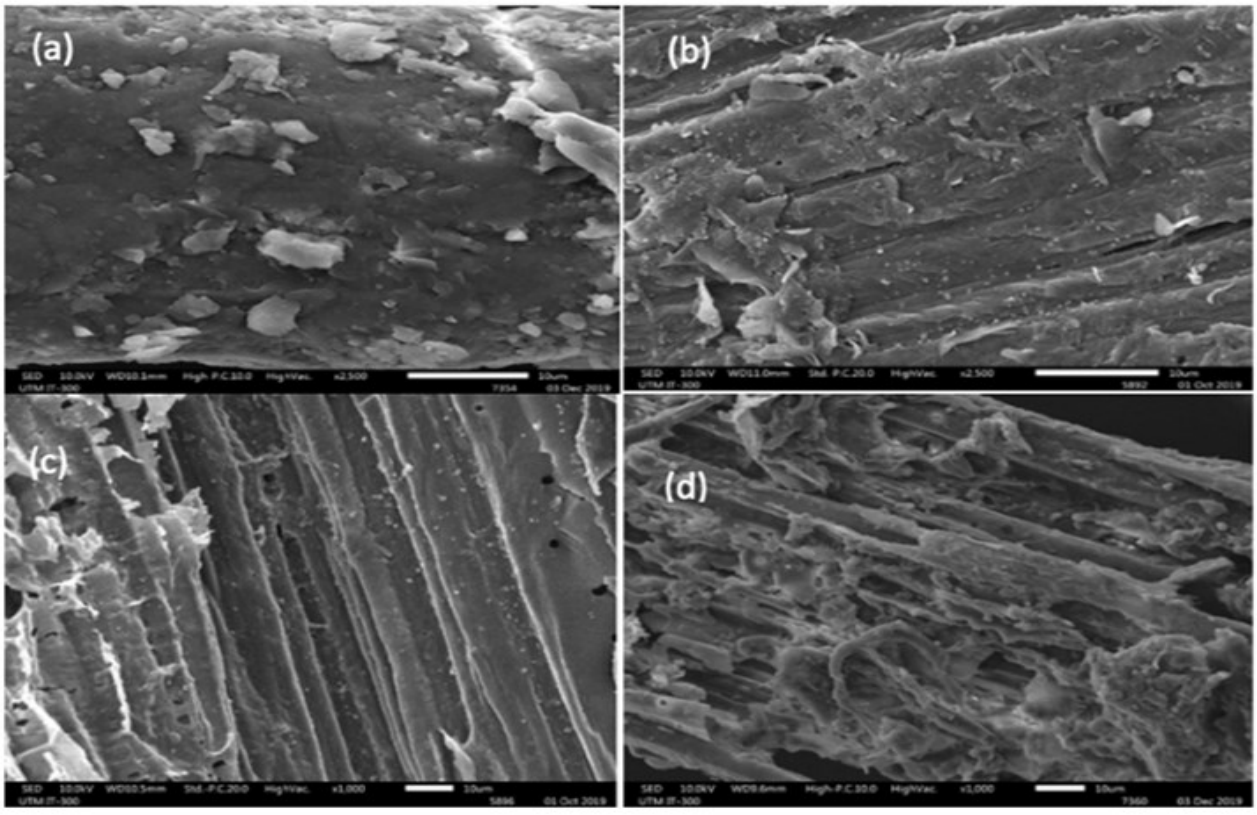

Figure 7. SEM images of EFB at 500X magnification; (a) EFB raw (b) OTE 40 g/m^3, and (c) OTE 60 g/m^3 (d) OTE 80 g/m^3.

surfaces of OTE samples. These morphological changes in OTE samples is due to the decomposition of lignin at the surface of EFB because of ozone gas exposure. Based on the figure, the ozone concentration gives significant effect on the biomass surface as the surface becomes rougher. The ozone gas interaction with OTE surface causes the lignin linkage breakdown and exposes the crystalline portions of the cellulose [48].

The surface morphology changes of the OTE samples are parallel with the FTIR analysis discussion which are associated with the changes in functional group and bonds due to the destruction of lignin. Besides, the wax at the surface of OTE was reduced as ozone concentration increased [48]. Based on Figure 7 (c,d), SEM images of ozonated EFB reveal the formation of porous structures, due to the disruption of physical structures. The structural changes are attributed to the lignin degradation and hemicellulose solubilization [46].

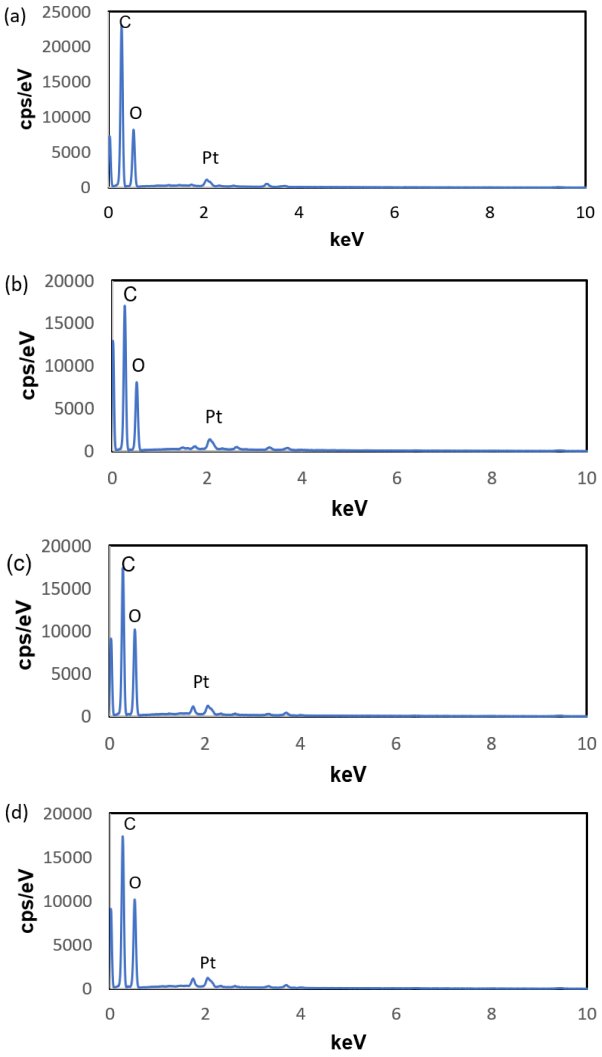

Figure 8. EDX elemental composition data of (a) EFB raw, (b) OTE at 40 g/m^3, (c) 60 g/m^3, and (d) 80 g/m^3.

3.3.4 Energy dispersive X-ray (EDX)

The EDX analysis in Figure 8 shows the elemental ratio for raw EFB and OTE at 40, 60, and 80 g/m^3. Based on the figure, the carbon ratio of raw EFB is higher compared to ozone treated EFB due to ozone gas oxidized and decomposed lignin by breaking the lignin bonds. Thus, the carbon content of EFB decreases. The ozonated EFB increases the oxygen content of the sample by converting the lignin component into hydroxyl, carbonyl, and carboxyl groups. In addition, other elements, such as: K, Ca, and Si which are known as major elements in ash composition, also decrease compared to the raw EFB due to breaking down of structural surface of EFB diminishing these elements owing to the ozonation process.

The OTE samples and raw EFB were analyzed using EDX analysis for its ratio of oxygen-to-carbon (O/C) as shown in Figure 9. The O/C ratio increased as ozone concentration increased from 0.32 to 0.61 due to the formation of atomic oxygen from ionized air with the surface of OTE. The increase in O/C ratios obtained concurs with the study reported by Klarhöfer *et al.* [45], where it stated that the decomposition of lignin and oxidation reaction led to formation of hydroxyl, carboxyl and carbonyl groups [48].

4. Conclusion

This study indicates that reaction parameters have significant effect on the chemical and structural changes and TRS recovery in ozonolysis of EFB. The additional mixing application in the reactor enhances the interaction be-

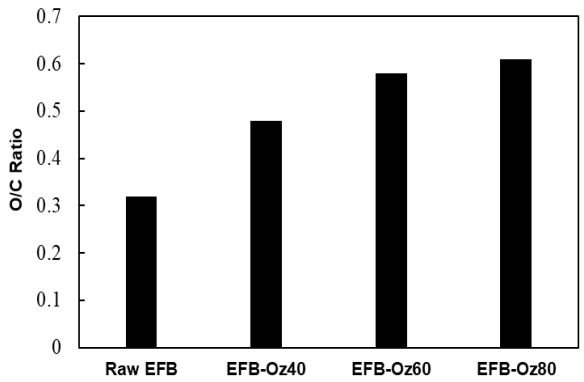

Figure 9. O/C ratio of (a) EFB raw, (b) OTE at 40 g/m^3, (c) 60 g/m^3, and (d) 80 g/m^3.

tween EFB particles with ozone, thus increases lignin degradation and TRS yield. The highest delignification obtained was 95.7 wt.% and TRS yield was enhanced to 84.9% at moisture content of 40 wt.% with 60 g/m^3 ozone concentration within 1-hour of reaction time. Both NMR and FTIR spectra confer major peaks attributing to lignin disappearance and higher lignin degradation can be achieved with ozonolysis. The results infer that the ozonolysis pre-treatment is able to modify the surface properties of EFB, and that ozonolysis pre-treatment could improve the glucose recovery for downstream bio-based conversion. The results also provide better understanding of ozonolysis of EFB for future up-scaling application and development.

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