The Improvement of Sugar and Bioethanol Production of Oil Palm Empty Fruit Bunches (*Elaeis guineensis* Jacq) through Microwave-Assisted Maleic Acid Pretreatment

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Microwave assisted maleic acid (MA) pretreatment is a potential method for biomass processing. The aim of the present study was to determine the optimum conditions for pretreatment of oil palm empty fruit bunch (OPEFB) for bioethanol production, as well as to understand the changes of functional groups in the pretreated OPEFB. The optimum pretreatment conditions with the highest reducing sugar yield (RSY) (47.21%) were 190 °C for 2.5 min with 1% MA. The temperature had more influence on the chemical components and RSY than the other pretreatment parameters. More severe pretreatment conditions tended to dissolve more sugar into the hydrolysates. Structural changes occurred in the OPEFB pretreated with MA and sulfuric acid, which were observed by the absence of certain functional groups. The simultaneous saccharification and fermentation (SSF) of MA-pretreated OPEFB produced ethanol with a concentration of 18.9 g/L and yield of 0.43 g ethanol/g cellulose after pretreatment, or equivalent to 76.6% of theoretical maximum yield. Microwave assisted MA pretreatment of OPEFB successfully increased the RSY about 300% compared to the untreated one. The MA pretreatment removed hemicellulose and lignin. The highest ethanol concentration and yield were produced after 48 h of SSF of the OPEFB pretreated with MA.

Keywords: Maleic acid; Microwave heating; Oil palm empty fruit bunch; Reducing sugar yield; Bioethanol production

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INTRODUCTION

Oil palm empty fruit bunch (OPEFB) is an industrial waste produced from oil palm processing (Anggraini and Roliadi 2011; Lim *et al*. 2015). OPEFB is collected by separating it from fresh fruit bunch, which contains 20% OPEFB (Mohammad *et al*. 2012). Indonesia and Malaysia are the two biggest palm oil producers in the world. In 2014, approximately 39.8 million metric tons of OPEFB was produced alongside 33 million tons of crude palm oil (Wright and Wiyono 2013). The OPEFB must be properly handled to reduce its negative effects on the environment. A common use of OPEFB is as an organic mulch to support oil palm growth or fertilizer by burning it in fields (Millati *et al*. 2011). This biomass contains hollocellulose (62.6% to 80.3%), lignin (27.60% to 32.50 %) (Syafwina *et al*. 2002; Akbar 2007; Pratiwi *et al*. 2013; Ishola *et al*. 2014; Kristiani *et al*. 2015), extractives (3.7%) (Menon and Rao 2012), and ash (1.63%) (Pratiwi *et al*. 2013). Considering its abundance and high hollocellulose content, OPEFB can be converted into valuable products, such as pulp, paper, fiberboard, fiber reinforcement, and bioenergy, such as bioethanol. OPEFB is an ideal biomass feedstock for bioethanol production (Syafwina *et al*. 2002; Akbar 2007; Jung *et al*. 2011; Millati *et al*. 2011; Menon and Rao 2012; Pratiwi *et al*. 2013; Ishola *et al*. 2014; Kristiani *et al*. 2015).

Of the three steps in bioethanol production from lignocellulosic biomass, pretreatment has the most significant effect on the hydrolysis and fermentation performances (Achinas and Euverink 2016). Pretreatment breaks down the recalcitrant structures in biomass to increase the accessibility of cellulose, which acts as a sugar source (Loow *et al*. 2015). Lignin and the crystalline structure of cellulose are known to have a high recalcitrance during cellulose conversion. Many pretreatments eliminate the recalcitrance of OPEFB, such as aqueous ammonia soaking (Jung *et al*. 2014), sulfuric acid (Jung *et al*. 2013), oxalic acid (Anita *et al*. 2012), biological methods (Risanto *et al*. 2012), and glycerol-sulfuric acid (Fatriasari *et al*. 2017). To optimize the cellulose conversion rate, an effective pretreatment is required. Microwave heating can be utilized to pretreat biomass. Compared with conventional heating, microwave heating has many advantages, such as a simple operation, rapid processing, covering of the heated object, high uniformity, low energy input, selective heating, and no waste is produced (Gong *et al*. 2010; Loow *et al*. 2016a; Lanigan 2011; Fatriasari 2014).

To obtain a better hydrolysis efficiency, microwave heating can be combined with chemicals, such as acids and alkaline compounds (Gong *et al*. 2010). Maleic acid (MA) is an organic acid that can be combined with microwave heating. This acid produces less inhibitors compared with sulfuric acid (SA). Hemicellulose has been hydrolyzed successfully without the formation of a large amount of furfural (Kim *et al*. 2012). No detoxification step is required during OPEFB fermentation for ethanol production (Jung *et al*. 2014). The effect of microwave heating on lignin and hemicellulose removal, as well as the disruption of biomass structures, has been studied before (Azuma *et al*. 1985; Hu and Wen 2008; Gong *et al*. 2010; Chen *et al*. 2011; Nomanbhay *et al*. 2013; Fatriasari *et al*. 2016). Maleic acid is an organic dicarboxylic acid that is more selective in hydrolyzing β-(1,4)-bonds than that of sulfuric acid. Microwave assisted oxalic acid (OA) pretreatment of OPEFB was also conducted as a parallel study by the authors.

In acid pretreatment, temperature, residence time, and acid concentration have been found to determine the pretreatment efficiency (Loow *et al.* 2016b). To determine the optimum microwave pretreatment conditions, the effect of the temperature, irradiation time, and MA concentration was studied. The effect of each factor was evaluated using a one-factor-at-a-time (OFAT) method to minimize the amount of trial runs and to facilitate easier studying of each factor. Pretreatment of Malaysian OPEFB using microwave heating at 190 °C with 1% (w/v) MA for 3 min ramping to the set temperature with no holding time resulted in a 60.9% enzymatic digestibility and 61.3% theoretical ethanol yield after 48 h of simultaneous saccharification and fermentation (SSF) in the study by Jung *et al*. (2013). In a previous study by the authors, there was no investigation into the sugar content in the hydrolysates, changes in the functional groups, or observation of the combined severity factor (CSF). The effectiveness of the pretreatment conditions can be evaluated by comparing the CSF with the effects of the temperature, irradiation time, and acid concentration during pretreatment on the enzymatic hydrolysis performance (Kim *et al*. 2013). Thus, this study provided more detailed information on both the enzymatic digestibility and ethanol yield compared with the previous study by Jung *et al*. (2014). These results were also compared with the digestibility of OPEFB pretreated with OA and SA. A smaller formation of inhibitors with a high sugar production became the main objectives of this study. The functional groups of the untreated OPEFB and MA- and SA-pretreated OPEFB were also compared.

EXPERIMENTAL

Materials

OPEFB fibers collected from Cibadak, Sukabumi, West Java-Indonesia with a moisture content (MC) of 6.94% were used in this study. They were ground into a 40- to 60-mesh size powder and stored in sealed containers at room temperature. This study used analytical grade MA (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The other chemicals used were also of analytical grade and used without further purification.

Methods

Microwave assisted maleic acid (MA) pretreatment

Pretreatment was done with the OFAT method to evaluate the effect of the irradiation time, temperature, and MA concentration. In the first experiment, heating was performed at a variety of temperatures that ranged from 160 °C to 200 °C with a 2.5-min irradiation time and 1% (v/v) MA concentration. In the second stage, the biomass was heated for different irradiation times at the optimum temperature determined from the first stage and 1% MA. Finally, the MA concentration was varied from 0.5% to 1.5% at the optimum temperature and time determined from the first two stages.

Three grams of OPEFB powder (6.94% of MC) with a solid to liquid ratio of 1:10 was immersed in a 1% (v/v) MA solution in a Teflon tube. The slurry was degassed at a 20-bar vacuum for 5 min before the pretreatment. There were ten tubes used for each run. The tightly-sealed tubes with stirrers were heated in a digestion microwave (Milestone START D, Milestone Inc, Bergamo, Italy) at 1000 W. One of the sealed reaction tubes was equipped with a thermocouple to control the temperature during heating. The temperature of the process was observed on a monitor attached to the microwave. The heating was maintained with 195-rpm magnetic stirring, 12 min of pre-heating, and 10 min of cooling.

After the heating reaction, the Teflon tubes were cooled in an ice bath for 20 min to 30 min to release the heat quickly. Subsequently, the slurries were filtered with 200-mesh filter paper using vacuum filtration (GAST DOA-P504-BN, Cole-Parmer, Vernon Hills, IL, USA). Afterwards, they were washed with distilled water until the pH was neutral, which was indicated by a blue color on litmus paper. To keep the pulp (solid fraction) fresh for enzymatic hydrolysis and further analysis, it was stored in a freezer in sealed plastic containers. The liquid fraction was centrifuged at 5 °C and 10,000 rpm for 10 min. The filtrate was separated and kept in a refrigerator at 10 to 11°C until further analysis of the reducing sugar content, absorbance of dark-colored compounds, and pH. The pulp recovery was calculated by comparing the initial biomass weight and final oven dried (OD) pulp weight. The pretreatment was performed in triplicate. The OPEFB was also pretreated using 1% (v/v) SA at 190 °C and 2.5 min with the same method detailed above.

Enzymatic hydrolysis of the oil palm empty fruit bunch (OPEFB) pulp

As much as 0.3 g of OD OPEFB pulp were further hydrolyzed by cellulase (Meicellase from *Trichoderma viride*, Meiji Seika Co., Ltd., Tokyo, Japan) in an Erlenmeyer flask. The cellulose has enzyme activity of 200 FPU/g. First, about 20 mL of 0.1 M sodium citrate buffer (pH 4.8) was added to the OPEFB substrate. Then, the substrate was added with 200 µL of 2% (w/v) sodium azide and cellulase enzyme with enzyme loading of 40 FPU/g dry substrate. Finally, 0.1 M sodium citrate buffer (pH 4.8) was added to get the final total weight of 30 g. After that, the flasks were tightly closed and then incubated at 50 °C for 72 h in an incubator shaker (WIS-30R, Wisecube, Gangwon-Do, Seoul, Korea) at 150 rpm.

Before the reducing sugar analysis using the dinitrosalicylic acid (DNS) method (Miller 1959), the hydrolysates were centrifugated at 10,000 rpm for 10 min at room temperature. A sample of 0.5 mL of centrifuged hydrolysate was added to 1.0 mL of distilled water and 3.0 mL of DNS reagent. Subsequently, the mixtures were mixed, boiled for 5 min, and transferred into an ice bath for 20 min. A 0.2 mL sample of the mixture was added to 2.5 mL of distilled water and mixed well by vortexing. The absorbances of the samples were then measured by an ultraviolet visible (UV VIS) Hitachi U-2001 spectrophotometer (Hitachi Instruments Inc., Tokyo, Japan) at a wavelength of 540 nm to calculate the reducing sugar concentration (Adney and Baker 2008). A series of glucose standard solutions were also prepared and their absorbances were measured to build a regression equation. The coefficient of determination (R^2) of the equation was higher than 0.99. Samples having absorbances higher than 0.8 were diluted to the value in the range 0.2 to 0.8. The reducing sugar yield (RSY) per biomass (g/100 g) was calculated by taking into consideration the OPEFB weight loss that occurred during microwave assisted MA pretreatment. Meanwhile, the liquid fractions were measured for dark-colored compounds (absorbance at a 490-nm wavelength with the UV VIS Hitachi U-2001) (Warrand and Janssen 2007; Whistler and Daniel 1985), pH (Eutech pH meter, Eutec Instrument Pte Ltd, Ayer Rajah Crescent, Singapore), and reducing sugar concentration (DNS method mentioned above). Enzymatic hydrolysis and the same analyses were performed on the samples obtained from the OPEFB pretreated with SA at the optimum MA pretreatment conditions. All of the experiments were conducted in triplicate, and the means and standard deviations of the data were presented.

Combined severity factor (CSF)

The severity of the pretreatment was determined to evaluate whether there was any relationship between the severity during pretreatment and the characteristics of the liquid hydrolysates, as well as the enzymatic digestibility of the insoluble fractions. The severity factor (SF) was determined using Eq. 1 (Goh *et al*. 2012),

$$\log (R_0) = \log (t * \exp (T - T_{100})) / 14.7 \tag{1}$$

where t is the irradiation time (min), and T is the reaction temperature (°C).

Because the pretreatment used acid, the pH of the solution was taken into consideration, and the CSF was determined using Eq. 2 (Lee *et al*. 2010; Loow *et al*. 2016b).

$$\text{CSF} = \log (R_0) - \text{pH} \tag{2}$$

Chemical composition of the pretreated OPEFB

The chemical compositions of the untreated and MA-pretreated OPEFB were analyzed, which included the moisture (TAPPI T264 cm-97 1997), ash (Sluiter *et al.* 2008a), extractive (Sluiter *et al*. 2008b), acid soluble lignin (ASL) and acid insoluble lignin (AIL) (Templeton and Ehrman 1995), hollocellulose (sodium chlorite delignification) (Wise *et al*. 1946), and α-cellulose contents (Rowell *et al.* 2012). The hemicellulose content was calculated by subtracting the holocellulose content from the α-cellulose content (Punyamurthy *et al.* 2013). The chemical composition of the OPEFB pretreated with SA at the optimum MA pretreatment conditions was also measured for comparison with the MA-pretreated OPEFB.

Fourier transform infrared (FTIR) spectroscopy analysis

The infrared (IR) spectra patterns of the SA- and MA-pretreated OPEFB (190 °C, 2.5 min, 1% v/v) and untreated OPEFB were obtained by Fourier transform infrared (FTIR) spectroscopy on a Tensor 37 spectrometer (Bruker, Billerica, MA, USA). Two-milligram samples were mixed with 200 mg of potassium bromide (KBr, spectroscopy grade), which were then pressed into pellets to form disks. The IR spectra were recorded with a total of 16 cumulative scans per sample in the absorption mode over the range 4000 to 600 cm^{-1} using OPUS software (Bruker).

Microorganism and inoculum preparation

The yeast used in this research was *Saccharomyces cerevisiae* InaCC Y93 obtained from Indonesia Culture Collection (InaCC), Indonesian Institute of Research (Cibinong, Bogor, Indonesia). The yeast was maintained on a potato dextrose agar slant and was transferred to a 500-mL Erlenmeyer flask containing 5% YPD pre-culture medium (10 g/L yeast extract, 20 g/L peptone, and 50 g/L dextrose). The cells were incubated by shaking at 120 rpm and 35 °C for 16 h to 20 h until an O.D.600 of 0.8 was reached. The cells were recovered by centrifugation at 4 °C and 8000 rpm for 15 min, and then resuspended in 1/10th of the volume of 0.9% NaCl. These cells were used as an inoculum for the SSF.

Simultaneous saccharification and fermentation (SSF)

The SSF was conducted based on the method by Dowe and McMillan (2008) with slight modifications. Samples of 15.3 g of acid pretreated OPEFB was put in an Erlenmeyer flask. The flask was supplemented with a YP medium stock (200 g/L peptone and 100 g/L yeast extract) and inoculated with the yeast inoculum at 10% (v/v). The pH of the media was maintained by adding 1 M sodium citrate buffer diluted to 50 mM (pH 4.8). Then, the cellulase enzyme (Meicellase, enzyme loading 40 FPU/g dry substrates) was added to the flask. Finally, sterilized water was added to the flasks until the final volume was 50 mL to replace the water that was lost when the flasks were autoclaved. The flasks were covered with needle-pierced silicon stoppers to allow for the release of CO_2. The SSF was conducted at 38 °C for 72 h under agitation (120 rpm) in an incubator shaker. Samples were taken after 0 h, 24 h, 48 h, and 72 h of fermentation to measure the reducing sugar and ethanol concentrations.

SSF with pre-hydrolysis

The washed insoluble solid fraction of the MA-pretreated OPEFB was used for the pre-hydrolysis experiment. Pre-hydrolysis was performed at 50 °C, 120 rpm for 4 h prior to SSF. After that, the temperature was reduced to 38 °C and the pre-hydrolysis OPEFB was inoculated with yeast suspension. The time of yeast inoculation was defined as 0 h. Samples were taken after -4 h, 0 h, 24 h, 48 h, and 72 h of fermentation to measure the reducing sugar and ethanol concentrations.

Ethanol analysis

Analysis of the ethanol content was performed on a Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) with a flame ionization detector (FID) equipped with an RTX-WAX column (Length: 30 m, Inner Diameter: 0.25 mm, Film Thickness: 0.25 µm; Restek, Bellefonte, PA, USA). The initial column temperature was 35 °C. The injection and detector temperatures were maintained at 200 °C and 210 °C, respectively. One-microliter samples were injected under the following conditions: pressure of 130.6 kPa, total flow of 83.5 mL/min, column flow of 1.96 mL/min, linear velocity of 40.0 cm/s, purge flow of 3.0 mL/min, and split ratio of 40.0. The sampling rate was 40 ms with hydrogen and air flow rates that were maintained at 40.0 mL/min and 400.0 mL/min, respectively. Ethanol (EtOH) concentration in the solution was calculated using formula in Eq. 3 (Hermiati 2012). The concentration of ethanol produced during fermentation for "t" hours was corrected by the concentration of ethanol produced at the beginning of fermentation or at "0" hour, as in Eq. 4. Then, the yield of ethanol in g per g of cellulose was determined by formula in Eq. 5 and the percentage of theoretical yield of ethanol was determined by the formula in Eq. 6.

$$\text{EtOH (g/L)} = \text{EtOH (v/v) \%} \times 10 \times 0.789 \tag{3}$$

$$\text{EtOH (g/L)} = \text{EtOH}_t - \text{EtOH}_0 \tag{4}$$

$$\text{Ethanol yield (g/g cellulose)} = \frac{\text{g of ethanol produced}}{\text{g of cellulose in SSF solution}} \tag{5}$$

$$\text{Ethanol yield (\% of theoretical)} = \frac{\text{Ethanol yield (g/g)}}{\text{Theoretical of ethanol yield (g/g)}} \times 100 \tag{6}$$

RESULTS AND DISCUSSION

Chemical component analyses of the OPEFB show that the material contain 43.0% α-cellulose, 12.7% hemicellulose, 17.8% AIL, 1.30% ASL, 1.91% extractives, and 4.26% ash. Microwave assisted MA pretreatment of the OPEFB was conducted in three stages. In the first stage we evaluated the effects of the temperature on the reducing sugar yield, while in the second and third stages we evaluated the effects of duration of heating and acid concentration on reducing sugar yield, respectively. Based on these evaluations, we determined the optimum condition for the pretreatment.

Effect of Temperature

Table 1 shows the pulp recovery and chemical components of the OPEFB pretreated by microwave heating for 2.5 min with an MA concentration of 1%. When the heating temperature increased, the amount of pulp recovered decreased. The chemical component loss after the pretreatment might have contributed to the decrease in pulp recovery.

Increasing the temperature to 190 °C tended to increase the α-cellulose content in the pretreated OPEFB, and at 200 °C the α-cellulose content decreased. The highest α-cellulose content was found in the samples pretreated at 190 °C.

Table 1. Effect of the Heating Temperature during Microwave Pretreatment with an Irradiation Time of 2.5 min and 1% MA on the Pulp Recovery and Chemical Composition of the OPEFB

Temperature (°C)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
160	80.32±1.01	3.30±0.56	2.79±0.03	21.63±1.41	52.51±1.95	16.61±2.12
170	70.32±2.97	5.38±0.10	2.55±0.13	18.69±2.37	51.75±5.14	14.66±4.42
180	66.97±1.11	7.25±1.16	3.13±0.15	19.47±2.00	49.02±3.80	16.09±0.18
190	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
200	50.40±0.75	10.96±0.69	2.49±0.45	18.58±2.07	44.04±2.67	12.04±5.52

The decrease in the α-cellulose content in the samples pretreated at 200 °C might have been related to the degradation of the crystalline cellulose. At this temperature, a high weight loss occurred in the pretreated OPEFB, which was attributed to the loss of hemicellulose, lignin, and cellulose that occurred with the increase in temperature. Microwave heating caused fiber disruption, which has been reported previously, for example in pretreated bamboo (Fatriasari *et al*. 2016). Hemicellulose has lower molecular weight than cellulose. Besides, it also has branched chains, while cellulose has ordered linear structure of β-1,4 glycosidic chains. These make hemicellulose easier to be broken down during pretreatment. The chemical component losses from the pretreated samples tended to increase when increasing the temperature (Fig. 1a). The utilization of MA in the microwave pretreatment mainly increased the hemicellulose degradation instead of the lignin degradation, and the highest hemicellulose loss was obtained in the samples pretreated at 200 °C. The hemicellulose and lignin removal (Fig.1a) improved the cellulose accessibility to cellulase enzymes during enzymatic hydrolysis. MA has been proven as an effective acid for hydrolyzing hemicellulose without resulting in significant furfural formation (Kim *et al*. 2012), so that the process can produce high xylose yield and low sugars degradation products (Lu and Mosier 2007; Lu and Mosier 2008; Kootstra *et al*. 2009). This acid could hydrolyze biomass due to its higher solution potential as opposed to mineral acids (Lee and Jeffries 2011). Results in these reports indicate that removal of hemicellulose due to MA acid pretreatment at high temperature resulted in this study is in line with previous studies.

Extractives that acted as non-structural components were also dissolved and facilitated in improving the enzyme accessibility. In addition to the chemical component and weight losses, microwave assisted acid pretreatment can generate low molecular weight compounds from the degradation of polysaccharides and lignin contained in the biomass. The pretreatment mostly degrades hemicellulose and only partially cellulose that leads to the formation of different products, such as pentose and hexose sugars, sugar acids, aliphatic acids (primarily acetic acid, formic acid and levulinic acid), and furan aldehydes (5-hydroxymethylfurfural (HMF) and furfural). Some of these compounds could cause brown or dark color in the soluble fractions of the pretreated biomass. The brown or dark color is formed, for example when furfural or HMF is exposed to more severe conditions that make them polymerize, or when the phenolics are oxidized to quinones. Therefore, the formation of brown or dark color from these compounds can be used as an indicator of glucose, xylose, and lignin degradation caused by the pretreatment method employed. Figure 1b showed that the intensity of the absorbance of the brown compounds increased with an increase in temperature. This tendency was in agreement with the hemicellulose loss from the OPEFB (Fig. 1a). As reported before, the increase of heating temperature of microwave-assisted hydrolysis of cassava pulp tends to solubilize hemicellulose gradually (Hermiati *et al.* 2011). The study showed that when the temperature was increased, there was also an increase of absorbance at 490 nm, which corresponded with the loss of hemicellulose (galactan, xylan, rhamnan, arabinan, and mannan) from cassava pulp.

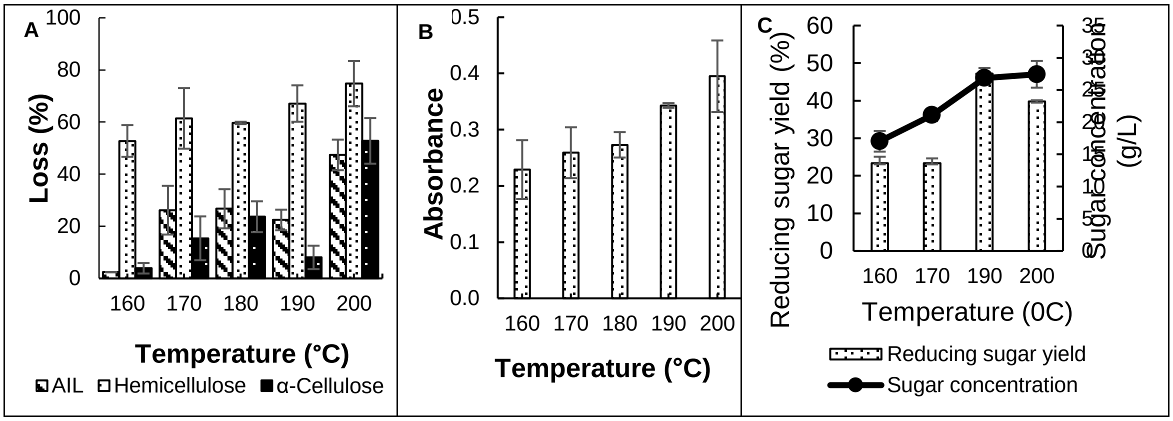

Fig. 1. Effects of the heating temperature during microwave pretreatment of OPEFB with 1% MA and an irradiation time of 2.5 min on the: (A) AIL, hemicellulose, and α-cellulose losses; (B) absorbance of dark-colored compounds; (C) RSY after enzymatic saccharification; and reducing sugar concentration in the liquid fraction

The RSY of the pretreated OPEFB after enzymatic hydrolysis is shown in Fig. 1c. The RSY tended to increase with an increase in temperature up to 190 °C, and then it decreased at 200 °C. This was in agreement with the cellulose content shown in Table 1 and the hemicellulose and lignin removal (Fig. 1a). The highest RSY after enzymatic hydrolysis (47.21%) was achieved in a sample pretreated at 190 °C. Microwave heating caused substrate softening and increased the substrate porosity, which were attributed to the removal of amorphous compounds (lignin and hemicellulose) and fiber disruption (Conde-Mejía *et al*. 2012). To determine what happened to the polysaccharides that were dissolved in the liquid fraction, the sugar concentration of the liquid fraction was also measured. Figure 1c showed that the reducing sugar concentration in the liquid fraction tended to increase when the temperature increased to 190 °C, and then it remained the same. This result revealed that there were more sugars formed when the heating temperature was increased to a certain point, which in this case was 190 °C. After that, the reducing sugar production did not increase, which was probably because of the increased degradation of these sugars into lower molecular weight compounds, such as furfural and 5-HMF. Considering the sugars obtained from both the liquid fraction and enzymatic hydrolysis, the temperature of 190 °C was chosen for the second pretreatment step, in which the irradiation time was varied.

Effect of the Irradiation Time

The effect of the irradiation time on the chemical components and pulp recovery of the pretreated OPEFB are shown in Table 2 and Fig. 2a. Longer irradiation times tended to result in higher α-cellulose contents, except for the irradiation time of 10 min (Table 2). Direct interaction between the substrate and irradiation heating source allows every part inside of the biomass to be accessed. Additionally, the removal of the amorphous zone contributed to a relative increase in the cellulose proportion. The pulp recovery (Table 2) tended to decrease with longer pretreatment times. The disruption of the substrates, carbohydrates, and lignin complexes, which was indicated by the removal of lignin and hemicellulose, has been reported in previous studies (Li *et al*. 2010; Fatriasari *et al*. 2016). The hydrogen bonds in the cellulose also interfered during heating (Li *et al*. 2010).

Table 2. Effect of the Irradiation Time during Microwave Pretreatment of the OPEFB at 190 °C and 1% MA on the Pulp Recovery and Chemical Composition of the OPEFB

Irradiation Time (min)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
2.5	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
5	65.51±2.31	5.29±0.36	1.40±0.06	20.97±0.96	53.92±1.71	12.13±1.18
7.5	63.58±1.23	9.07±0.01	1.24±0.01	20.74±0.84	55.05±0.99	11.16±0.71
10	63.50±0.65	9.85±0.29	1.04±0.11	20.03±0.74	49.36±5.87	20.56±5.30
12.5	57.15±0.35	10.73±0.05	1.33±0.06	16.62±4.05	56.89±2.97	13.50±2.55

The cellulose contents of the OPEFB heated for 5 min to 12.5 min had only slight differences compared with the OPEFB pretreated for 2.5 min. This indicated that a longer irradiation time was not necessary for this microwave-assisted acid pretreatment. The more severe heating conditions until 7.5 min caused higher chemical component (Fig. 2a) and total biomass weight losses (Table 2).

Figure 2a showed that the irradiation time of 2.5 min resulted in mild pretreatment conditions and mainly removed hemicellulose. The irradiation time can be prolonged until 7.5 min for high losses of hemicellulose and lignin. However, longer irradiation times also caused higher absorbance values of dark-colored compounds at 490 nm (Fig. 2b). The higher amount of dark-colored compounds in the liquid fraction with the longer irradiation time indicated a further degradation of sugars, except for at the irradiation time of 2.5 min. Furthermore, the RSY also decreased greatly with an increase in the irradiation time (Fig. 2c). Irradiation for 2.5 min at 190 °C produced as much as 47.2% reducing sugar after enzymatic hydrolysis, while prolonging the irradiation time further reduced the sugar yields.

The high α-cellulose loss was not expected, because it resulted in a smaller source of carbohydrates that can be converted into reducing sugars. The RSY per biomass tended to increase slightly when the irradiation time increased from 5 min to 12.5 min (Fig. 2b), and the highest RSY was only 20.6%. Even though the high hemicellulose and lignin losses occurred after prolonging the irradiation time, the RSYs were still low and lower than those obtained in the previous stage at 190 °C and 2.5 min (47.2%). It is proposed that hemicellulose and lignin only contributed to improving the hydrolysis performance. Based on the results of the experiments in this stage, the optimum irradiation time for the pretreatment stage was 2.5 min. The sugar concentration in the liquid fraction increased by increasing the irradiation time (Fig. 2c). It was proposed that severe pretreatment conditions caused the depolymerization of polysaccharides into sugars, which were soluble in the liquid fraction.

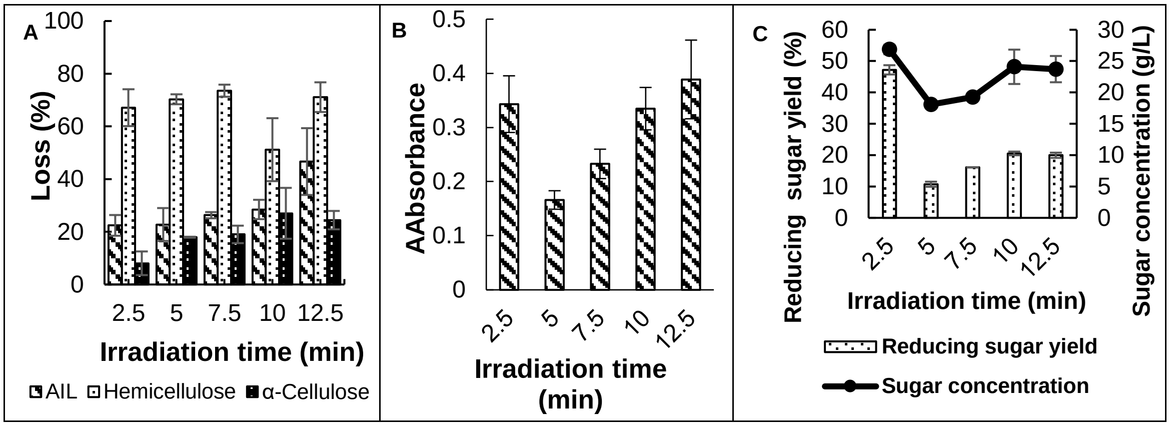

Fig. 2. Effect of the irradiation time during microwave pretreatment of the OPEFB at 190 °C and 1% MA on the: (A) AIL, hemicellulose, and α-cellulose losses; (B) absorbance of dark-colored compounds; (C) RSY after enzymatic saccharification; and) reducing sugar concentration in the liquid fraction

Effect of the MA Concentration

The final stage of experimentation studied the MA concentration effect on the RSY per biomass using the optimum irradiation time (2.5 min) and temperature (190 °C). The acid pretreatment can dissolve hemicellulose and decrease the cellulose crystallinity (Keshwani 2009). As was mentioned before, the hemicellulose removal was followed by other chemical component changes. The increase in the MA concentration caused a lower pulp recovery, as shown in Table 3, which was related to the chemical component loss of the pretreated OPEFB. When the MA concentration increased to 1%, the α-cellulose content increased, and then a decrease in the cellulose content occurred when the MA concentration increased to 1.5%. The highest α-cellulose content and pulp recovery were found in the OPEFB pretreated with 1% MA. The table also showed that when the MA concentration increased, the hemicellulose content decreased.

The hemicellulose loss increased when the MA concentration was increased from 0.5% to 1.0%, but then slightly decreased when the MA concentration was further increased to 1.5%. Meanwhile, the lignin losses were almost the same at all of the acid concentrations (Fig. 3a). Figure 3a also makes it clear that the MA pretreatment could remove both hemicellulose and lignin. However, it removed more hemicellulose. The absorbance value increased as the MA concentration increased from 0.5% to 1.0%, and then did not increase when the MA concentration increased to 1.5% (Fig. 3b).

Table 3. Effect of the Acid Concentration during Microwave Assisted MA Pretreatment at 190 °C with an Irradiation Time of 2.5 min on the Pulp Recovery and Chemical Composition of the OPEFB

Acid Concentration (%)	Pulp Recovery (%)	Extractives (%)	ASL (%)	AIL (%)	α-Cellulose (%)	Hemicellulose (%)
0.5	67.99±1.29	6.07±0.27	0.33±0.01	19.29±2.16	48.52±6.76	20.89±6.95
1	68.80±3.62	11.40±2.08	2.66±0.58	20.07±1.02	57.50±2.81	12.77±2.72
1.5	62.71±0.43	8.41±0.10	0.32±0.00	20.90±0.18	54.91±4.76	15.96±4.89

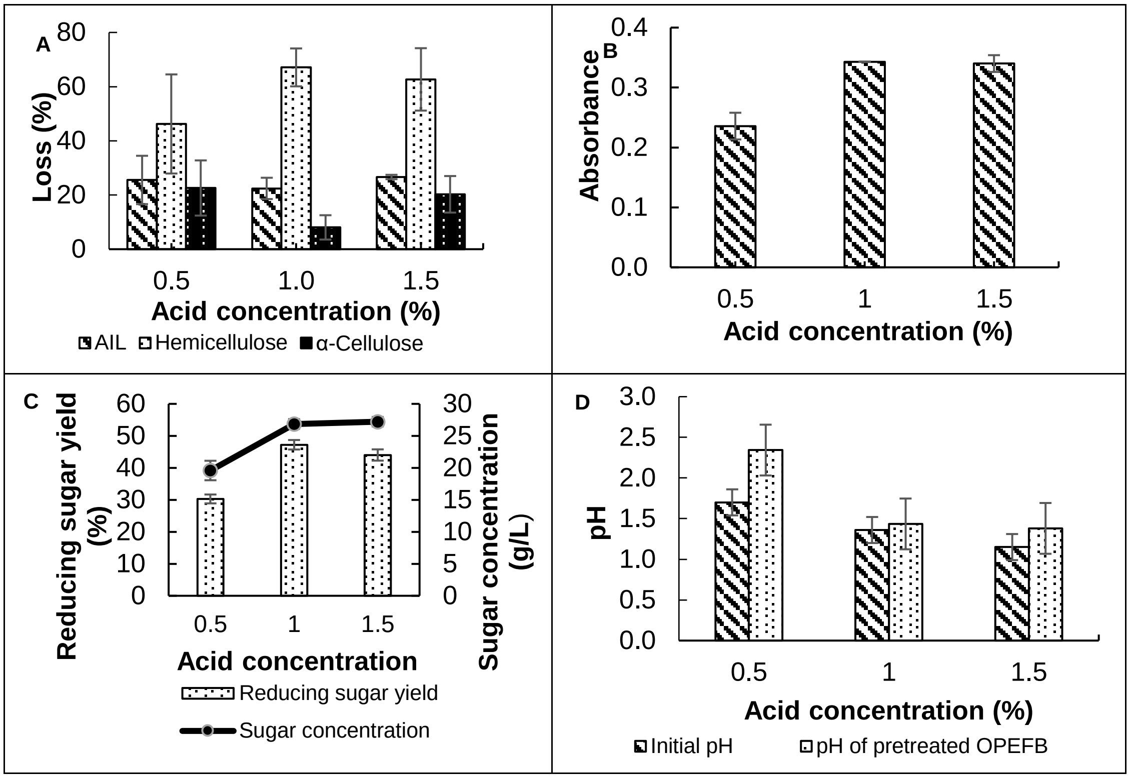

Fig. 3. Effect of the acid concentration during microwave assisted MA pretreatment of the OPEFB at 190 °C with an irradiation time of 2.5 min on the: (A) AIL, hemicellulose, and α-cellulose losses; (B) absorbance of dark-colored compounds; (C) RSY after enzymatic saccharification and reducing sugar concentration in the liquid fraction, and (D) pH of the liquid fraction after pretreatment

The RSY per biomass increased when the MA concentration increased to 1%, and then slightly decreased when the MA concentration was increased to 1.5% (Fig. 3c). This was related to the cellulose content presented in Table 3 and hemicellulose removal in Fig. 3a. A slightly different trend was observed for the reducing sugar concentrations in the liquid fractions, where the sugar concentration obtained from the pretreatment increased up to 1% MA, but then it was stayed the same when the MA concentration was increased to 1.5% (Fig. 3c). Based on the reducing sugar obtained from the liquid fraction and enzymatic saccharification, it was concluded that the optimum pretreatment conditions were 190 °C, 2.5 min, and 1% MA. These pretreatment conditions were in agreement with a similar study with a ramping time of 3 min (Jung *et al.* 2014). By using 60 FPU of cellulase and 30 CBU of β-glucosidase per g of glucan during enzymatic hydrolysis, an enzymatic digestibility as high as 60.9% was obtained by Jung *et al*. (2014). The utilization of a lower enzyme loading (40 FPU/g of substrate) in this study most likely caused the lower RSY (47.21%) compared with the results of Jung *et al*. (2014).

The changes in the pH of the biomass suspensions before and after pretreatment (Fig. 3d) supported the conclusion that there was a more intensive degradation of carbohydrates at higher acid concentrations. At low acid concentrations, the pH of the liquid fraction was much higher than that of the initial pH, while at higher acid concentrations the difference in the pH before and after pretreatment was very small. At low acid concentrations, some hydrogen ions from the acid were used for hydrolysis of the biomass, which increased the pH of the suspension. At higher acid concentrations, the same thing occurred, but there were also some hydrogen ions from the degradation products of the carbohydrates, such as acetic acid and formic acid (Harmsen *et al*. 2010). Therefore, the pH after the pretreatment was almost the same as it was before the pretreatment.

Evaluation of the CSF during Pretreatment

The severity factor (SF) combines the effects of the reaction temperature and time to compare them with the autohydrolysis processing of the materials (Lee *et al*. 2010). It is also an approach that evaluates the combination of effects on certain parameters. This comparison usually gives better results and has a better correlation of the changes to the composition of the pretreated biomass. With the SF, the different conditions of the pretreatment could be compared more easily (Goh *et al*. 2012). When the effect of the pH of the acid was included, then the term CSF was used.

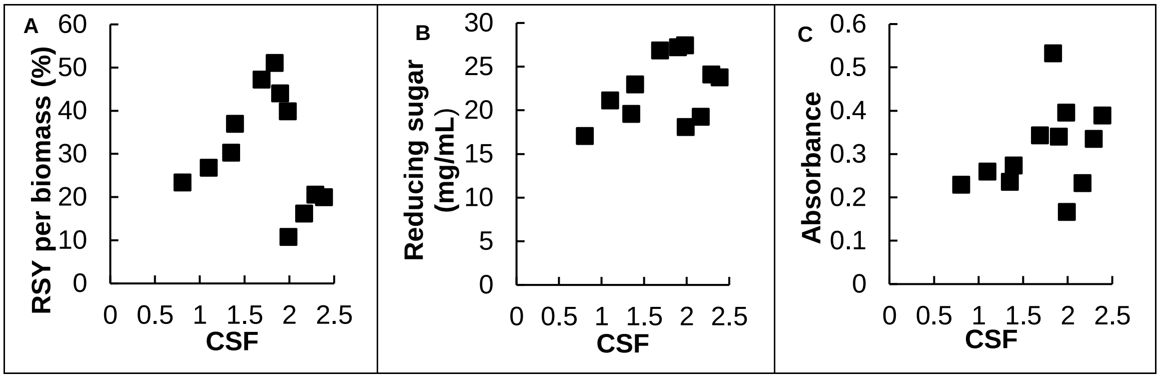

Fig. 4. Effect of the CSF on the (a) RSY per biomass, (b) reducing sugar concentration of the liquid fraction, and (c) absorbance of dark-colored compounds

The effects of the CSF at various pretreatment conditions on the RSY, sugar concentration, and dark-colored compounds in the liquid fraction are presented in Fig. 4. The RSY after enzymatic saccharification, reducing sugar concentration in the liquid fractions, and absorbance at 490 nm tended to increase with an increase in the CSF up to a certain point, and then decreased. This indicated that the more severe the conditions were, the more polysaccharides, especially hemicellulose, that were hydrolyzed and became soluble. This caused an increase in the reducing sugar in the soluble fractions (Fig. 4b). The increase in severity caused further degradation of the sugars into lower molecular weight compounds, such as furfural and 5-HMF (Fengel and Wegener 1989), and thus caused a decreased sugar concentration in the soluble fraction. Meanwhile, when the hemicellulose removal from the solid biomass increased, the susceptibility of the biomass to cellulase also increased. As such, the RSY after enzymatic saccharification also increased with an increase in the CSF up to a certain point (Fig. 4a). The decrease in the RSY after this point was also probably because of the degradation of some polysaccharides in the solid biomass into lower molecular weight compounds. The effect of the CSF on the absorbance of dark-colored compounds was similar to the effects on the RSY and sugar concentration in the liquid fraction. The CSF range that could produce more reducing sugar (RSY 37% to 47%; sugar concentration in the liquid fraction 23 mg/mL to 27 mg/mL) was 1.5 to 2.0. In the authors' previous study, the pretreatment of OPEFB using MA and OA in a liquid hot water (LHW) reactor did not clearly show the effect of the severity on the RSY when MA was used, but a similar trend was seen when OA was used. The range of the CSF that could produce a higher RSY (50% to 60%) was 1.6 to 1.8 (Sari *et al*. 2016).

Effect of the MA and Sulfuric Acid Pretreatments on the Chemical Components, Sugar Yield, and Functional Groups of the OPEFB

A comparison of the microwave pretreatments with SA and MA was presented in Table 4. The use of SA caused a lower pulp recovery (56.8%) than for MA (68.8%). This was supported by the higher loss of chemical components from the SA-pretreated OPEFB. The cellulose, hemicellulose, and AIL losses caused by the microwave assisted SA pretreatment were as high as 24.1%, 73.1%, and 39.4%, while the microwave assisted MA pretreatment caused losses of 8.04%, 67.1%, and 22.4%, respectively. These results showed that MA prevented the degradation of cellulose in the biomass better than SA.

Table 4. Comparison of the Results of the Pretreatments Using MA and SA

Acid Pretreatment at a Concentration of 1%, 190 °C, and 2.5 min	Pulp Recovery (%)	Chemical Component Content (%)			Chemical Component Loss (%)			RSY after Enzymatic Saccharification (%)	
		α-Cellulose	Hemicellulose	AIL	α-Cellulose	Hemicellulose	AIL	48 h	72 h
MA	68.80±3.62	57.50±2.81	12.77±2.72	20.07±1.02	8.04±1.93	67.12±7.00	22.44±3.93	17.69±0.56	47.21±1.48
SA	56.85±0.94	57.43±0.30	12.63±0.75	18.97±1.43	24.10±0.40	73.12±1.60	39.42±4.57	51.03±2.40	37.29±0.60

The results of the enzymatic saccharification of the OPEFB pretreated by the two acids showed interesting phenomena. The RSY of the OPEFB pretreated by MA increased when the duration of hydrolysis was longer, while the opposite trend occurred for the OPEFB pretreated by SA. This indicated that the MA pretreatment required longer durations of hydrolysis to reach higher RSYs compared with the SA pretreatment. This could have been related to the chemical component loss, especially the AIL loss, which was much higher in the OPEFB pretreated by SA. Less lignin in the biomass makes it more susceptible to cellulase enzymes, which enhances the degradation of cellulose and production of reducing sugar. Despite this, the formation of reducing sugar was faster in the OPEFB pretreated by SA, the possibility of the formation of some inhibitors by this pretreatment should also be considered. The high inhibition of secondary degradation products, mainly furfural that resulted from the SA pretreatment was previously reported by Kootstra *et al*. (2009).

The FTIR spectroscopy analysis was used to identify the functional groups in the OPEFB pretreated with SA and MA. Based on the FTIR analysis presented in Fig. 6, the MA pretreatment caused the loss of certain functional groups at 2854 cm^{-1}, which was identified as the asymmetric stretching vibration of CH_2 in cellulose (Koutsianitis *et al*. 2015).

Even though the main effect of acid pretreatment is hemicellulose removal, the results of this study showed that structural changes also occurred in the cellulose after the MA pretreatment. The more severe pretreatment conditions with MA than with SA, showed by a higher CSF, might have affected this finding. In the parallel study on OA-pretreated OPEFB done by the authors, this band also disappeared after pretreatment (Solihat *et al*. 2017).

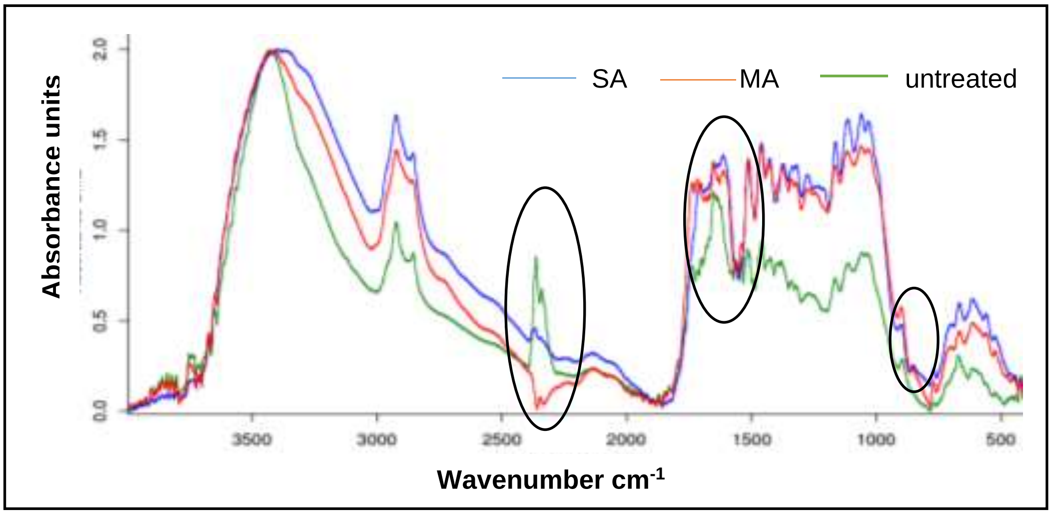

Fig. 5. FTIR spectra of the OPEFB pretreated by SA and MA from 4000 to 600 cm^{-1}; Green line: untreated OPEFB; Red line: OPEFB pretreated with 1% MA at 190 °C for 2.5 min; Blue line: OPEFB pretreated with 1% SA at 190 °C for 2.5 min; Circles indicate the finger print regions

Generally, the spectra of the SA- and MA-pretreated OPEFB had similar patterns. They showed increases in the peak intensities at each band compared with that of the untreated OPEFB. This might have been because of the removal of recalcitrant compounds covering the cellulose.

Compared with the untreated OPEFB, there was a slight increase in the bands at 3300 cm^{-1} (O-H valence vibration of cellulose) and 2921 cm^{-1} (symmetric vibration of CH$_2$ groups in cellulose) for both pretreated OPEFB. These findings correlated with the cellulose content in the pretreated OPEFB (Table 1), which was higher than in the untreated OPEFB. The band at 2854 cm^{-1} assigned to the asymmetric C-H$_2$ valence vibration was not detected in the pretreated OPEFB.

Some bands in the finger print region of 1750 cm^{-1} to 1500 cm^{-1} changed in intensity. The finger print regions are marked on Fig. 5 with circles. The bands that were identified are summarized in Table 5. Even though there was a high hemicellulose loss, the band at 1735 cm^{-1} associated with the unconjugated C=O in xylan was still detected in all of the samples. The vibration of this band is attributed to carbonyl (C=O) stretching of carboxyl groups in hemicelluloses, lignin, and extractives, as well as esters in lignin and extractives (Bouafif *et al*. 2008; Faix 1991).

The lignin structure was identified as the bands around 1600 cm^{-1} (C=O stretching), 1513 cm^{-1} (aromatic skeletal vibration), 1460 cm^{-1} (C-H deformation) (Fengel and Wegener 1989), 1255 cm^{-1} (guaiacyl ring), and 1339 cm^{-1} (C-O vibration in syringyl derivatives) (Pandey and Pitman 2003). The aforementioned peaks in the pretreated OPEFB had higher intensities than those in the untreated OPEFB. This was related to the chemical bond disruption effect of the MA and SA pretreatment, especially on the hemicellulose and lignin. The lignin could be distinguished and still remained in the pulp. The lignin was also found in bamboo that underwent a microwave pretreatment (Fatriasari *et al*. 2016) and yellow poplar pretreated with OA (Kundu and Lee 2015), which had a lower intensity after pretreatment.

Table 5. Assignments of the IR Bands of the Untreated and Pretreated OPEFB

	Untreated OPEFB	OPEFB Pretreated with SA	OPEFB Pretreated with MA	Assignments
	Maximum Band Position (cm^{-1})			
1	3429	3398	3421	Hydrogen bond (O-H) stretching absorption; O(3)H….O(3) intermolecular in cellulose (Pandey and Pitman 2003; Isroi *et al*. 2012)
2	2854	-	-	Asymetric C-H$_2$ valence vibration in cellulose (Koutsianitis *et al*. 2015)
3	1738	1701	1735	Unconjugated C=O in xylan (Pandey and Pitman 2003)
4	1561	1611	1610	C=O stretching; S > G; G condensed; G etherified in lignin (Faix 1991~~)~~
5	1513	1513	1512	Aromatic skeletal vibration; G > S Aromatic skeletal vibrations plus (Pandey and Pitman 2003; Faix 1991)
6	1458	1459	1458	C-H deformation in lignin and cellulose (Pandey and Pitman 2003)
7	1339	1318	1337	C-H vibration in cellulose (Pandey and Pitman 2003)
				C-O vibration in syringyl derivates in lignin (Pandey and Pitman 2003)
8	896	851	898	C-O-C stretching at β-glicosidic linkage or C-H deformation in cellulose (Nelson and O'Connor 1964)
9	-	769	769	C-H$_2$ vibration in cellulose Iα in cellulose (Isroi *et al*. 2012)

The peak near 895 cm^{-1}, which was assigned to β-glycosidic linkages, was clearly detected in all of the samples (Koutsianitis *et al*. 2015). The intensity of this band in the OPEFB pretreated with MA was higher than in the OPEFB pretreated with SA, which was in agreement with the slightly higher cellulose content in the MA-pretreated OPEFB. This finding was supported by previous studies on sugarcane bagasse pretreated with microwave heating and SA and OPEFB pretreated with fungal-phosphoric acid (Chen *et al*. 2011; Isroi *et al*. 2012). The C-H$_2$ vibration in cellulose Iα (band in 769 cm^{-1}) was seen more clearly in the pretreated OPEFB than in the untreated OPEFB, and had almost identical intensities in the MA- and SA-pretreated samples. This was because of the removal of the hemicellulose and lignin that covered the cellulose in the pretreated OPEFB.

Bioethanol Production *via* SSF

The washed insoluble solid (pulp) of the pretreated OPEFB obtained from the optimum pretreatment conditions (1 % MA at 190 °C for 2.5 min) was used for the SSF. These optimum pretreatment conditions were chosen based on the RSY after enzymatic hydrolysis of pretreated OPEFB. The pulp pretreated by microwave assisted SA also underwent SSF for comparison purposes. Figure 6a shows the reducing sugar and ethanol concentrations that were obtained during 72 h of SSF. Both of these pretreatments showed similar trends in the reducing sugar and ethanol concentrations after SSF. After 24 h of SSF, the reducing sugar concentrations produced from the MA- and SA-pretreated OPEFB were 4.57±0.76 g/L and 2.89±2.08 g/L, respectively, and then the reducing sugar concentration produced decreased to 2.72±0.03 g/L and 0.34±0.19 g/L after 48 h of SSF, respectively. The higher reducing sugar concentration achieved in the first few hours of SSF was due to the action of the cellulase (Akhtar *et al*. 2017). The concentration then decreased because of the consumption of sugar by the yeast. Interestingly, an increase in the reducing sugar in the late phase of fermentation occurred in SA treatments. In the late phase of fermentation, the reducing sugar concentration of SA-pretreated OPEFB increased to 3.32±1.8 g/L. This was probably caused by the lower yeast activity during the late phase of fermentation. The sugar release rate from the enzymatic hydrolysis was faster than the sugar consumption rate by the yeast (Zhu *et al*. 2015). The continuation of cellulase activity whereas yeast fermentation has finished was also an indication of remaining reducing sugar in the late phase of fermentation (Ballesteros *et. al.* 2004).

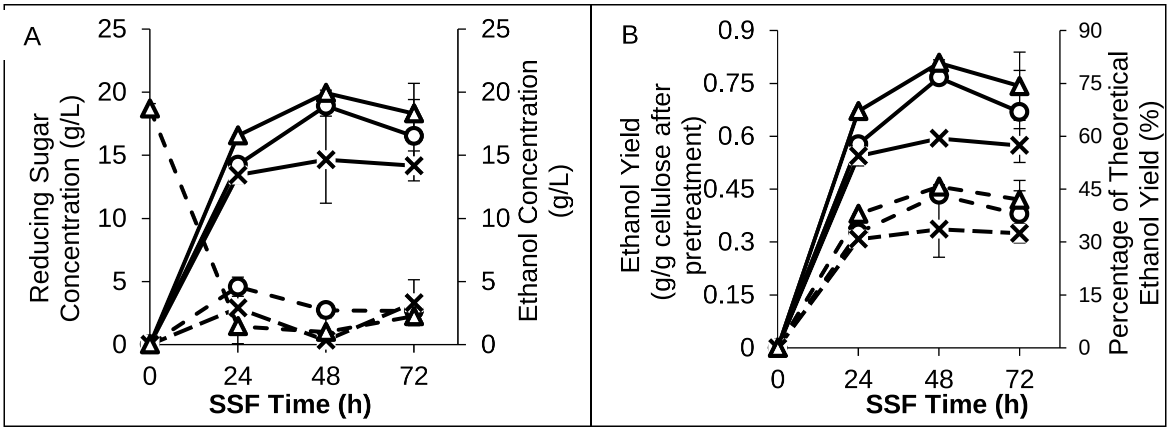

Fig. 6. Reducing sugars (**- - - -**) and ethanol concentrations (——) (A) and ethanol yield (**- - - -**) and percentage of theoretical (———) (B) during simultaneous saccharification of microwave-assisted acid pretreated OPEFB (○ = 1% maleic acid, × = 1% sulfuric acid, Δ = 1% maleic acid with prehydrolysis)

The SSF performed using 15.3 g of pretreated OPEFB (in 50 mL working volume) resulted in an ethanol concentration of 18.9 g/L and ethanol yield of 0.43 g/g cellulose after MA-pretreatment, which corresponded well with the theoretical value of 76.6%. This highest ethanol concentration was obtained after 48 h of SSF. Ethanol concentration then decreased until 16.5 g/L at 72 h of SSF. Similar trend with SSF result, the highest ethanol concentration was achieved after 48 h fermentation using OPEFB with 4 h pre-hydrolysis step. Moreover, there was no remarkable difference in the ethanol production when comparing the microwave pretreatments using MA and SA. From Fig. 6 it was observed that the microwave assisted MA pretreatment produced higher ethanol yield and concentration than microwave assisted SA pretreatment. The highest ethanol concentration of 14.6 g/L and yield of 0.34 g ethanol/g cellulose after pretreatment, or equivalent to 59.4% of theoretical was achieved after 48 h SSF using microvave- SA pretreated OPEFB. Based on these results, it was suggested that MA can be used as an alternative to SA during pretreatment.

The pretreatment process is crucial for achieving effective hydrolysis of substrates into sugars, which can be used directly or fermented by microorganisms in the SSF medium (Triwahyuni *et al*. 2015). Compared with the untreated OPEFB, which had an ethanol yield of 0.37% (data not shown), the microwave assisted acid pretreatment enhanced the ethanol yield by 76.6% of theoretical. This indicated that pretreated lignocellulosic materials resulted in higher ethanol yield than did untreated materials. The removal of hemicellulose and lignin from the pretreated OPEFB was one of the factors responsible for the increase in the material digestibility.

Effect of Pre-Hydrolysis on the Production of Reducing Sugar and Ethanol

One of the purposes of the pre-hydrolysis step before SSF is to hydrolyze the solid material faster, and thus a more homogenous and higher glucose concentration was available for ethanol fermentation. In this study, the pretreated OPEFB was pre-hydrolyzed at 50 °C and 120 rpm for 4 h prior to SSF. After that, *S. cerevisiae* was added into the SSF flask under sterile conditions. The pre-hydrolysis step was conducted with the same concentration of substrate in the SSF. After 4 h of pre-hydrolysis, the viscosity of the SSF hydrolysate was reduced (data not shown). This reduction was probably related to the action of the enzyme, which hydrolyzed the OPEFB during pre-hydrolysis.

Figure 6a shows the effects of microwave assisted MA and SA pretreatment of the OPEFB on the reducing sugar and ethanol concentrations. A reducing sugar concentration of 18.7 g/L was quickly obtained after 4 h of hydrolysis. After the addition of yeast, the sugar decreased because of the consumption of sugars by the yeast, which resulted in a 12.2 g/L ethanol concentration after 24 h of SSF. Although high concentration of reducing sugar was formed during pre-hydrolysis, thus readily available in the pre-hydrolized SSF broth, it did not give significant effect on the ethanol concentration in that particular sample after the fermentation. Additionally, both the SSF and pre-hydrolysis followed by SSF (PSSF) produced similar amounts of ethanol at the end of the processes. When applying PSSF, the highest ethanol concentration was 19.9 g/L and ethanol yield was 0.46 g/g cellulose, which was equivalent to 80.8% of theoretical yield. Meanwhile, the highest concentration of ethanol from the SSF was 18.9 g/L with ethanol yield of 0.43 g/g cellulose, which corresponded with 76.6% of theoretical yield (Fig. 6b). This indicated that applying PSSF only slightly increased the ethanol yield obtained from SSF. Nevertheless, ethanol productivity obtained from the PSSF was much higher than that from SSF during 24 and 48 hours, and only slightly higher during 72 hours of fermentation (Table 6).

Table 6. Ethanol Productivity (g/L/h) during Simultaneous Saccharification and Fermentation of Microwave-Assisted Maleic Acid Pretreatment of OPEFB

Saccharification and Fermentation methods	Duration of Saccharification and Fermentation (h)		
	24	48	72
SSF	0.59±0.01	0.39±0.00	0.23±0.04
SSF with pre-hydrolysis (PSSF)	0.69±0.00	0.42±0.00	0.25±0.03

In this study, samples of 15.3 g of pretreated OPEFB in total of 50 mL working volume was used to achieve a good mixing during SSF. The method by Dowe and McMillan (2008) also recommends the use of a lower cellulose content (3% to 6%, w/w) to avoid a high viscosity. This condition probably affected the increase of the ethanol yield in the PSSF. Meanwhile, Hoyer *et al.* (2013) reported that pre-hydrolysis did not have a major effect when a high ethanol yield and concentration were already achieved without a separate pre-hydrolysis step. As long as the enzymes were capable of hydrolyzing the substrate during SSF efficiently, a pre-hydrolysis step was not necessary (Paschos *et al.* 2015). However, study by Anita *et al.* (2017) reported that pre-hydrolyis step increased ethanol concentration and shortened fermentation time of pretreted OPEFB. SSF with 3 h pre-hydrolysis (PSSF) produced higher ethanol concentration (18.52±0.06 g/L with

60.90±0.19% yield during 48 h) than SSF without pre-hydrolysis which produced almost similar titer of ethanol during 72 h of fermentation. Another study also reported the different effects of a pre-hydrolysis step on different lignocellulosic materials. For example, the utilization of PSSF for 12 h at 40 °C resulted in an ethanol yield of 79.5% from a 15% solid loading of alkaline pretreated-cashew apple bagasse, which was lower than the ethanol yield achieved from SSF (92.7%) (de Barros *et al*. 2017). Meanwhile, a pre-hydrolysis step for 4 h and 22 h at 48 °C prior to SSF at 32 °C and using 13.7% (25% of total solid) of the whole slurry of steam-pretreated spruce remarkably increased the overall ethanol yield to 66.0% and 72.0%, respectively (Hoyer *et al*. 2013).

The comparison of ethanol production from OPEFB in this study with the results of some previous studies of OPEFB pretreatment, saccharification, and fermentation was presented in Table 7. In term of theoretical yield, the results of this study were comparable with the results of alkali pretreatment (Christia *et al*. 2016; Sudiyani *et al*. 2013) and acid impregnation steam explosion pretreatment (Siramon *et al*. 2017). The present results were lower than that of phosphoric acid pretreatment (Ishola *et al*. 2014) and combined sulfuric and sodium hydroxide petreatment (Bukhari *et al*. 2014), but higher than those of Jung *et al*. (2013, 2014) with similar pretreatment of OPEFB, using combined of microwave heating and acids. Jung *et al*. (2013, 2014) also showed that the ethanol yield increased when the whole slurry of pretreated OPEFB was used, instead of just the washed insoluble solid of pretreated OPEFB.

Table 7. Comparison of Ethanol Production of OPEFB using Various Pretreatments

Pretreatment	Saccharification and Fermentation	References
Sodium hydroxide pretreatment (8% NaOH at 100 °C for 10 to 90 min)	Fermentation by *M. indicus* resulted in 68.4% of the theoretical ethanol yield	Christia *et al*. 2016
Microwave assisted glycerol-sulfuric acid pretreatment	Ethanol concentration, ethanol productivity and ethanol yield after SSF for 72 h were 1.26 g/L, 0.02 g/L/h, 0.64 g/g	Fatriasari *et al*. 2017
Acid impregnation-steam explosion pretreatment	Ethanol yield from Separated Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) were 74.43% and 64.30%, respectively.	Siramon *et al*. 2017
Combined chemical pretreatment (1.0% (v/v) dilute H_2SO_4 at 125 °C for 90 min followed by 1% (w/v) NaOH at 100 °C for 60 min)	The fermentation of pretreated OPEFB by *Saccharomyces cerevisiae* within 24h resulted ethanol yield of 12.13 ± 0.99 g/L with theoretical yield of 89.1%.	Bukhari *et al*. 2014
Alkali pretreatment with 15% w/v NaOH for 40 min at 130 °C	Ethanol concentration obtained after hydrolysis of OPEFB by 7% v/v H_2SO_4 at 119 °C for 110 min and continued with fermentation with baker's yeast was 8.49 g/L.	Duangwang and Sangwichien 2015
Alkali pretreatment (10% NaOH)	Saccharification of pretreated OPEFB using modified cellulase enzyme followed by fermentation using local strain of *Saccharomyces cerevisiae* Mk for 36 h resulted in ethanol with concentration of 6.36% v/v, and yield of 69%.	Sudiyani *et al*. 2013

Microwave-assisted maleic acid pretreatment at 190 °C with 1 % (w/v) maleic acid for a short time (3 min ramping to the set temperature with no holding time)	SSF of the washed insoluble solid and whole slurry of pretreated OPEFB for 48 h resulted in 39% and 61.3 % theoretical yield of ethanol based on the initial amount of glucan in untreated EFB, respectively.	Jung *et al*. 2014
Phosphoric acid, fungal, and combination of fungal and phosphoric acid pretreatment	The highest theoretical ethanol yield after SSF of OPEFB pretreated by phosphoric acid, fungal, and combination of fungal and phosphoric acid pretreatment were 89.4, 27.9, and 62.8%, respectively.	Ishola *et al*. 2014
Microwave-assisted dilute sulfuric acid pretreatment (1% (w/v) with 3 min ramping to 190 °C	SSF of the washed insoluble solid and whole slurry of the pretreated OPEFB *using Saccharomyces cerevisiae* for 72 h resulted in 47.2% and 52.5% of theoretical ethanol yield based on total glucan in the untreated initial OPEFB. Activated carbon treatment of pretreated OPEFB slurry before SSF resulted in 87.5% ethanol yield based on the initial glucan content in untreated OPEFB after 48 h of SSF.	Jung *et al*. 2013
Aqueous ammonia soaking pretreatment (60 °C, 12 h, and 21% (w/w) aqueous ammonia)	SSF of pretreated OPEFB for 168 h with 5% (w/v) glucan loading and 60 FPU of cellulase and 30 CBU of β-glucosidase per gram glucan resulted in ethanol production of 18.6 g/L titer, ethanol theoretical maximum yield of 65.6%, ethanol productivity 0.11 g/L/h.	Jung *et a*l. 2011
One and two-stage hydrolysis using dilute-sulfuric acid (0.2, 0.8%) at 170-230 °C with holding time of 5 and 15 min.	Fermentation of first stage hydrolyzate by *Mucor indicus* resulted ethanol yields of 0.45%, while fermentation of second stage hydrolyzate by *Saccharomyces cerevisiae* resulted the corresponding 0.46 g ethanol g^{-1} sugar consumed.	Millati et al. 2011
Microwave assisted maleic acid pretreatment (1% acid, 190 °C, 2.5 min)	SSF of pretreated OPEFB for 48 h using *S. cereviceae* produced the ethanol with concentration of 18.91 g/L and yield of 0.43 g ethanol/g cellulose or equivalent to 76.62% of theoretical maximum yield. SSF of pretreated OPEFB with 4 h pre-hydrolysis (PSSF) resulted in ethanol concentration of 19.94 g/L and yield of 0.46 g ethanol/g cellulose or equivalent to 80.78±% of theoretical maximum yield.	This study

CONCLUSIONS

1. Microwave assisted maleic acid (MA) pretreatment of oil palm empty fruit bunch (OPEFB) (*Elaeis guineensis* Jacq) successfully increased the reducing sugar yield (RSY) after enzymatic hydrolysis. The MA pretreatment removed hemicellulose and lignin, which facilitated the accessibility of cellulase enzyme to the cellulose. The temperature had more of an influence on improving the RSY compared with the irradiation time and MA concentration.
2. After an evaluation of the RSY with three pretreatment stages, the optimum pretreatment conditions that resulted in the highest RSY (47.2%) was a temperature of

190 °C with an irradiation time of 2.5 min and an MA concentration of 1%. To obtain an RSY per biomass of up to 30%, it is suggested to use a CSF in the range of 1.5 to 2.0. Pretreatment using sulfuric acid (SA) caused a higher removal of lignin than from pretreatment using MA, with comparable α-cellulose and hemicellulose contents. More severe pretreatment conditions tended to hydrolyze more hemicellulose, as shown by the higher reducing sugar content in the liquid fraction of the more severely pretreated samples.

3. The highest ethanol concentration and yield (18.9 g/L and 0.43 g ethanol/g cellulose after pretreatment, or equivalent to 76.6% of theoretical maximum yield, respectively) were produced after 48 h of SSF of the OPEFB pretreated with MA. A pre-hydrolysis step prior to SSF only slightly increased the ethanol yield.

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