Microwave assisted dilute organic acid pre-treatment of oil palm empty fruit bunch to improve enzyme accessibility

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Abstract. The depletion of fossil fuel is intriguing for researchers to find sustainable energy. Oil palm empty fruit bunch (OPEFB) is abundant biomass in Indonesia which is potential to be converted into bioethanol because of its high cellulose content. Suitable pre-treatment could increase the effectiveness of cellulose conversion into sugars. The aim of this study is to evaluate the effectiveness of Microwave-assisted Oxalic Acid pre-treatment (MOxA) and Enzymatic Hydrolysis (EH) to improve enzyme accessibility through variation of heating temperature, irradiation time, and EH incubation time. The higher the microwave heating temperature, longer irradiation time, and longer EH incubation time, the higher the glucose yield per pulp (GY) and reducing sugar yield (RSY) were observed. After MOxA at 200°C-15 min, as much as 43.22 % GY per initial biomass was obtained by EH for 10 days. SEM images showed more ruptures on the surface area of OPEFB fibre after MOxA that supported in increasing of enzyme digestibility. The XRD spectra demonstrated that the crystallinity index (CI) increased after MOxA because pre-treatment reduced amorphous components in the biomass. In addition, the FTIR spectra showed that peak intensity at 1714 cm⁻¹ corresponded to hemicellulose was gradually reduced after MOxA while peaks corresponded to lignin was more discernible.

1. Introduction

Oil Palm Empty Fruit Bunch (OPEFB) is a biomass waste formed after palm oil production. In 2018, Indonesia produced 47 million tons of palm oil [1] and approximately it produced 10.81 tons of OPEFB as waste [2]. OPEFB contains 4.25% of ash, 1.95% of extractives, 17.66% of acid insoluble lignin, and 67.98% of holocellulose, which consists of 43.18% of α-cellulose and 24.80% of hemicellulose [3]. The high α-cellulose content in the OPEFB makes it a potential feedstock for bioethanol production. However, a major drawback in the conversion process is the recalcitrant of biomass because it impedes cellulose conversion into bioethanol. Therefore, the pre-treatment of OPEFB is needed as a prior step. Physical, chemical/physical-chemical, and biological treatments or a combination of them are common methods to breakdown a strong cell wall of biomass [4]. Microwave is a physical-chemical method that was reported as an effective method in degrading lignin and hemicellulose, eventually improved accessibility of cellulose to enzyme attack [5]. Besides, the microwave has short reaction time to degrade hemicellulose and lignin, hence it was reported as green technology pre-treatment [4].

Microwave irradiation has been combined with chemical solutions such as organic or inorganic acid to increase pre-treatment effectiveness. Fatriasari et al. [6] reported that microwave-sulphuric acid pre-treatment released 49.2% reducing sugars from jabon kraft pulp [6] while glucose 156 mg/g
was obtained from wheat and rye stillages after microwave-sulphuric acid pre-treatment [7]. Sulphuric acid is known as an effective acid to overcome the lignocellulose structure. However, there are some disadvantages of using sulphuric acid in the pre-treatment, for example, it generates toxic compounds that can inhibit the fermentation process [8]. To overcome this limitation, an organic acid such as oxalic acid has been evaluated as a promising alternative to sulphuric acid pre-treatment due to its selectivity on hemicellulose hydrolysis and less corrosive behavior [9]. In simultaneous fermentation with pre-treated corn cob pellets, ethanol concentration could reach 16.9 g/L when oxalic acid was used as a pre-treatment catalyst [10]. As much as 92% of glucose was converted from cellulose in bagasse after microwave-oxalic acid pre-treatment. Proper pre-treatment and enzymatic hydrolysis (EH) conditions play important roles in converting cellulose to sugars. Generally, the enzymatic reaction was conducted for 24 to 72 hours [11]. Longer incubation of enzymatic reaction resulted in higher reducing sugar yield (RSY), however, after reach optimum condition the RSY decreased due to sugar dehydration to side product [12,13]. Recently, Anita et al. [14] reported 34.6% of reducing sugar yield was obtained from microwave assisted oxalic acid pre-treatment (MOxA) of EFB which was then continued with EH for 72 hours [14]. Further research is needed to evaluate longer EH incubation time on releasing sugars. Besides observing sugar yield after pre-treatment, evaluating the change of structural properties is also important to assess the effectiveness of pre-treatment [15].

Therefore, in this study pre-treatment of EFB using various microwave temperatures, irradiation times, and EH incubation period were conducted to evaluate the effectiveness of MOxA pre-treatment determined by Glucose Yield (GY) and Reducing Sugar Yield (RSY) after saccharification. Alteration of ultrastructure, functional groups, and crystallinity in EFB fibre before and after pre-treatment was also studied.

2. Materials and Methods

2.1. Materials

The EFB used in this study was the same as that in the previous report. The EFB was obtained from Sukabumi, West Java with chemical components (in percentage): moisture 8.44±0.04, ash 2.91±0.03, extractive 2.69±0.10, acid insoluble lignin (AIL) 23.62±0.08, cellulose 41.23±0.77, and hemicellulose 26.55±0.57 [16]. Oxalic acid from Merck (Darmstadt, Germany) was used as organic acid for pre-treatment.

2.2. Methods

2.2.1. Microwave-assisted oxalic acid pre-treatment (MOxA) of EFB

The pre-treatment procedure was following the steps in the previous report [3]. Briefly, three grams of dry weight EFB was added with 1% oxalic acid in the microwave Teflon tube with solid to liquid ratio of 1:10, and subsequently degassed at -20 bars for 5 minutes. The closed tube was put in the microwave oven (START D Microwave Digestion System, Milestone, Sorisole, Bergamo, Italy). The parameters of microwave pre-treatment conditions were set as follows: stirring at 195 rpm, temperatures at 160, 180, and 200°C, irradiation times for 5, 10, 15 minutes excluded preheating for 12 min and cooling for 10 minutes. After the process finished, the tube was put in an ice bath for approximately 30 minutes. Then, the solid fraction was separated from the liquid fraction through filter paper by vacuum filtration (GAST DOA-P504-BN, USA). The solid fraction or pulp was washed with distilled water until neutral condition. The pulp was weighed and was analysed for moisture content. Some pulp from each treatment was dried for its characterization. The rest of the pulp was stored in a freezer below 0°C before used for further processes or analyses. The pulp recovery (PR) was calculated using Equation 1 [14]. The experiment was carried out in duplicate.

\[ PR \, (%) = \frac{(100 - \frac{PMC \times 100}{ODI}) \times 100}{PR} \]
where, PMC is moisture content of the pulp after pre-treatment, P is weight of pulp after pre-treatment, ODI is oven dry weight of initial biomass.

2.2.2. Enzymatic hydrolysis and sugar analysis

The MOxA residues or pulp were subjected to Enzymatic Hydrolysis (EH) by Meicellase from *Trichoderma viride* (Meiji Seika, enzyme activity 200 FPU/g). The reaction was conducted in glass bottles. Each glass bottle was filled with 1 g (oven dry weight) of each pulp, 10 mL of 0.05 M citrate buffer pH 4.8, 1 mL of 2% sodium azide, and 60 mL of 1% Meicellase in DI water. Finally, 0.1 M citrate buffer pH 4.8 was added into the glass bottle to get 100 g of total weight. The bottles were tightly closed and incubated at 50°C for 5 and 10 days in an incubator shaker (WIS-30R Wise Cube, witeg Labortechnik GmbH, Wertheim, Germany) at 150 rpm [15]. After it is completed, the samples were heated in boiling water for 5 minutes to inactivate the enzyme. After cooling down to room temperature the hydrolysate was separated from the residue by filtration.

Glucose concentration after enzymatic saccharification was analysed using the Glucose CII test kit (Wako Junyaku, Co., Osaka, Japan), and was quantified by a Paradigm P2 Spectramax microplate reader (Molecular Devices LLC, San Jose, California, USA) at a wavelength of 505 nm. Glucose yield based on the weight of pulp and based on the weight of initial biomass was calculated using Equation 2 and 3, respectively, while cellulose conversion was calculated using Equation 4 [15].

\[
Glucose \text{ yield } (\% \text{ pulp}) = \frac{(GC \times 0.1)}{ODP} \times 100
\]

(2)

where, GC stands for glucose concentration in the hydrolysate (g/L), 0.1 is volume of the hydrolysate (L), and ODP is oven dry weight of pulp sample (g).

\[
GY \text{ (\% initial biomass)} = \frac{(GC \times 0.1)}{ODP} \times \frac{PR}{100}
\]

(3)

where, GC is reducing sugar concentration (g/L), 0.1 is a total final volume of EH (L), ODP is oven dry weight of pulp sample (g), and PR is pulp recovery (%).

\[
Cellulose \text{ conversion } (\%) = \frac{(GC \times 0.1 \times 0.9)}{(ODP/ODI) \times 100} \times 100
\]

(4)

where, GC is glucose concentration in the hydrolysate (g/L), 0.1 is volume of the hydrolysate (L), 0.9 is conversion factor of glucose to cellulose, CT is cellulose content of the initial biomass (%), and ODP is oven dry weight of pulp sample (g).

The reducing sugars was analyzed by DNS method [17] and the absorbance was measured by Hitachi U-2001 spectrophotometer (Hitachi Corp., Tokyo, Japan) at a wavelength of 540 nm. The reducing sugars yield (RSY) was calculated based on the weight of pulp and the weight of initial biomass using the calculation method for GY as in Equation 2 and 3, by only changing the glucose concentration by reducing sugar concentration.

2.2.3. Morphological analysis of OPEFB

The dried untreated and pre-treated OPEFB were put on a 10 mm diameter specimen stub. Prior to load the stub in the scanning electron microscope (SEM), the samples were coated with 400-500 Å thick of gold using coating unit Ion Coater IB2. Then, the stub was loaded into a Hitachi SU-3500 SEM (Hitachi Corp., Tokyo, Japan). The images were captured at 1000 magnification and 20 KV accelerating voltage, while the work distance of SEM was maintained at 5 mm.

2.2.4. Functional groups analysis of OPEFB

The changes of functional groups in OPEFB before and after pre-treatment were observed by Fourier Transform Infra-Red (FTIR) Universal Attenuated Total Reflectance (UATR) Spectrometer (Spectrum
two Perkin Elmer, Shelton, USA). About 0.1 mg of dried sample was placed on a diamond plate and force gauge (FG) was pressed by hand until the obtained spectra appear (approximately FG not more than 80). The spectra were recorded at a wavelength of 4000-500 nm, resolution 16 cm⁻¹, and 32 scans.

2.2.5. Crystallinity analysis of OPEFB

Before crystallinity measurement, the samples were dried in a freeze dryer (Alpha 1-2 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) for about 9 hours. Then, the samples were placed on sample plate X-Ray Diffractometer (XRD) (Rigaku Smartlab Instrument, Japan) with Cu (λ=1.54 Å) as a radiant source. The analysis was conducted at a scanning angle of 10-40° with a scanning speed of 2.0°/min step size of 0.02°, the energy of 40.0 kV, and an electric current of 30.0 mA. The crystallinity index (CI) was automatically calculated by using the PANalytical X-pert Pro Diffractometer software.

3. Results and Discussion

3.1. Effect of MOxA on PR of OPEFB

The PR of OPEFB pretreated by MOxA pre-treatment was in the range of 59-70%, where the highest PR was obtained after MOxA pre-treatment at 160°C for 5 minutes (70.57%) and the lowest was achieved from pre-treatment at 200°C for 15 minutes (59.68%). The effect of temperature and irradiation time on PR can be seen in Figure 1. The temperature has a higher effect on pulp recovery compared to irradiation time. There was approximately up to 9% of PR decrease due to 20°C increase in temperature, while it was only about 3% of PR decrease due to 5 min increase of irradiation time. It might be due to the significant effects of thermal pre-treatment on degrading large molecules in OPEFB, such as polysaccharide and lignin, into small molecules [14]. Besides, organic acid pre-treatment was reported effective to hydrolyse and solubilize hemicellulose in biomass, so that the solid fraction was reduced after pre-treatment [18]. The highest PR of OPEFB after MOxA in this study was slightly lower compared to PR of OPEFB after microwave assisted 1% maleic acid pre-treatment at 160°C for 2.5 minutes (80.32%) [19].

![Figure 1. Pulp recovery of OPEFB after microwave assisted oxalic acid at 160, 180, and 200°C for 5, 10, and 15 minutes](image)

3.2. Glucose yield obtained after MOxA pretreatment

The solid fraction of OPEFB after MOxA was subjected to 5 and 10 days EH and the glucose yields (GY) based on the weight of pulp are presented in Figure 2a and GY per initial biomass in Figure 2b. It can be seen that MOxA had a positive effect on both GY per pulp and per initial biomass. For instance, only 9.99% GY was obtained from OPEFB without pre-treatment after 5 days EH. The GY was rapidly increased to 44.10% (per pulp) and 31.17% (per initial biomass) after pre-treatment at 160°C for 5 minutes. Generally, the GY per pulp and per initial biomass continuously increased as increasing in temperature. However, at heating temperature 160 and 180°C GY was decreased after 10 minutes duration of heating. The GY per pulp was increased by 10.15 % after pre-treatment at 160°C.
for 10 minutes and it was slightly decreased to 53.48% after 15 minutes duration of heating. The effects of extending the duration of heating at temperature of 180°C on GY per pulp have the same trend as those of heating at 160°C. After extending 5 minutes of heating from 5 to 10 minutes, increasing GY from 48.10 to 60.2% was observed, but extending another 5 minutes of heating was decreased the GY to 57.4%. On the contrary, GY obtained from pre-treatment at temperature 200°C was steadily elevated as the increase of heating time. A long heating time at high temperature was typically more effective in reducing biomass recalcitrant [20]. The same trend was also observed in GY per initial biomass (Figure 2b) which shows the increase of GY from 31.17 up to 43.22%. The cellular matrix of biomass that has been disrupted by MOxA pre-treatment was sent to EH for making cellulose accessible to the enzyme. The highest GY, 61.83% or 618.3 mg/g pre-treated OPEFB, was released after MOxA at 200°C for 15 minutes. This result proved that MOxA effectively increased enzyme susceptibility of OPEFB and disintegrated the lignocellulosic structure [21]. Ultimately, it facilitated the conversion of cellulose to glucose. Previous study indicated that the steam explosion and microwave irradiation (SE–MI) for 5 minutes at 180°C were favourable to obtain 72.1 g per 100 g glucose and xylose in feedstock [22]. Another study reported that 75% of reducing sugar yield was achieved from Norway spruce after sulphuric acid-microwave irradiation at 200°C for 5 minutes [23]. The GY obtained in this study was higher than the previous study by Mikulski and Kłosowski who achieved glucose concentration 156 mg/g dry weight of wheat after dilute sulphuric acid-microwave pre-treatment for 15 minutes [7].

The effect of long enzymatic reaction (10 days) on GY was also evaluated in this study because one of the important factors in bioethanol production from lignocellulosic biomass is selecting the optimal duration of EH. It was obviously seen in Figure 2a and 2b that extending duration of EH to 10 days increased GY as much 5-11% compared to GY after 5 days of EH. The highest GY (72.35% based on the weight of pulp and 43.22% based on the weight of initial biomass) was obtained after MOxA 200°C for 15 minutes. EH is a heterogeneous reaction in binding enzyme with substrate to convert cellulose into glucose. In this study, GY obtained corresponded with longer hydrolysis time where the longer EH incubation time, the higher glucose yield released. The trend indicated that the enzyme was more susceptible to longer reaction times. This finding was similar to the EH of OPEFB after organosolv pre-treatment [24]. Nevertheless, the increase of GY and RSY from 5 to 10 day EH was lower than the GY and RSY obtained from 5 days EH. Therefore, prolonging the EH until 10 days did not give too much benefit in the glucose or sugars recovery from OPEFB.

The cellulose conversion in this study was in the range of 68.04 - 94.33%. The highest cellulose conversion in Figure 2c (94.33%) was obtained at 200°C – 15 minutes after 10 days EH, which was the same conditions for obtaining the highest GY. Moreover, the tendency of cellulose conversion due to increase of pre-treatment temperature and heating time as well as the duration of EH (Figure 2c) was correlated with that of GY.

Reducing sugar yield (RSY) is another common parameter to evaluate the effectiveness of pre-treatment and EH. After 5 days EH untreated OPEFB only resulted in 16.07% RSY, while the MOxA pre-treated OPEFB resulted in 51.84-59.89% RSY per pulp (Figure 3a) or 31.83-39.60% RSY per initial biomass (Figure 3b). The RSY was steadily increased by 5-10 % after extending another 5 days of EH (Figure 3a and 3b). These increases were much lower than the RSY obtained from the first 5 days EH. The highest RSY (69.84% per pulp) was obtained from enzymatic saccharification of OPEFB pre-treated by MOxA at 200°C for 15 minutes after 10 days EH but the highest RSY per biomass (46.13 %) was obtained from that pretreated at 160°C for 10 minutes. However, the trend of RSY due to variations of irradiation times and heating temperatures was similar with that of GY, which showed that the increase of temperature and irradiation time tended to increase the GY as well as RSY. However, the irradiation time of longer than 10 minutes at 160 and 180°C decreased the RSY. It was probably due to the degradation of sugars into lower molecular weight compounds, such as furan aldehydes [19].
Figure 2. (a) Glucose yield per pulp, (b) glucose yield per initial biomass, and (c) cellulose conversion obtained from enzymatic saccharification of untreated and microwave assisted oxalic acid (MOxA) pre-treated OPEFB at different temperatures and heating times.

Figure 3. (a) Reducing sugar yield (RSY) per pulp and (b) RSY per initial biomass obtained from enzymatic saccharification of untreated and microwave assisted oxalic acid (MOxA) pretreated OPEFB at different temperatures and heating times.
3.3. Morphological changes on EFB fiber after MOxA pretreatment

SEM is a common tool to investigate the effect of pre-treatment on surface, morphology, and microstructure analysis of biomass [20]. However, the samples for SEM are needed to be conductive to avoid sample damage by the electron beam. The gold coating was used in this study to make the surface conductive. Another drawback before SEM capture image is an unreasonable conclusion of SEM picture due to the high surface tension of samples, thus drying of the samples before loading them on the SEM plate is needed. Oven drying was not recommended, because it could be resulted in significant surface deformation [25]. Therefore, freeze drying was used in this study before SEM analysis.

The morphological changes after MOxA were observed in the OPEFB surface. Micrographs of the surface of untreated EFB in Figure 4a show that some silica bodies, the white dot, still embedded, but some of them were removed resulted in perforated craters on the surface. This was probably due to the effect of reducing the sizing process of raw OPEFB using a hammer mill. The removal of silica bodies and disruption of surface are more obvious in samples exposed to higher heating temperatures and longer irradiation time. The silica bodies were gradually disappeared at high temperature (Figure 4b-f) and almost completely vanished after heating at temperature and duration of higher than 180ºC, 15 minutes (Figure 4g-j). Besides that, cracks of fibre were more pronounced at samples exposed to longer heating time and higher heating temperatures. When the silica bodies were removed and the circular craters were cracked, the enzyme could easily penetrate into the OPEFB fibre to breakdown the cellulose into cello-oligomers, and finally into glucose. In addition, at the highest temperature and longest duration of heating, more disrupted surface area was typically seen. The SEM images were in agreement with GY obtained (Figure 1a): the higher the temperature, the higher the GY was obtained because the enzyme was more accessible to attack the lignocellulose structures. Therefore, it can be concluded that high temperature and long heating time during MOxA pre-treatment has a significant effect on the disruption of morphological structures of OPEFB, so that it enhances enzyme susceptibility and eventually higher amount of glucose is released. This complies with previous studies that observed rougher biomass surface after microwave-acid pre-treatment [26,27].

![Figure 4](image-url) Fibre surface images of OPEFB before pre-treatment (a) and after treatment of MOxA at 160ºC for 5 min (b); 160ºC for 10 min (c); 160ºC for 15 min (d); 180ºC for 5 min (e); 180ºC for 10 min (f); 180ºC for 15 min (g); 200ºC for 5 min (h); 200ºC for 10 min (i); 200ºC for 15 min (j).

3.4. Crystallinity Index (CI) measurement by XRD

Crystallinity Index (CI) is a parameter to indicate the relative amount of crystalline and amorphous region of a cellulosic material [25]. CI is one of factors that can influence enzyme accessibility to the biomass during EH. Higher CI means that the biomass contained more cellulose or cellulose of crystalline structure due to the loss or solubilization of some amorphous parts of biomass, such as lignin and hemicellulose. The increase of biomass susceptibility to enzyme was due to the removal of those amorphous parts from the biomass rather than because of the increase of CI. The XRD analysis was performed for every sample. However, only representatives of the diffraction spectra of OPEFB...
were shown in Figure 5. There are two major peaks at 2θ of 14-18º and 20-25º which correspond to amorphous portion and crystalline portion, respectively. The major peak at 14-18º was slightly shifted in samples pretreated at higher temperature due to removal of hemicellulose and lignin, which are the major amorphous components in the biomass [28]. The shifting of peaks of crystalline region was also observed in the spectra that was believed to increase biomass digestibility [29].

![Figure 5. XRD pattern of OPEFB before and after treated at 160ºC, 180ºC, and 200ºC for 10 min](image)

Table 1 shows that CI gradually increased as increase of heating temperature and irradiation time which indicates degradation of amorphous substance. Untreated OPEFB has CI of 13.96% and there was 5% increased after microwave irradiation at 160ºC-5 minutes. The highest CI (21.33 %) was obtained after pretreatment of OPEFB at 180ºC-10 minutes, while CI of 20.08% was acquired from pretreatment at 200ºC-15 minutes, the condition that the highest GY was achieved. In general, the result of XRD in this study was in correlation with data of PR, GY, and SEM image when higher temperature and longer duration of heating during microwave-acid pretreatment induced the deconstruction of larger molecules in the OPEFB fiber to make the fiber more vulnerable for cellulose degradation. This finding was in agreement with previous research such as dilute acid pretreatment of *Phalaris aquatica* L. [30], sulphuric acid pretreatment of sugarcane bagasse [31], and acetic acid pretreatment of *Eucalyptus* chips [18].

**Table 1.** Crystallinity index (CI) of untreated and microwave-oxalic acid pre-treated OPEFB at various temperatures and heating times

| Pre-treatment conditions | Crystallinity Index (%) |
|-------------------------|-------------------------|
| Untreated               | 13.96                   |
| 160ºC                   |                         |
| 5 min                   | 18.87                   |
| 10 min                  | 18.45                   |
| 15 min                  | 20.54                   |
| 180ºC                   |                         |
| 5 min                   | 19.34                   |
| 10 min                  | 21.27                   |
| 15 min                  | 19.60                   |
| 200ºC                   |                         |
| 5 min                   | 20.29                   |
| 10 min                  | 21.33                   |
| 15 min                  | 20.08                   |
3.5. Functional groups of OPEFB

Although the main effect of pre-treatment is removal of hemicellulose and lignin, the changes of other functional groups were also observed by FTIR. Structural changes of OPEFB before and after all MOxA conditions were observed. The representative FTIR spectra are presented in Figure 6, while the summary of FTIR bands from all samples is available in Table 2. The peak intensity corresponds to C=O ester linkage lignin-xylan and CH deformation in hemicellulose, peaks at bands 1714 and 1370 cm\(^{-1}\) (no 3 and 9) were gradually decreased after MOxA. Moreover, the decrease of peak intensities was also obviously seen at bands 1646 cm\(^{-1}\) (4) and 1241 cm\(^{-1}\) (12) that were associated with lignin structure. It can be inferred that organic acid pre-treatment is effective in degrading lignin and hemicellulose compounds. However, some peaks associated with lignin (1600 (5), 1507 (6), 1456 (7), 1270 (11) cm\(^{-1}\)) were more noticeable after pre-treatment that may suggest releasing and co-precipitating of lignin on the pulp. This result was similar with pulp of *Eucalyptus* after acetic acid pretreatment [18]. More pronounced bands at 1425 (8), 1323 (10), 1105 (14), 1054 (15), and 897 (17) cm\(^{-1}\) which represent stretching vibration of –CH\(_2\) shear, C-H, C-O of secondary alcohol, C-C, and β-glycosidic linkage in cellulose were clearly discernible, implying an increase of the crystallinity of cellulose. The FTIR result is confirmed by XRD data that higher temperatures reduce amorphous substance in OPEFB, and eventually increases the CI.

![FTIR spectra of OPEFB before and after pre-treatment at 160, 180, and 200°C for 10 min](image)

**Figure 6.** FTIR spectra of OPEFB before and after pre-treatment at 160, 180, and 200°C for 10 min

| No | Untreated 5 min | Absorption bands wavenumber (cm\(^{-1}\)) | Assignment |
|----|----------------|------------------------------------------|------------|
| 1  | 3335           | 3335, 336                               | O-H stretching of cellulose and hemicellulose [3] |
|    | 160°C 5 min    | 3336                                     |            |
|    | 160°C 10 min   | 3335                                     |            |
|    | 160°C 15 min   | 3334                                     |            |
|    | 180°C 5 min    | 3335                                     |            |
|    | 180°C 10 min   | 3335                                     |            |
|    | 180°C 15 min   | 3336                                     |            |
|    | 200°C 5 min    | 3335                                     |            |
|    | 200°C 10 min   | 3336                                     |            |
|    | 200°C 15 min   | 3336                                     |            |
| 2  | 2909           | 2906, 2909                              | C-H symmetric stretching of cellulose and hemicellulose [32] |
|    | 1706           | 1708, 1704                              |            |
|    | 1708           | 1704, 1704                              |            |
| 3  | 1714           | 1704, 1704                              | C=O unconjugated stretching of xylan [33] |
|    | 1704           | 1704, 1704                              | -CHO, C=O, -COO stretching of lignin [34] |
| 4  | 1646           | -                                        |            |
Comparison of functional groups changes before and after EH on OPEFB fibre after MOxA at 200°C-15 minutes was also observed (Figure 7). This condition was selected for comparison due to the highest GY was released at that condition, indicating most of the cellulose was converted into glucose. In Figure 7, although there was no functional group transformation after EH, distinction of peaks intensity was obviously observed and was marked by red asterisks. Depicting symmetric C-H bond and C-O-C stretching in cellulose were visibly appear at 2927 cm⁻¹ and 1114 cm⁻¹ after EH, while hemicellulose peak at 1714 cm⁻¹ was diminished. Peaks corresponding with lignin at 1600, 1507, 1456, and 1220, and 834 cm⁻¹ were sharper. This finding might be attributed to expose of linkage of lignin after degrading amorphous cellulose substance (band 1425 cm⁻¹ and 897 cm⁻¹) and hence lignin peaks were more visible after EH [40]. Previous research stated that the presence of lignin impeded enzymatic digestion of cellulose, thereby limiting conversion of cellulose to sugars [41]. Based on the results of this study, it can be concluded that hemicellulose was easier to be degraded than was lignin. However, the determination of syringyl/guaiacyl (S/G) ratio of lignin in OPEFB pulp before and after EH in the future is needed to evaluate the effectiveness of MOxA in degrading lignin.
Figure 7. FTIR spectra of OPEFB after pretreatment at 200ºC for 15 min and after enzymatic hydrolysis (EH) of the pretreated OPEFB.

4. Conclusions
This study revealed that microwave assisted oxalic acid (MOxA) pre-treatment can be used as an alternative method to increase enzyme accessibility in enzymatic hydrolysis (EH) of OPEFB. Glucose yield (GY) obtained after saccharification of OPEFB was increased about 4 times when the biomass was pre-treated by microwave heating at 160ºC for 5 minutes, while the highest GY per initial biomass (43.22%) were obtained from that pre-treated at 200ºC for 15 minutes. The higher microwave heating temperature and the longer the irradiation period, the higher the GY and CI were observed. In addition, the ultrastructure of OPEFB fibre had more ruptures at high temperatures and longer irradiation time which could better facilitate in increasing enzyme penetration into the OPEFB fibre and eventually improved hydrolysis of cellulose to glucose. MOxA pre-treatment reduced amorphous components in the OPEFB, such as hemicellulose, lignin, and amorphous cellulose, which was indicated by increasing of CI from 13.96% to 21.33% after MOxA pre-treatment at 200ºC for 10 minutes. Alteration of the functional groups shown by FTIR spectroscopy also confirmed and supported the results of GY, morphological analysis, and CI determination.

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