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Research Article

Pretreatment of Oil Palm Empty Fruit Bunch (OPEFB) at Bench-Scale High Temperature-Pressure Steam Reactor for Enhancement of Enzymatic Saccharification

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ABSTRACT. Upscaling of biomass pretreatment from laboratory scale to a bench-scale reactor is one of the important steps in the application of the pretreatment for pilot or commercial scale. This study reports the optimization of pretreatment conditions, namely reaction temperature and time, by one factor at a time (OFAT) method for the enhancement of enzymatic saccharification of oil palm empty fruit bunch (OPEFB). OPEFB was pretreated using high temperature-pressure steam reactor with different reaction temperatures (160, 170, 180, 190, 200 °C) and times (10, 20, 30, 40, 50 min). The effectiveness of the pretreatment was determined based on chemical compositions of raw OPEFB and OPEFB pulp and sugar production from enzymatic saccharification of the OPEFB pulp. Solubilized components from OPEFB, such as glucose, xylose, formic acid, acetic acid, 5-hydroxymethyl furfural (HMF), and furfural in the hydrolysate that generated during steam pretreatment were also determined. Pretreatment at 180 °C for 20 min provides the highest sugar yields (97.30% of glucose yield per initial cellulose and 88.86% of xylose yield per initial hemicellulose). At the optimum condition, 34.9% of lignin and 30.75% of hemicellulose are successfully removed from the OPEFB and resulted in 3.43 delignification selectivity. The relationship between severity factor and by-products generated and the sugars obtained after enzymatic saccharification are discussed. The pulp of OPEFB at the optimum condition was also characterized for its morphological characteristic by scanning electron microscopy (SEM) and crystallinity by X-ray diffractometry (XRD). These pulp characteristics are then compared with those of the raw OPEFB. The steam pretreatment causes some fiber disruptions with more defined and opened structures and increases the crystallinity index (CrI) by 2.9% compared to the raw OPEFB.

Keywords: Steam pretreatment, bench-scale reactor, enzymatic saccharification, OPEFB

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1. Introduction

Lignocellulosic biomass is mainly constituted by three different biopolymers (i.e. cellulose, hemicellulose, and lignin) with minor components (i.e., extractives, proteins, chlorophyll, and ashes) (Taherzadeh & Karimi, 2007; Kim *et al.*, 2013; Galia *et al.*, 2015). Cellulose as polysaccharide constituents of lignocellulosic biomass can be converted to glucose by hydrolysis through biological, chemical or enzymatic process and be further used as a source of ethanol or other useful chemicals. However, the presence of lignin, hemicellulose, and extractives, which serves as a protective barrier against enzyme and microbial degradation, has been a hurdle for the efficient utilization of lignocellulosic biomass on the commercial scale (Santos *et al.*, 2012). A pretreatment in the biorefinery process can be defined as the process to deconstruct the linkages and disrupt the structure of the cell walls, so that enzyme can easily access to cellulose (Rigual *et al.*, 2018).

An effective pretreatment should recover as high as possible of all carbohydrates in the biomass. Additional positive features should be low energy demand and operational cost associated with construction material, feedstock size reduction, and treatment of process residues (Laser *et al.*, 2002). Pretreatment also must obtain maximum production of valuable hemicellulose derived products with limited generation of undesired degradation compounds; maximum production of valuable by-products from lignin; cellulose-containing solids with high susceptibility towards enzymatic hydrolysis; and minimum generation of processing wastes (Galbe & Zacchi, 2007; Romani *et al.*, 2011).

Indonesia is one of the largest palm oil producing countries in the world. In 2019 Indonesia produced 34.7 million tons of CPO (Crude Palm Oil) which is 14% higher than in 2018 (Timorria, 2019). The growth of oil palm plantation area in Indonesia has also continued to

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increase. In 2018, the area of palm oil plantation is 14.33 million hectares with an estimated increase to 14.68 million hectares in 2019 (Direktorat Jenderal Perkebunan, 2018). One ton of fresh fruit bunch (EFB) contains as much as 230 kg or 23% of OPEFB. Therefore, about 7 million tons of OPEFB was produced every year in Indonesia. The high content of polysaccharides, which is about 50–80%, makes OPEFB as a potential biomass to be used as raw material for bioethanol production (Anita *et al.*, 2019).

A large number of pretreatment methods have been investigated and developed during the last 10 years. There are several types of pretreatments, such as mechanical, physical, thermal, chemical, and combinations of these (Chen *et al.*, 2017; Kumar & Sharma, 2017; Kumari & Singh, 2018). Pretreatment is intended to remove hemicellulose, reduce cellulose crystallinity, and increase the porosity and the surface area of the material. Steam or hydrothermal pretreatment refers to the use of steam or water to pretreat biomass, and the process occurred is an auto-hydrolysis of chemical components in the biomass. This process has several potential advantages, such as no requirement for chemicals (using water as a solvent), no special requirement for non-corrosive reactor materials, and low operation cost (Laser *et al.*, 2002). Steam or hydrothermal pretreatment has some major objectives, one of them is to solubilize the hemicelluloses into soluble oligomer (Lee *et al.*, 2009). Other main objectives are to increase enzyme accessibility on cellulose for enzymatic hydrolysis and to avoid the formation of inhibitors, such as furan derivatives (5-hydroxymethyl furfural (5-HMF) and furfural), organic acids (acetic, formic, and levulinic acid), and phenolic compounds (Jeong & Lee, 2015; Kim *et al.*, 2011).

Most of the reports regarding auto-hydrolysis process in the pretreatment of biomass for bioethanol production were obtained from studies using small scale batch-operating equipment, such as laboratory flasks. In order to verify the technology, it is important to implement the process in a bench-scale reactor. This will provide better data for assessment and for scale-up to a demo- or pilot- or full-scale process. Therefore, in this research, we investigate steam pretreatment of OPEFB using high temperature-pressure steam reactor with larger capacity (100 g of biomass and 3 L of water). The objective of this study was to identify the optimum conditions of the pretreatment, including temperature and time reaction.

2. Materials and Methods

2.1 Materials

OPEFB was obtained from Cikasungka Plantation of PT Perkebunan Nusantara VIII, Bogor, West Java Province, Indonesia. The biomass was ground into small particles and dried, then it was sieved until it was passed through a 60 mesh sieve and stored in a sealed plastic bag. All chemicals, such as ethanol, benzene, sulfuric acid, sodium hydroxide, sodium chlorite, acetic acid, acetone, citric acid

monohydrate, used in this study were analytical grade and were purchased from MERCK (Germany).

2.2 Analysis of chemical components of OPEFB

TAPPI methods were used to determine the chemical component of raw OPEFB and OPEFB pulp such as moisture content (TAPPI, 1997), ash (TAPPI, 2002), and extractive contents (TAPPI, 1997). The lignin (acid insoluble lignin) was determined based on NREL methods (Sluiter *et al.*, 2008). The holocellulose and α -cellulose were determined according to Wise *et al.*, (1946), and Rowell *et al.*, (2019). The hemicellulose content was obtained by subtracting the holocellulose content with the α -cellulose content (McMillan, 1994).

2.3. Steam pretreatment

The steam pretreatment was conducted in a high temperature-pressure reactor (Yasujima Type SB-0405S, Japan) having capacity of 0.2 kg (oven dry weight / ODW) of biomass per batch (Fig. 1). The reactor consists of six units of heaters with thermometer to adjust the temperature of the heater and protect the heater from overheating, a cooling water jacket system to cool down the equipment after the pretreatment process has been completed, and a control panel to control the operation of the equipment and display the pressure and temperature during the process.

The reactor was equipped with a spring balancer to open and close the lid of the equipment easily, a pressure gauge that shows the pressure during the process, a pressure sensor that measure the pressure inside the main unit of the reactor, and an internal temperature sensor that measures and maintains the temperature inside the main unit of the reactor. A stainless-steel basket (38 cm diameter, 15 cm height), located above the steam generates by the heaters, is provided to put the biomass.

One hundred g (ODW) of OPEFB were loaded into a stainless-steel basket, designed specifically for the steam reactor. Deionized water (3L) was added to the reactor to generate high temperature and pressure steam when it was heated. This amount of water was used as suggested by the reactor manufacturer. In the first step we optimized the temperature. Therefore, the temperature of the reactor was elevated up to the desired temperatures (160, 170, 180, 190, 200 °C) and kept constant at that temperatures for 10 min. In the second step, we used the optimum temperature obtained from the first step experiment and the heating was conducted for 10, 20, 30, 40, 50 mins. After the pretreatment has been completed, and the reactor was cooled down to about 50 °C, the pulp of OPEFB was taken out of the reactor and collected in a cloth bag. The pulp was then washed thoroughly with deionized water until it reached neutral pH to remove acids and free sugars. The pulp recovery was determined based on Eq. (1) (Anita *et al.*, 2019).

$$\text{Pulp recovery (\%)} = \left(\frac{((100 - \text{MCP})/100) \times \text{WP}}{\text{WB}} \right) \times 100 \quad (1)$$

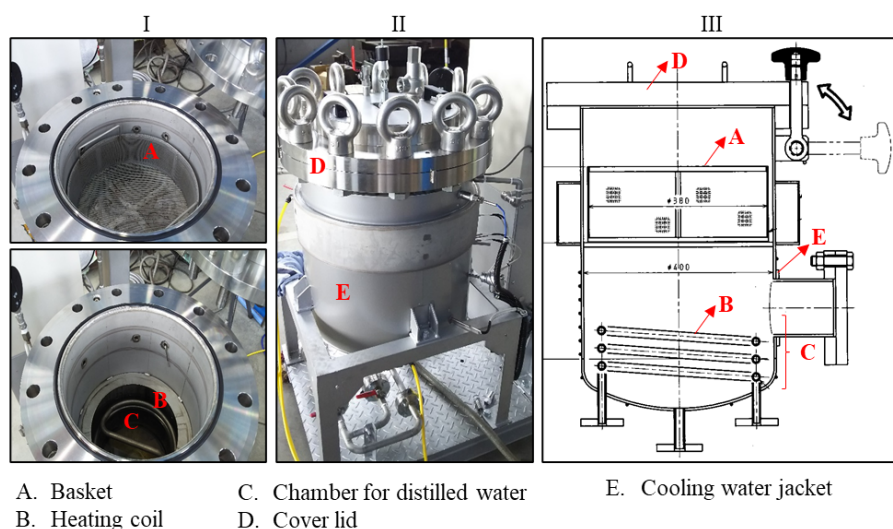


Fig 1. High Temperature-Pressure Steam Reactor for steam pretreatment. I: inside the reactor, II: outside the reactor, III: the schematic diagram

where MCP is moisture content of pulp (%), WP is weight of pulp (g), WB is oven dry weight of initial biomass (g). Before further analysis, the pulp was placed in a sealed container, and then stored in a freezer. The water that used for generating steam was collected from the reactor. It was centrifuged to separate the remaining solid from the hydrolysate, then the acidity of the hydrolysate was measured by pH meter (IONIX). The above experiments were performed in duplicate.

In this study, we determined the severity factor (expressed as $\log R_0$) to quantify the intensity of the steam pretreatment of OPEFB. The severity of the pretreatment was used to observe possible relationship between the severity during auto-hydrolysis and characteristics of the hydrolysate. The severity factor (SF) was calculated using Eq. (2) as proposed by Overend *et al* (1987).

$$R_0 = t \times \exp\left(\frac{T-100}{14.75}\right) \quad (2)$$

where t is the reaction time (min), T is the temperature ($^{\circ}\text{C}$), 100 is the reference temperature, and 14.75 is the fitted value of the arbitrary constant.

The ratio of lignin loss to cellulose loss during pretreatment was calculated to determine the delignification selectivity (DS) (Fatriasari *et al.*, 2014; Solihat *et al.*, 2017). The component loss was calculated following Eq. (3).

$$\text{Component loss (\%)} = \frac{RW - (CC \times PR)}{RW} \times 100 \quad (3)$$

where RW is the chemical composition of lignin, cellulose, or hemicellulose in the raw material (%), CC is chemical composition of lignin, cellulose, or hemicellulose in the pulp (%), and PR is the pulp recovery after pretreatment (%)

2.4. Enzymatic saccharification

The pulp of OPEFB was further hydrolyzed using CTEC 2 enzyme (Novozymes, Bagvaerd, Denmark) (enzyme

activity 160 FPU/mL) with enzyme loading 40 FPU/g substrate. The enzymatic hydrolysis was conducted in a 100 mL Erlenmeyer flask, each containing 0.3 g (ODW) of OPEFB pulp, and 15 mL of 0.05 M citrate buffer, pH 4.8, 200 μL of 2% sodium azide, and 80.1 μL of enzyme. Eventually, 0.05 M citrate buffer was added to obtain total final weight of 30 g. After this step, the flasks were tightly closed. The flasks were then incubated at 50 $^{\circ}\text{C}$ for 72 h in an incubator shaker (Wisecube, WIS-30R) at 150 rpm. Enzymatic saccharification was performed in duplicate. At the end of saccharification the hydrolysate in the flask was heated in boiling water bath for 5 min to inactivate the enzyme, then it was cool down and put in a refrigerator before further used and analysis.

2.5. Sugars and inhibitors analysis

Sugars (xylose and glucose) and by-products, such as 5-HMF, acetic acid, and furfural content in the hydrolysates of pretreatment and sugars obtained from enzymatic saccharification were analyzed using High Performance Liquid Chromatography (HPLC) (Shimadzu, Japan). All the sample solutions were centrifuged, then they were filtered through a 0.45 μm Millipore membrane. As much as 20 μL of sample was injected into HPLC apparatus which was equipped with Coregel 87H3 column and a Refractive Index Detector RID-10A (Shimadzu, Japan). A solution of H_2SO_4 (5 mM) was used as the mobile phase with a flow rate of 0.6 mL/min, and the column oven temperature was maintained at 80 $^{\circ}\text{C}$. Running time of sample was 40 min. The results were calculated by internal normalization of the chromatographic peak area. Sugars and other by-products analyzed were identified by comparing the relative retention times of sample peaks with standards.

2.6. Determination of crystallinity index

The crystallinity of raw OPEFB and OPEFB pulp was measured by XRD-7000 MaximaX (Shimadzu, Japan). The dried samples were scanned in 2θ range from 10 $^{\circ}$ to 90 $^{\circ}$ using Cu radiation generated at 30 kV and 30 mA. The

crystallinity index (CrI) of samples was calculated according to the XRD peak height method, shown in Eq. (4) (Yu & Wu, 2010)

$$\text{CrI (\%)} = \frac{I_{\text{crystalline}} - I_{\text{amorphous}}}{I_{\text{crystalline}}} \times 100 \quad (4)$$

where $I_{\text{crystalline}}$ is the intensity of crystalline regions ($2\theta = 22^\circ\text{-}23^\circ$) and $I_{\text{amorphous}}$ means intensity of amorphous regions ($2\theta = 18^\circ\text{-}19^\circ$)

2.7. Morphological characteristics

Scanning electron microscopy (SEM) image was taken for both raw and pulp samples of OPEFB. Dried samples were gold coated and observed under SEM-EDX JEOL JSM-6510LA (JEOL Ltd, Japan). SEM images of samples were recorded at 500 magnification using an accelerating voltage of 10 kV and work of distance (WD) of 10 mm.

2.8. Functional groups observation

Attenuated Total Reflectance (ATR)-Fourier Transform Infra-Red (FTIR) Analysis (Spectrum two-Perkin Elmer-USA) was used to characterize the functional groups of raw OPEFB and OPEFB pulp. About 0.1 mg of OPEFB sample was placed on a diamond plate and then IR spectra were recorded in absorption mode with a scan count of 4 per sample and a resolution of 4.0 cm^{-1} in the wave number range of 4000 to 400 cm^{-1} at room temperature using spectrum two Perkin Elmer software.

3. Results and Discussions

3.1. Optimization of temperature operation during steam pretreatment

In order to assess the efficiency and selectivity of each pretreatment condition, the pulp recovery was determined and the chemical composition of OPEFB pulp was

compared with that of the raw OPEFB as presented in Table 1. Increase of reaction temperatures from 160 to 200 °C causes decrease of pulp recovery from 89.70 to 60.70% (Table 1), which was due to the removal or solubilization of some chemical components of biomass during pretreatment. Similar with other pretreatment methods, steam pretreatment can also generate low molecular weight compounds such as pentose and hexose sugars, organic acids (acetic, levulinic, and formic acid), furan aldehydes (5-HMF and furfural) from the degradation of polysaccharides and phenolic compounds from the degradation of lignin contained in the biomass through auto-hydrolysis process (Jeong & Lee, 2015). The auto-hydrolysis was catalyzed by hydronium ions generated from auto-ionization of water and by acetic acid generated by hydrolysis of hemicellulose (Girio *et al.*, 2010). Some of the degraded products were solubilized in the water that were used to generate steam in the reactor, while the insolubilized parts remained in the solid fraction that contributed to the pulp recovery. The three major components of OPEFB (cellulose, hemicellulose and lignin) were solubilized and contributed to the pulp recovery after pretreatment, Hemicellulose and lignin contents in the biomass decreased steadily from 23.35 to 17.55% and from 20.64 to 19.17%, respectively, with the increase of temperature. On the other hand, cellulose content increased from 49.83 to 54.44% with the increase of temperature. The increase of cellulose in the biomass composition was due to the marked loss of hemicellulose and lignin from the biomass. Nevertheless, all the three components had been solubilized during the pretreatment, which was shown by the loss of the three components during pretreatment (Table 2).

This result shows that temperature give more effect on auto-hydrolysis of hemicellulose and lignin than that on cellulose. Cellulose has a strong crystalline structure, while hemicellulose has a random, amorphous, and branched structure that makes it more susceptible to hydrolysis than cellulose (Taherzadeh & Karimi, 2008).

Table 1

The effect of heating temperature during steam pretreatment of oil palm empty fruit bunch with reaction time of 10 min on pulp recovery and chemical composition of biomass

T (°C)	Pulp recovery (%)	Chemical composition (%)				
		Ash	Extractives	AIL*	Hemicellulose	α -celullose
Raw	-	2.41 ± 0.02	4.32 ± 0.25	23.85 ± 0.10	21.53 ± 0.83	46.48 ± 0.76
160	89.70 ± 0.06	0.78 ± 0.01	4.14 ± 0.44	20.64 ± 0.50	23.35 ± 0.44	49.83 ± 0.62
170	83.62 ± 1.63	0.69 ± 0.01	4.21 ± 0.05	21.84 ± 0.35	20.77 ± 0.16	52.97 ± 0.06
180	74.29 ± 3.78	0.52 ± 0.02	6.45 ± 0.25	20.96 ± 0.74	21.06 ± 0.39	53.12 ± 0.11
190	67.29 ± 0.67	0.53 ± 0.02	8.22 ± 1.29	19.53 ± 0.03	17.67 ± 0.16	55.22 ± 1.66
200	60.70 ± 0.05	0.56 ± 0.02	9.57 ± 0.21	19.17 ± 0.28	17.55 ± 0.44	54.44 ± 0.42

*AIL: Acid insoluble lignin

Table 2

The effect of heating temperature during steam pretreatment of oil palm empty fruit bunch with reaction time of 10 min on pH, chemical losses, and delignification selectivity

T (°C)	pH	Component losses (%)			Delignification selectivity (%)
		AIL*	Hemicellulose	α -celullose	
160	6.53 ± 0.02	22.36 ± 4.97	2.70 ± 1.77	3.84 ± 0.66	5.82
170	6.21 ± 0.61	23.41 ± 2.72	19.35 ± 0.95	4.70 ± 1.96	4.98
180	5.54 ± 0.02	34.77 ± 1.03	27.31 ± 5.03	15.11 ± 4.14	2.30
190	4.55 ± 0.24	45.88 ± 0.46	44.78 ± 0.04	20.04 ± 3.20	2.24
200	4.40 ± 0.02	51.20 ± 0.67	50.51 ± 1.21	28.90 ± 0.61	1.77

*AIL: Acid insoluble lignin

Similar results regarding the losses of hemicellulose and lignin after hydrothermal pretreatment were observed on other biomass, such as *Tamarix ramosissima* (Xiao *et al.*, 2013) and oil palm (Zakaria *et al.*, 2015) which only had partial degradation instead of complete removal of hemicellulose component by hydrothermal pretreatment. However, several studies reported that most of the hemicellulose could be removed by auto-hydrolysis pretreatment which are conducted by Lee *et al.* (2009), Medina *et al.* (2016), and Aguilar-Reynosa *et al.* (2017) from coastal bermuda, OPEFB, and corn residue, respectively. This might be due to the difference in treatment severities and the types of biomass used by different researchers. The more severe conditions (higher temperature operation and/or longer reaction time) caused higher removal of hemicellulose. Each type of biomass has different hemicellulose content and different lignin carbohydrate complex binding. The lower the hemicellulose content and the looser lignin carbohydrate complex binding, the higher the hemicellulose could be removed from the biomass.

The pH profile during auto-hydrolysis indicates severity of steam pretreatment and related to the solubility of the hemicelluloses into the liquid phase, in turn, allows for the recovery of carbohydrates as soluble mono and oligo-sugars (Ertas *et al.*, 2014). During steam pretreatment, pH condition should be maintained around 4 to minimize the production of sugar degradation products. Table 2 shows the effect of auto-hydrolysis conditions on acidity of the filtrate during pretreatment at 160 – 200 °C for 10 min. The pH of the filtrate after auto-hydrolysis at 170 to 200°C decreases significantly from 6.25 to 4.34. The decrease of pH in liquid phase is due to auto-hydrolysis at higher temperature and longer reaction time decomposing hemicelluloses and cellulose, which induced the formation of organic acids, such as acetic, levulinic, and formic acid, by degradation and dehydration. Acetic acid is generated by hydrolysis of the acetyl groups on hemicelluloses and commonly is observed with the release of xylose. The levulinic acid can be produced from glucose as the final products, during hydrothermal treatment. Formic acid is a by-product, in the production of levulinic acid from glucose. The concentration of levulinic acid and formic acid are increased, with increasing reaction temperature and time. These organic acids will help to decrease pH, during hydrothermal treatment (Jeong & Lee, 2015).

Besides the structural component of OPEFB, non-structural component such as extractives was also dissolved and facilitated in improving the enzyme accessibility on carbohydrate (Fatriasari *et al.*, 2018). The higher reaction temperature, the higher extractives content in OPEFB pulp (Table 2). Some fragments of lignin and polysaccharides with lower molecular weights were re-precipitated on the pulp samples which referred to as pseudo-extractives. These compounds have similar structures and behaviours with extractives (de Carvalho *et al.*, 2015). This phenomenon was also reported in acid pretreatment of OPEFB (Solihat *et al.*, 2017), and hydrothermal pretreatment of eucalyptus (de Carvalho *et al.*, 2015). The number of inorganic compounds in OPEFB is presented as ash content. Generally, increasing reaction temperature tends to reduce the ash content in OPEFB and there is a significant decrease in ash content compared to raw OPEFB (Table 1). A decrease in ash content is due to solubilization of some minerals contained

in the biomass to the hydrolysate when the biomass components are degraded during the high temperature and pressure of pretreatment.

Delignification selectivity (DS) is one of the most influential factor to observe the effectiveness of delignification process during steam pretreatment (Romani *et al.*, 2011). An effective pretreatment is indicated from the ability to remove lignin and preserve cellulose during pretreatment. Therefore, higher DS values indicate more effective pretreatment process while a low DS value means a relatively high loss of cellulose occurred during pretreatment compared to lignin (Fatriasari *et al.*, 2014; Solihat *et al.*, 2017). Table 2 also shows that the higher the temperature, the lower the DS value. It means that more severe pretreatment tends to remove lignin and cellulose simultaneously. The similar effect can also be observed in hemicellulose loss. The increase of temperature has more effects on hemicellulose than on lignin and cellulose. Compared to cellulose, hemicellulose has lower molecular weight with branched structure of β -1,4 glycosidic chains thus breaking down process of hemicellulose occurs easier.

Even though the highest DS value from pretreatment was obtained at 160°C, the highest glucose yield was achieved at 180°C during saccharification process (Fig 2). It is well known that lignin can provide barrier for enzyme performance to access carbohydrate. However, its removal is not a single factor that affects glucose production during saccharification. The removal of hemicellulose and lignin (Table 2) improves the cellulose accessibility to cellulase enzymes during enzymatic saccharification.

Fig. 2 showed the effect of temperature on glucose and xylose yield based on initial cellulose, hemicellulose, and biomass at reaction time of 10 min. At the lowest reaction temperature (160°C), the glucose yield was 61.46% per initial cellulose or 33.85% per initial biomass while the xylose yield was 65.14% per initial hemicellulose or 17.85% per initial biomass. The glucose yield reaches the maximum (94.31% per initial cellulose or 54.56% per initial biomass) up to temperature of 180 °C, after that the yield tends to decrease. However, the highest xylose yield (83.79% per initial hemicellulose or 19.88% per initial biomass) was obtained at 170°C.

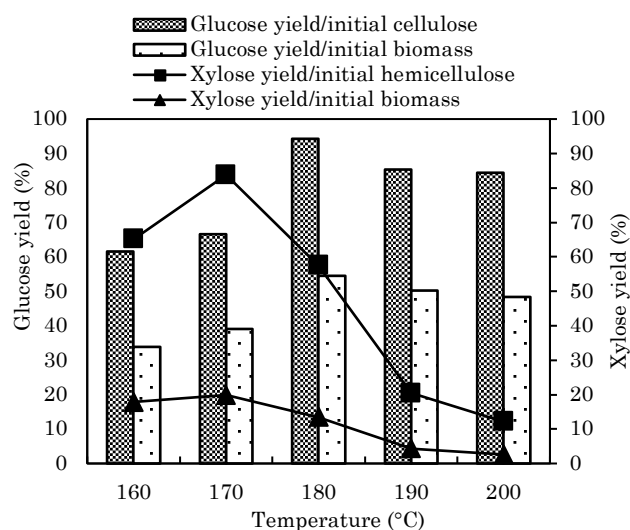


Fig. 2 Glucose and xylose yields after enzymatic saccharification under variation of reaction temperature in steam pretreatment of oil palm empty fruit bunch

Table 3

The effect of reaction time during steam pretreatment of OPEFB at 180 °C on pulp recovery and chemical composition of the OPEFB pulp

Reaction time (min)	Pulp recovery (%)	Chemical composition (%)				
		Ash	Extractive	AIL	Hemicellulose	α -cellulose
10	74.29 \pm 3.78	0.52 \pm 0.02	6.45 \pm 0.25	20.96 \pm 0.74	21.06 \pm 0.39	53.12 \pm 0.11
20	76.24 \pm 0.99	0.58 \pm 0.00	5.47 \pm 0.04	20.37 \pm 0.04	19.55 \pm 0.44	54.77 \pm 0.28
30	68.07 \pm 0.42	0.51 \pm 0.00	6.00 \pm 0.27	19.30 \pm 0.03	18.34 \pm 0.11	57.90 \pm 0.07
40	68.47 \pm 0.57	0.58 \pm 0.03	6.41 \pm 0.08	19.48 \pm 0.82	18.37 \pm 0.67	56.51 \pm 0.05
50	64.42 \pm 1.21	0.42 \pm 0.16	6.85 \pm 0.28	20.11 \pm 0.88	17.56 \pm 0.39	56.09 \pm 0.11

*AIL: Acid insoluble lignin

Table 4

The effect of reaction time during steam pretreatment of OPEFB at 180 °C on pH hydrolysate, chemical losses, and delignification selectivity

Reaction time (min)	pH	Chemical losses (%)			Delignification Selectivity (%)
		AIL	Hemicellulose	α -cellulose	
10	5.54 \pm 0.02	34.77 \pm 1.03	27.31 \pm 5.03	15.11 \pm 4.14	2.30
20	4.50 \pm 0.06	34.90 \pm 0.98	30.75 \pm 2.45	10.17 \pm 0.70	3.43
30	4.26 \pm 0.22	44.92 \pm 0.44	42.02 \pm 0.71	15.21 \pm 0.42	2.95
40	4.17 \pm 0.10	44.07 \pm 2.82	41.57 \pm 2.61	16.76 \pm 0.76	2.63
50	4.02 \pm 0.13	45.67 \pm 3.39	47.44 \pm 2.14	22.27 \pm 1.61	2.05

*AIL: Acid insoluble lignin

Increasing reaction temperature from 180 to 200°C decreases glucose yield from 94.31 to 84.35% per initial cellulose. It can be caused by hemicellulose and cellulose loss at higher reaction temperature as presented in Table 2. Based on the result of glucose yield from this enzymatic saccharification, we continued the next step of the optimization experiment at 180 °C.

The glucose yields obtained after enzymatic hydrolysis of OPEFB pretreated at 180, 190, and 200 °C were higher than the theoretical yields calculated based on the amount of glucose that could be generated from cellulose in the initial OPEFB. This is probably due to the hydrolysis of some glucans in hemicellulose remained in the OPEFB pulp. Based on the thermal stability, cellulose and glucan-based hemicelluloses are the most thermal stable chains, followed by xyloglucan, arabinoxylan, arabinogalactan, galactomannan, glucomannan and finally xylan as the least stable (Werner *et al.*, 2014). The less thermal stable hemicellulose chains are easier to be hydrolyzed during pretreatment, and they are solubilized in the liquid fraction, while the more thermal stable hemicellulose chains, such as xyloglucans, are still remained in the OPEFB pulp. These glucans were probably hydrolyzed during enzymatic hydrolysis, and the glucose resulted from these glucans contribute to its yields.

3.2. Optimization of reaction time during steam pretreatment

Data on the effects of reaction time during steam pretreatment on chemical composition and pulp recovery of OPEFB are presented in Table 3. A longer reaction time decreased the pulp recovery from 74.29 to 64.42%. It can be understood, because the longer reaction time caused more degradation and solubilization of chemical components in the OPEFB. However, increase of reaction time has less effects than does increase of reaction temperature on the decrease of pulp recovery. Hemicellulose and lignin contents tend to decrease with

increase of reaction time, while the cellulose content is vice versa. The chemical component was calculated as percentage of each component in the biomass. Therefore, the decrease of one component will increase the percentage of the others. The cellulose content increases with increases of reaction time from 10 until 30 min (53.12 to 57.90%). However, it decreases up to 56.09% at more severe conditions or longer heating times. This implied that partial cellulose degradation begins to take place at longer reaction time. These results were in agreement with another report (Shamsudin *et al.*, 2012), which showed that the hemicellulose and lignin content of OPEFB pulp were gradually decreased with increased of reaction time from 15 to 60 min. On the other hand, cellulose content of OPEFB was increased after the steam pretreatment at 140°C for 15, 30, and 45 min, and was decreased when the reaction time was prolonged to 60 min. The degradation of hemicellulose and lignin was possibly beneficial to the subsequent enzymatic hydrolysis of the cellulose-rich biomass, because the hemicellulose and lignin could provide physical barriers during cellulose hydrolysis and prevent access of the enzymes to the cellulose surface (Jeong & Lee, 2015). The effects of reaction time on extractives and ash content are similar with those of reaction temperature. The extractives tend to increase with increase of reaction time, while ash content is vice versa. The increase of extractives was probably due to re-precipitation of lower molecular weight of fragments on the surface of the biomass pulp that dissolved in the organic solvents used in the analysis of extractive content (de Carvalho *et al.*, 2015).

Chemical losses from OPEFB pretreated at 180°C with variation of reaction time are presented in Table 4. Compared to the effect of temperature, reaction time of steam pretreatment has less effect on chemicals losses during pretreatment. The hemicellulose and lignin losses increase from 27.31 to 47.44% and from 34.77 to 45.67%, respectively confirming the extensive removal caused by pretreatment. There is also some cellulose degradation

during the pretreatment at more severe condition (22.27%), which decrease the potential sugars recovery from the enzymatic saccharification of the OPEFB pulp.

The high cellulose loss can result in a smaller source of carbohydrates that can be converted into sugars (Fatriasari *et al.*, 2018). However, compared to Table 2, it shows that cellulose loss is significantly affected by higher temperature rather than by longer reaction time. A similar trend was also reported by Goh *et al.* (2012). It can be concluded that temperature has a more positive and direct effect on biomass components removal and degradation compared to reaction time. The acidity of the filtrate became higher with an increase of the reaction time from 5.45 to 4.02 (Table 4). However, increasing reaction time above 30 min did not increase the acidity. As a result, the hemicellulose loss after the pretreatment for 30-50 min did not significantly decrease as well (Table 4). The DS reach maximum in reaction time of 20 min. It means that pretreatment condition at temperature of 180 °C for 20 min is more selective in preserving cellulose compared to degradation of lignin.

Some factors that influence on enzymatic hydrolysis of biomass are cellulose crystallinity and swelling, lignin content and its decomposition products, and the complex shield of hemicellulose and lignin that cover cellulose (Kim *et al.*, 2013). After pretreatment at 180 °C for 20 min, more than 89.83% of the cellulose can be recovered in the solid phase while the other 10.17% might be appeared as cello-oligosaccharides and glucose in the liquid phase (Table 4). Glucose and xylose yield from enzymatic saccharification were increased with increasing of reaction time until 20 min. The highest glucose (97.30% per initial cellulose or 59.43% per initial biomass) and xylose (88.86% per initial hemicellulose or 19.43% per initial biomass) yields are obtained at 180°C for 20 min (Fig 3). Increasing reaction time above 20 min, decreased the glucose yield to 59.10% per initial cellulose and xylose to 45.02% per initial hemicellulose.

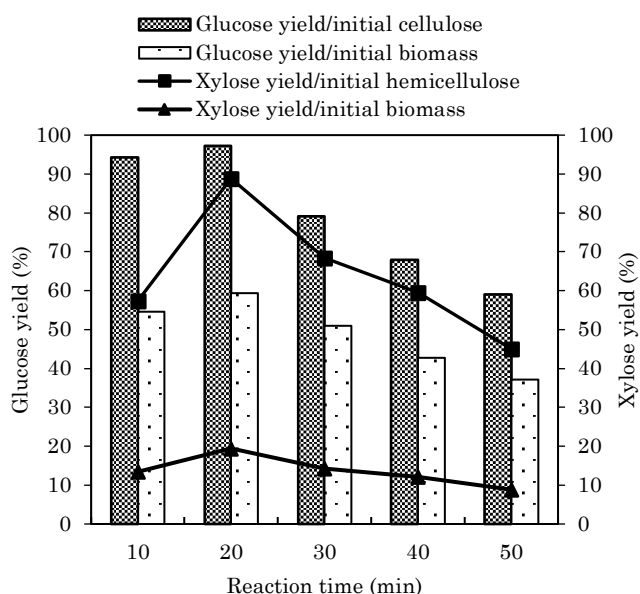


Fig. 3 Glucose and xylose yields after enzymatic saccharification under variation of reaction time in steam pretreatment of oil palm empty fruit bunch

Based on these results, the reaction time that selected as the optimum condition is 20 min. The lignin and hemicellulose removal affect this tendency. It means that prolonging reaction time in steam pretreatment is not required. During pretreatment, lignin is often modified and resulted in demethylation and solubilization (formation of simple/oligomeric phenolics). These phenolics are the most important inhibitor to enzymatic saccharification, being formed at inhibitor levels when biomass pretreatments are carried out at high solid level (Tejirian & Xu, 2011). Besides that, these phenolic acids are redeposited on the surface of the pulp acting as a barrier for enzymatic saccharification (Anderson *et al.*, 2007). Hodge *et al.*, (2008) reported that the primary causes of inhibition of the enzyme action are the soluble compounds such as acetic acid and phenolic compounds.

3.3. The effect of severity factor (SF) during steam pretreatment on by products in the hydrolysates and sugar yields after enzymatic saccharification

In this study we have tried to correlate the SF with the generation of by-products and with the results of enzymatic saccharification of OPEFB. SF combines the effects of the reaction temperature and time to compare them with the auto-hydrolysis processing of the materials (Hodge *et al.*, 2008). SF become an approach to observe changes in the composition of the pulp of biomass thus the different conditions of the pretreatment could be compared more easily (Fatriasari *et al.*, 2018). Fig 4a shows that the depolymerization of hemicellulose was increased with increase of SF, shown by the increase of concentration of sugars, especially xylose, in the hydrolysates. At low SF (2.8) glucose and xylose concentrations were not detected. They were started to be detected at SF about 3.5. A tremendous increase of xylose was occurred when the SF was increased from 3.8 to around 4. Methods working close to neutral conditions such as steam pretreatment solubilize most of the hemicellulose due to hydronium ions and the acids released from the hemicellulose. In this study, glucose and xylose concentrations in the hydrolysates were quite low, the highest were 0.26 and 0.82 g/L, respectively. Since the total volume of the hydrolysate was 3L, the total amount of glucose and xylose in the hydrolysates were approximately 0.78 and 2.46 g, respectively. These amounts were much lower than the amount of hemicellulose loss from the biomass (50% losses or about 10.75 g) due to its solubilization in the hydrolysate. Thus, it could be suggested that major amount of hemicellulose was still in the form of its oligomers. These results were in agreement with previous studies that showed that steam pretreatment did not result in total conversion to monomer sugars, because hemicelluloses are depolymerized and converted into soluble oligomers, which is the major reaction products (Galbe & Zacchi, 2007; Lee *et al.*, 2009).

Beside oligosaccharides, sugars and aldehydes, the liquid fraction has compounds such as soluble lignin, furfural, HMF, levulinic acid, and formic acid (Aguilar-reynosa *et al.*, 2017). Except formic acid, which was detected at much lower SF, the degradation products of carbohydrates were started to be detected at SF above 3.5 (Fig 4b).

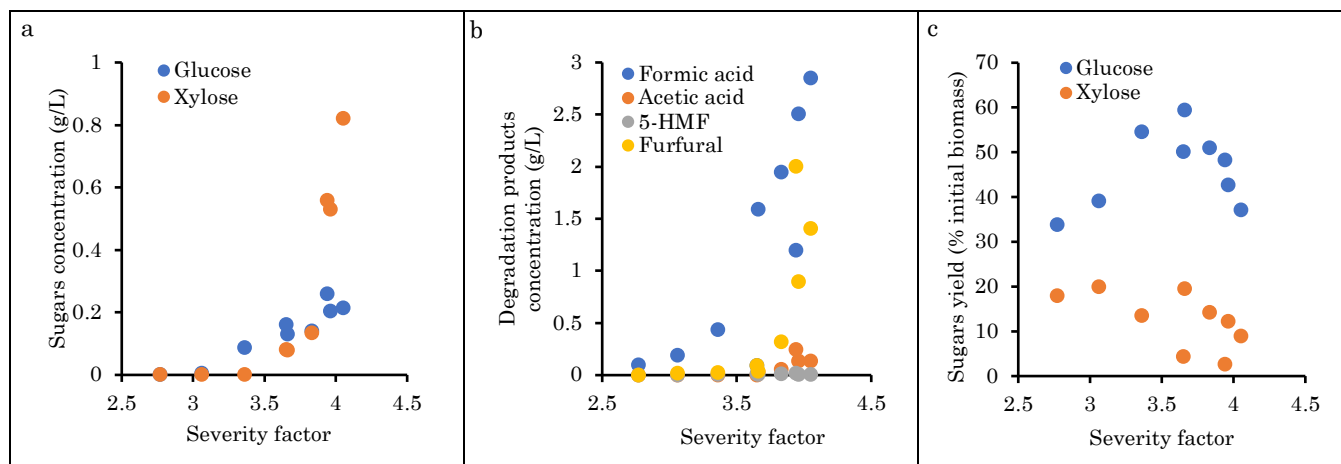


Fig. 4 Relationship between severity factor and by-products in the hydrolysates (4a and 4b) and sugars obtained after enzymatic saccharification (4c) of pulp of oil palm empty fruit bunch

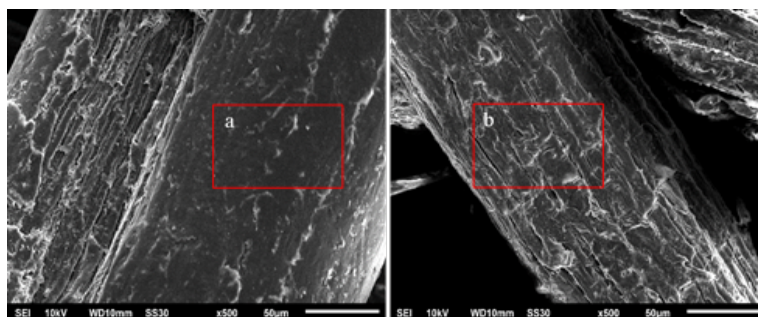


Fig. 5 Scanning electron microscopic view of (a) raw and (b) pulp oil palm empty fruit bunch (180°C for 20 min)

These suggested that some sugar monomers in the hydrolysates started to degrade to lower molecular weight compounds at SF above 3.5. A relatively small part of the glucan has already been converted to monomeric glucose during the pretreatment. This indicated that further degradation of sugars has occurred by its long-duration exposure to high reaction temperature which influence higher concentration of by product in the liquid fraction.

Some furans by products of conversion of sugars, such as furfural and 5-HMF, resulted from degradation of pentoses and hexoses, respectively were observed in the liquid fraction under severe conditions. There was very little concentration of 5-HMF found in the hydrolysate, while furfural was found in higher concentration (2 g/L) as the severity increases up to about 4 (Fig. 4b). It can be occurred due to the different degradation rate between glucose to 5-HMF and xylose to furfural. Xylose is more easily degraded to furfural, than glucose is to 5-HMF, during hydrothermal treatment (Jeong & Lee, 2015). Acetic acid was detected and generated from the release of the acetyl groups present in the hemicelluloses. Acetic acid presence in the liquor increases to 0.13 g/L at the highest severity (SF 4.05). These by products as mentioned above may have inhibitory effects on the growth of ethanologenic species and further might result in a decline of ethanol productivity (Anita *et al.*, 2019; Hsu *et al.*, 2010). However, furfural concentration obtained from all pretreatment conditions in this study was below 3 g/L; therefore, the possible antagonistic behaviour to cell growth has not

generated (Ethajib *et al.*, 2015). Similar tendencies of increasing degradation products due to increase of SF in the range of 3.06-4.24 were previously reported during hydrothermal pretreatment of oil palm frond (Zakaria *et al.*, 2015). However, hydrothermal pretreatment of OPEFB at higher SF (4.54-5.72) revealed different tendencies of degradation products concentrations due to increase of SF. At this range only acetic acid concentration was increased, while the others are decreased (Zakaria *et al.*, 2015). Fig 4c shows glucose and xylose yields obtained after enzymatic saccharification of OPEFB pretreated at different SF. Xylose yield was not increased greatly up to SF of 3.36, then it was decreased at higher SF. Glucose yield was increased from 33.85% at SF of 2.77 up to 59.43% at SF about 3.4-3.7, then they were decreased at higher SF. The optimum condition obtained is in agreement with the result of optimization using the OFAT method, discussed in the previous section. This suggests that SF could be used as an alternative parameter to determine optimum condition of steam pretreatment for enzymatic saccharification of OPEFB. However, the use of SF for determination of optimum condition of steam or liquid hot water pretreatment for other biomass should be further elucidated, because for biomass such as corn stover, pretreatment temperature has higher influence than does SF (Zhou *et al.*, 2010). The scale of the experiment could also contribute to the SF value that results in optimum condition. Several studies reported the optimum condition using steam pretreatment of OPEFB

in laboratory scale. The temperature and reaction time at optimum conditions in those studies are vary, but the SF is similar, which is about 3.54 (Anderson *et al.*, 2007; Tejirian & Xu, 2011; Medina *et al.*, 2016). This value is slightly lower than the SF that results in the highest glucose yield in our study, which is 3.66. This is probably related to the thermal efficiency of the process. This study used much bigger reactor with more biomass than those used in previous reports by other researchers. Larger scale pretreatment as in this study could affect the thermal efficiency of the pretreatment due to more heat loss during the process.

3.4. Morphology, crystallinity and functional groups of raw OPEFB and OPEFB pulp

SEM analysis was conducted to determine the morphological feature changes and surface characteristic of the OPEFB. SEM images of OPEFB before and after steam pretreatment at the optimum conditions are shown in Fig. 5. Comparison of SEM images shows a significant change to the biomass structure after being subjected through the steam pretreatment. The surface of raw OPEFB (Fig. 5a) is smooth and has a compact structure with no visible pores in the structure (red box). After steam pretreatment, the surface of OPEFB pulp (Fig 5b) shows many disruptions with more defined and opened structure within the fiber structure as signed by red boxes. Pretreatment can also increase the surface area of OPEFB fiber.

Disruption of OPEFB fiber structures are due to several reasons, such as pretreatment and enzyme hydrolysis, to which the fibers had been subjected during preliminary handling (pretreatment) and during hydrolysis process. It can also be affected by removal of hemicellulose and lignin during pretreatment (Table 2 and 4). It facilitates the accessibility of enzyme in the fiber structure, and furthermore will enhance glucose production during enzymatic hydrolysis (Solihat *et al.*, 2017).

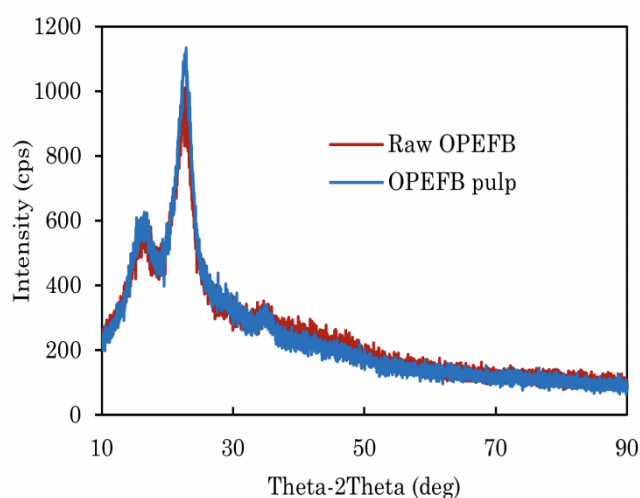


Fig. 6 X-ray diffraction spectra of raw OPEFB and OPEFB pulp (180 °C for 20 min)

The X-ray diffraction (XRD) analysis and the crystallinity index determination were carried out in the raw and OPEFB pulp to analyze their crystalline structure changes. The crystallinity index of raw OPEFB (67.12%) was lower than that of OPEFB pulp (70.02%) (Fig. 6). It can also be observed in the diffractogram in which the intensity of OPEFB pulp in the crystalline regions ($2\theta = 22^\circ\text{-}23^\circ$) increases, while the diffraction intensity of the amorphous region of OPEFB pulp ($2\theta = 18^\circ\text{-}19^\circ$) decreases. The removal of amorphous fractions such as lignin and hemicellulose caused this increase of crystallinity. Hemicelluloses and lignin have amorphous and branched structures, while cellulose has a strong crystalline structure. A major part of cellulose (around 2/3 of the total cellulose) is in the crystalline form. The increase of the crystallinity of biomass pulp is caused by the loss of the amorphous region (hemicellulose, lignin, and some cellulose) of the biomass during pretreatment (Hermiati *et al.*, 2020). Therefore, crystallinity index can reflect the degradation of amorphous fractions in lignocellulosic biomass during pretreatment (Zhuang *et al.*, 2016). The increase of crystallinity index of OPEFB after pretreatment was also reported in previous studies of Fatriasari *et al.*, (2018) and Hermiati *et al.*, (2020).

Crystallinity of cellulose is one of the factors influencing enzymatic hydrolysis besides lignin and hemicellulose contents and their distribution, porosity, and particle size (Pu *et al.*, 2013; Kuila, 2011). In auto-hydrolysis process, temperature has significant impact on the amorphous and crystalline cellulose degradation. The minimum temperature required to breakdown the glycosidic bonds in the chain segments within the amorphous portion of cellulose appeared to be approximately 150 °C, whereas for the crystalline portion of cellulose was 180 °C which glucose monomer starts to be produced. This difference in the hydrolysis behaviour between amorphous and crystalline cellulose was attributed to the ultra-structural differences in the amorphous and crystalline portions of cellulose (Yu & Wu, 2010). The more amorphous regime loses, make cellulase easier to penetrate to cellulose and hydrolyze the cellulose to glucose. OPEFB crystalline structure inhibits the enzymatic hydrolysis of cellulose, so that the enzyme needs some time to hydrolyze all the cellulose. The crystallinity index of cellulose plays a major role in determining the rate of the enzymatic hydrolysis reaction. A fully amorphous sample will be hydrolyzed much faster than partially crystalline cellulose. It can be concluded that amorphous domains in a partially crystalline cellulose sample are hydrolyzed first, leaving crystalline parts to be hydrolyzed at the end (Hall *et al.*, 2010; Yoshida *et al.*, 2008).

The changes of the functional groups in the OPEFB were analyzed with FTIR-ATR method. The result is presented in Fig. 7. The spectrogram pattern of the raw OPEFB and OPEFB pulp was almost the same, but the transmittance of OPEFB pulp was stronger than that of raw OPEFB. Based on the FTIR analysis, the pretreatment did not result in the formation of new functional groups but only changed the intensity of the absorption bands and causes band shifts. The identified functional groups and their sources are summarized in Table 5.

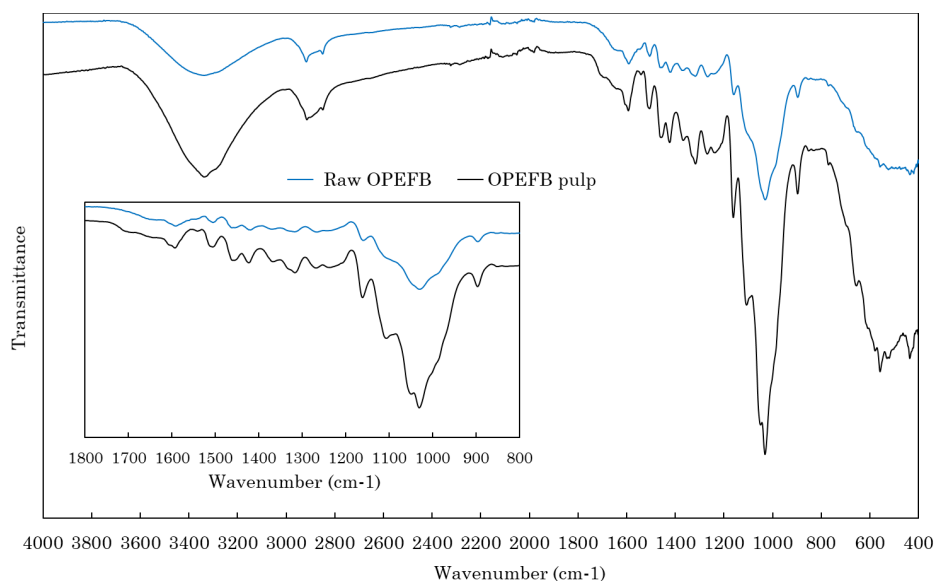


Fig. 7 FTIR spectra of raw OPEFB and OPEFB pulp (180 °C for 20 min)

Table 5 Assignments of characteristic peaks in FTIR spectrogram

Wavenumber (cm ⁻¹)	Assignments of characteristic peaks
3330	O–H stretching of the hydrogen bonds of cellulose
2917	C–H stretching within the methylene of cellulose
2849	Asymmetric stretching vibration of CH ₂
1593	Absorbed O-H and conjugated C=O
1506	Aromatic skeletal vibration
1456	C-H deformation in lignin and carbohydrates
1422	Aromatic skeletal vibrations combined with C–H in plane deformation
1316	O-H bending of alcohol group in the cellulose
1266	Guaiacyl ring stretching vibration
1161	C-O-C vibration
1105	Aromatic skeletal and C-O stretching
1048	C–O stretching in cellulose and hemicellulose
1031	Plane deformations of aromatic C-H, C-O deformations in primary alcohols, and C=O stretching vibrations
897	C–O–C stretching at β-glycosidic linkages in cellulose and hemicellulose

Pretreatment of the OPEFB resulted in an increase of the band at 3330 cm⁻¹ and the band at 2917 cm⁻¹. This implied that most of the crystalline cellulose in the OPEFB was disrupted by auto-hydrolysis reaction at the pretreatment temperature of 180°C (Hsu *et al.*, 2010). The band in the region of 2849 cm⁻¹ was only found in the raw OPEFB. It assigned to asymmetric stretching vibration of CH₂ in the CH₂-OH group of cellulose. The band at 2849 cm⁻¹ in the pulp OPEFB shows that the peak decrease, indicating some ruptures in the methyl and methylene of cellulose molecules (Qing *et al.*, 2015). It was correlated with the cellulose loss in the pulp sample at 180°C for 20 min (Table 4). The band at 1316 cm⁻¹ in the pulp of OPEFB, indicating O-H bending of alcohol groups in the cellulose, was stronger than that in the raw OPEFB due to the breakdown of ester bond linkages between lignin and carbohydrate, thus making the cellulose more exposed (Solihat *et al.*, 2017).

As compared to the raw OPEFB, lignin band at 1506 cm⁻¹ (aromatic skeletal vibration of lignin) is significantly enhanced in the OPEFB pulp. This phenomenon can be

reasonably explained by hemicelluloses removal or lignin release and re-deposited on the surface of the fiber (Xiao *et al.*, 2011). Thus, the features of lignin are distinguished and remained in the solid residue. Therefore, the increase in temperature made these peaks sharper. This observation also proves that the structure of the lignin is hardly disrupted by auto-hydrolysis process.

The small band at 897 cm⁻¹ representing amorphous cellulose was observed in raw OPEFB and OPEFB pulp, showing one of the differences of crystallinity between raw material and biomass pulp. OPEFB pulp showed increase of amorphous cellulose peak intensity when the pretreatment temperature achieved 180°C. It indicated that more amorphous cellulose was achieved by hydrothermal pretreatment. Another band around 1,105 cm⁻¹ responsible for crystalline cellulose was observed in OPEFB pulp. Stronger peaks at around 1,105 cm⁻¹ were generated after pretreatment, suggesting an increasing proportion of crystalline cellulose in biomass pulp due to the removal of amorphous cellulose compared with raw biomass. The steam pretreatment increases the

crystallinity of cellulose which is consistent with the crystallinity profiles shown in Fig 6. The wide band at around $1,031\text{ cm}^{-1}$ diverged into two small peaks at $1,031\text{ cm}^{-1}$ and $1,048\text{ cm}^{-1}$ after pretreatment. These bands can be assigned to different bonds vibration coming from lignin, cellulose, and hemicellulose. Regarding the bands at $1,161\text{ cm}^{-1}$, OPEFB pulp shows stronger peaks than raw OPEFB suggesting a more significant compositional change of cellulose and hemicellulose in OPEFB pulp.

6. Conclusion

This study showed that steam pretreatment using a high temperature-pressure reactor was a promising pretreatment method to enhance the enzymatic saccharification of biomass, such as OPEFB. This bench scale study also shows that SF could be used as an alternative parameter in the determination of the optimum condition of steam pretreatment for enzymatic saccharification of OPEFB. The steam pretreatment causes some disruptions with more defined and opened structure within the fiber structure and dissolved the hemicellulose and lignin. It facilitates the enzyme to penetrate the biomass more easily, and eventually improved the saccharification of cellulose to sugars. Using this steam pretreatment, we successfully remove 34.9% of lignin and 30.75% of hemicellulose from the OPEFB and yielded 59.43% of glucose based on initial biomass, which is equal to the conversion of 97.30% cellulose in the initial biomass. The degradation products (furfural and 5-HMF, formic acid, and acetic acid) in the hydrolysate are observed under very severe conditions. The results of this bench scale experiment hopefully can be used for conducting pilot-scale verification tests, although it has some limitations. The reactor in this experiment can not be adjusted to the desired pressure. The steam pressure is affected by and correlated with the specified pretreatment temperature used in this study. Further investigation is needed to test the feasibility and practicability of steam pretreatment for the full utilization of OPEFB in terms of the material and energy balances.

Declarations

Authors contribution

Fahriya Puspita Sari: Conceptualization, methodology, data analysis, data curation, writing – original draft preparation, review & editing, project leader. Faizatul Falah: data curation. Sita Heris Anita: Methodology, validation. Kharisma Panji Ramadhan: Methodology, data curation. Widya Fatriasari: Methodology, writing original draft-reviewing, editing, validation, supervision. Euis Hermiati: Methodology, validation, writing-original draft preparation-reviewing, editing, supervision.

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