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DELIGNIFICATION OF OIL PALM EMPTY FRUIT BUNCH USING PERACETIC ACID AND ALKALINE PEROXIDE COMBINED WITH THE ULTRASOUND

Heri Hermansyah[1*], Dwini Normayulisa Putri[1], Andiko Prasetyanto[1], Zhofran Bintang Chairuddin[1], Meka Saima Perdani[1], Muhamad Sahlan[1], Masafumi Yohda[2]

[1]*Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia*
[2]*Department of Biotechnology and Life Sciences, Faculty of Engineering, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo 184-8588, Japan*

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ABSTRACT

Lignocellulosic biomass has great potential as a low-cost source of fermentable sugar for the production of biofuels and high value organic acids. One potential biomass is oil palm empty fruit bunch, since it has high cellulose and hemicellulose content. However, its lignin content can hinder the access of cellulose and hemicellulose during the hydrolysis process. Therefore, an effective pretreatment for the delignification of lignocellulose biomass should be considered to reduce the lignin content. In this study, delignification of oil palm empty fruit bunch using peracetic acid and alkaline peroxide solution combined with the ultrasound method is investigated as a novel combination method of biomass pretreatment. The effect of pretreatment time was observed by using a peracetic acid solution for 1, 3, 5, 7 and 9 hours, followed by an alkaline peroxide solution for 4, 6, 8 and 10 hours. Based on the results, the best delignification was achieved by pretreatment using peracetic acid pretreatment for 3 hours, followed by alkaline peroxide pretreatment for 10 hours. Both pretreatments were assisted by the ultrasound method. The results show hemicellulose, cellulose and lignin content of 14.13%, 77.27% and 8.6% respectively. The lignin content was reduced by 68.73% and the cellulose content increased by 121.85%, relative to the untreated EFB. This result was considered as the best pretreatment, since the pretreatment time was shorter and high cellulose content together with low lignin content was achieved, which will improve the hydrolysis process.

Keywords: Delignification; Pretreatment; Oil palm empty fruit bunch; Ultrasound

1. INTRODUCTION

Lignocellulosic biomass has great potential as a low price raw material for the production of biofuels and high value products, such as bioethanol, enzyme and organic acid (Hermansyah et al., 2015; Hermansyah et al., 2018). One potential lignocellulose biomass is oil palm empty fruit bunch (EFB), since annually it is widely generated worldwide, especially in Indonesia, but unfortunately it has limited uses, only as an organic fertilizer or boiler fuel to generate electricity (Palamae et al., 2014).

Typically, EFB is composed of 24–65% cellulose, 21–34% hemicellulose, and 14–31% lignin (Chang, 2014). Based on its composition, EFB can potentially be used as a raw material for biofuels and organic acid production by utilizing the cellulose and hemicellulose content.

[*]Corresponding author's email: heri@che.ui.ac.id, Tel. +62-813-85399048, Fax. +62-21-7863515
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Hemicellulose and cellulose can be hydrolyzed into simple sugars for fermentation into biofuels and other products via microbial processes (Pattanamanee et al., 2012; Kim & Kim, 2013; Sklavounos et al., 2013).

Fortunately, EFB is a great source of cellulose and hemicellulose, since the lignin content can be removed, while the loss of hemicellulose and cellulose is kept to a minimum. Therefore, effective pretreatment for the delignification of EFB should be considered in order to improve the removal of lignin content.

In recent years, there have been many studies on the pretreatment of lignocellulosic biomass for the delignification process, either chemically or physically. Chemical pretreatment has been widely reported as a potential method for delignification, including acid and alkaline pretreatments (Mosier et al., 2005). In acid treatment, the bonds between cellulose, hemicellulose and lignin are broken down by H+ ions. The main objective of acid treatment is to solubilize the hemicellulose fraction of the biomass (Alvira et al., 2010) and change the structure of the lignin (Mosier et al., 2005) by solubilizing the acid soluble lignin. Otherwise, the main objective of alkali treatments is to remove nearly all the lignin and some of the hemicellulose. This treatment will have a great affect on the enzymatic hydrolysis of cellulose to sugars (Taherzadeh & Karimi, 2008). Furthermore, in some chemical treatments, the agents mentioned above are combined with oxidizing agents such as hydrogen peroxide and sodium hypochlorite water (Nazir et al., 2013) to assist the delignification and depolymerization processes.

Several researchers have succeeded in achieving the delignification of EFB by using chemical treatment. It has been delignified simultaneously and consecutively by using NaOH and H_2O_2, with lignin removal of 72% and 99% (Misson et al., 2009). Another chemical treatment has been attempted using peracetic acid solution. In this approach, approximately 53% of the lignin was removed, but nearly all the hemicellulose was retained (Palamae et al., 2014). On the other hand, a recent study has employed a sequential treatment using peracetic acid (PA) solution and alkaline peroxide (AP) solution, with around 92% of the lignin content from the EFB fiber removed (Palamae et al., 2017).

Besides using chemical treatment, physical treatment has also been studied to improve the results of lignocellulose biomass pretreatment. Previously, EFB has been successfully pretreated by using a sequential dilute acid and microwave alkali pretreatment, resulting in high delignification and a large amount of cellulose (Akhtar & Idris, 2017). In addition, ultrasound-assisted dilute aqueous ammonia pretreatment has also been investigated for the intensification of enzyme hydrolysis for corn cob, corn stover and sorghum stalk. It has been found that a combination of ultrasonic pretreatment can increase the accessibility of cellulose in the biomass and increase the enzymatic hydrolysis sugar yield (Xu et al., 2017). However, the combination of ultrasound and chemical pretreatment for EFB is still limited. Therefore, this study will modify the pretreatment method conducted by previous researcher which only using PA and AP solutions (Palamae et al., 2017). The aim of this study is to observe the performance of the delignification of EFB by employing a combination of chemical and physical treatments using PA and AP solutions and assisted by the ultrasound method. The study will focus on the time arrangement of the pretreatment in order to obtain the best delignification process.

2. METHODOLOGY

2.1. Materials

The EFB fiber was purchased from a local supplier (Palembang, Indonesia). Prior to use, it was washed and sun-dried and then dried in an oven at 70°C for 24 hours. The samples were then

ground and sieved using a sieve with a mesh number of 30 and 0.6 mm aperture before being stored in a container at room temperature.

The following chemicals were used: glacial acetic acid 100%, sodium hydroxide, and hydrochloric acid 37%, purchased from Merck KGaA (Darmstadt, Germany); sulfuric acid and acetone, purchased from Mallinckrodt (Phillipsburg, NJ, USA); and hydrogen peroxide 35%, purchased from PT. Wiloso Yasa Pratama (Jakarta, Indonesia).

2.2. EFB Pretreatment

The pretreatment process to perform the EFB delignification consisted of peracetic acid and alkaline peroxide pretreatment; both pretreatments were assisted by the ultrasound method. A schematic diagram of the pretreatment process is shown in Figure 1.

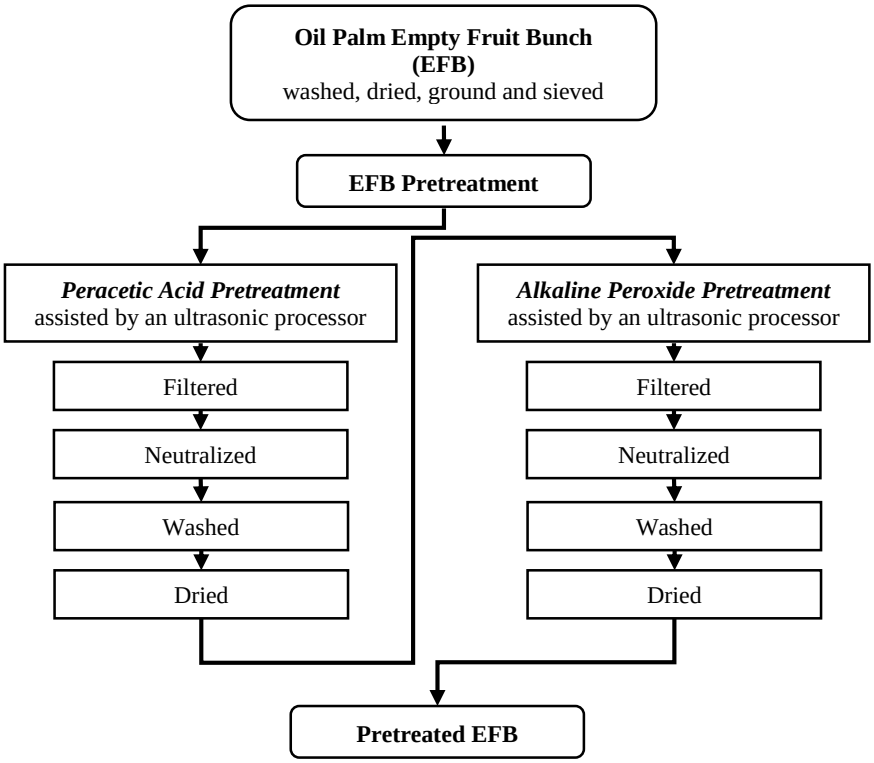


Figure 1 Schematic diagram of pretreatment process

2.2.1. Peracetic acid pretreatment

The PA solution was prepared by mixing glacial acetic acid with 30% w/w of hydrogen peroxide, at volumes of 600 mL and 400 mL respectively, and adding 15 mL of sulfuric acid as a catalyst. The solution was prepared at a room temperature of 35 ± 3°C and mixed for 72 h (Palamae et al., 2014). Delignification of the EFB fiber was conducted in a 2 L Duran glass bottle held in an ultrasonic processor (53 kHz, 90W). 75 g of the fiber was mixed with 1500 mL PA solution (20 mL of PA solution per gram of EFB fiber) and placed inside the ultrasonic processor set to 35°C, for 1, 3, 5, 7 and 9 hours. The resulting slurry was filtered through a muslin cloth. The pretreated EFB was then washed with distilled water, neutralized with 6 M NaOH, and then further washed with distilled water. The solids obtained were dried in an oven at 80°C for 48 h. The PA-pretreated solids, referred to as "delignified EFB fiber", were stored at room temperature.

2.2.2. Alkaline peroxide pretreatment

AP pretreatment was conducted in a 500 mL Duran glass bottle inside a 35°C ultrasonic processor (53 kHz, 90W). 10 g of delignified EFB fiber was mixed with 174 mL of sodium hydroxide (40% w/w) and 26 mL of hydrogen peroxide (35% w/w). The temperature was controlled at 35°C for 4, 6, 8 and 10 hours. The resulting slurry was then filtered through a

muslin cloth. Pretreated EFB was washed with distilled water, neutralized to a final pH of 5.5–6.0 with 6 M hydrochloric acid, and washed again with distilled water. The final EFB solids fraction was dried in an oven at 80°C for 48 h.

2.3. EFB Analysis

The content of the EFB fiber was determined by measuring the acetone extractable material, hemicellulose content, and acid insoluble lignin contents. The methods used were based on National Renewable Energy Laboratory (NREL), version 08-03-2012.

To measure the acetone extractives, 5 gr of EFB fiber (W_0) was extracted using acetone (80 mL) in a Soxhlet extractor. The extraction was performed at 90°C for 1 h; subsequently, fresh acetone was used to wash the EFB fibers for 30 min, which were then air dried at room temperature. The fibers were then oven dried at 105°C to a constant weight. The dried EFB fibers were then cooled in a desiccator and weighed (W_1). The acetone extractable contents E (%) of the EFB fibers were calculated using the Equation 1 (Li et al., 2004):

$$E\,(\%) = \frac{W_0 - W_1}{W_0} \times 100\,\% \tag{1}$$

In order to measure the hemicellulose, 0.3 g acetone extracted dry sample (W_2) was put into a 15 ml test tube and 3 ml of NaOH (0.5 mol/L) added. The mixture was then heated at 80°C for 3.5 h. Subsequently, the mixture was cooled to room temperature and filtered under vacuum. The solid residue was washed with distilled water until a pH level of 7 was reached. The residue was then oven dried at 105°C to a constant weight, and the, dried residue cooled in a desiccator and weighed (W_3). The hemicellulose content H (%) of the EFB fibers was calculated using Equation 2:

$$H\,(\%) = \frac{W_2 - W_3}{W_2} \times 100\,\% \tag{2}$$

To determine the acid insoluble lignin content, 0.2 g acetone extracted dry sample (W_4) was placed in a Erlenmeyer flask, and sulfuric acid (200 ml, 720 g/L) then poured gently into the flask. The flask was then reacted at 30°C for 1 h. Next, 56 mL of distilled water was added to the flask and then autoclaved at 121°C for 1 h. After cooling, the residues were filtered using Whatman no. 1 filter paper and washed with distilled water. The washed residue was then oven dried to a constant weight at 105°C, and the dried residue cooled in a desiccator and weighed (W_5). The contents of acid insoluble lignin L (%) in the original sample were calculated using Equation 3:

$$L\,(\%) = \frac{W_5}{W_4} \times 100\,\% \tag{3}$$

As acetone extractives, hemicellulose, acid insoluble lignin and cellulose are the only components found in EFB fiber (Di Blasi et al., 1999; Lin et al., 2010; Menon & Rao, 2012), the cellulose content C (%) was generally calculated from the other measured values, as follows:

$$C\,(\%) = 100 - H\,(\%) - L\,(\%) \tag{4}$$

On the other hand, the EFB morphology was analyzed using a transmission electron microscope (TEM) for untreated and pretreated EFB.

2.4. Calculation

The percentages of lignin removal, the decrease in hemicellulose and increase in cellulose were calculated using Equations 5–7:

$$Lignin\ removal\ (\%) = \frac{L\ in\ E_1 - L\ in\ E_{1+i}}{L\ in\ E_1} \times 100 \quad (5)$$

$$Decrease\ in\ hemicellulose\ (\%) = \frac{H\ in\ E_1 - H\ in\ E_{1+i}}{H\ in\ E_1} \times 100 \quad (6)$$

$$Increase\ in\ cellulose\ (\%) = \frac{C\ in\ E_{1+i} - C\ in\ E_1}{C\ in\ E_1} \times 100 \quad (7)$$

3. RESULTS AND DISCUSSION

3.1. Untreated EFB Content

Prior to the pretreatment, the EFB content was analyzed in order to measure the composition of cellulose, hemicellulose and lignin. Based on the results of the content analysis, as seen in Table 1, the untreated EFB contained 37.67% hemicellulose, 34.83% cellulose and 27.5% lignin. These levels are comparable with the general range of EFB composition, which are 24–65% cellulose, 21–34% hemicellulose and 14–31% lignin (Chang, 2014). According to the results, the cellulose and lignin content is in line with values previously reported, while the hemicellulose content is 3.67% higher (Chang, 2014).

3.2. Pretreated EFB Content

After PA pretreatment, followed by AP pretreatment assisted by ultrasound, the EFB content was again analyzed, the results of which are presented in Table 1.

Table 1 Pretreated EFB content

Sample	Pretreatment Condition	Composition (% w/w)			Lignin Removal	Decrease in Hemicellulose	Increase in Cellulose
		H	C	L	(%)	(%)	(%)
E1	None	37.67	34.83	27.50			
E2	PA (35°C, 1 h) AP (35°C, 4 h)	37.53	43.07	19.40	29.45%	0.37%	23.66%
E3	PA (35°C, 3 h) AP (35°C, 4 h)	27.07	53.73	19.20	30.18%	28.14%	54.26%
E4	PA (35°C, 5 h) AP (35°C, 4 h)	29.33	62.82	7.85	71.45%	22.14%	80.36%
E5	PA (35°C, 7 h) AP (35°C, 4 h)	6.20	71.70	22.10	19.64%	83.54%	105.86%
E6	PA (35°C, 9 h) AP (35°C, 4 h)	7.50	71.45	21.05	23.45%	80.09%	105.14%
E7	PA (35°C, 1 h) AP (35°C, 6 h)	23.83	59.92	16.25	40.91%	36.74%	72.04%
E8	PA (35°C, 3 h) AP (35°C, 6 h)	23.20	62.45	14.35	47.82%	38.41%	79.30%
E9	PA (35°C, 5 h) AP (35°C, 6 h)	19.10	65.00	15.90	42.18%	49.30%	86.62%
E10	PA (35°C, 7 h) AP (35°C, 6 h)	7.33	72.77	19.90	27.64%	80.54%	108.93%
E11	PA (35°C, 9 h) AP (35°C, 6 h)	9.83	74.77	15.40	44.00%	73.90%	114.67%
E12	PA (35°C, 1 h) AP (35°C, 8 h)	15.60	61.50	22.90	16.73%	58.59%	76.57%
E13	PA (35°C, 3 h) AP (35°C, 8 h)	22.33	65.47	12.20	55.64%	40.72%	87.97%
E14	PA (35°C, 5 h) AP (35°C, 8 h)	18.97	69.28	11.75	57.27%	49.64%	98.91%
E15	PA (35°C, 7 h) AP (35°C, 8 h)	18.60	65.80	15.60	43.27%	50.62%	88.92%
E16	PA (35°C, 9 h) AP (35°C, 8 h)	27.00	61.75	11.25	59.09%	28.32%	77.29%
E17	PA (35°C, 1 h) AP (35°C, 10 h)	17.07	62.88	20.05	27.09%	54.69%	80.53%
E18	PA (35°C, 3 h) AP (35°C, 10 h)	14.13	77.27	8.60	68.73%	62.49%	121.85%
E19	PA (35°C, 5 h) AP (35°C, 10 h)	4.00	77.60	18.40	33.09%	89.38%	122.80%
E20	PA (35°C, 7 h) AP (35°C, 10 h)	14.93	69.77	15.30	44.36%	60.37%	100.32%
E21	PA (35°C, 9 h) AP (35°C, 10 h)	25.13	64.47	10.40	62.18%	33.29%	85.10%

E1 - Untreated EFB; E2-21 - Pretreated EFB with different PA and AP pretreatment times, assisted by the ultrasonic processor (53 kHz, 90 W)

Based on these results, after pretreatment the lignin and hemicellulose content decreased in all the samples, whereas the cellulose content increased. The decrease in lignin content varied between 27.09% (sample E2) and 68.73% (sample E3), relative to the composition of untreated EFB. The same trend was observed in the hemicellulose content, which decreased between 33.29% (sample E6) and 89.38% (sample E4), again relative to the composition of untreated

EFB. On the other hand, the increase in cellulose content varied between 80.53% (sample E2) and 122.80% (sample E4).

These results mean that the modified method proposed in the study has successfully delignified the EFB, as proven by the reduction in lignin content and the increase in cellulose content. This is because pretreatment by the PA solution is highly selective in removing lignin, through several mechanisms including hydroxylation of aromatic rings, oxidative demethylation, oxidative ring opening, and epoxidation (Sundara, 1998; Song et al., 2013). In addition, pretreatment by AP solution plays a role in removing hemicellulose (Palamae et al., 2014). Moreover, the ultrasound-assisted method facilitates the disintegration and disruption of the EFB structure, meaning a larger surface area is exposed by the solutions (Xu et al., 2017). The results of a previous study also proved that the combination of chemical and mechanical/ physical methods was able to improve the performance of biomass pretreatment and also enzymatic hydrolysis (Aditiya et al., 2015).

3.3. Effect of Pretreatment Time on EFB Content

In the study, the effect of pretreatment time on EFB content was determined. A previous study by Palamae et al. (2017) established that the optimum conditions for EFB delignification were PA pretreatment for 9 hours at 35°C, followed by 12 hours of treatment with AP at 20°C, which achieved 92.02% lignin content removal. However, the pretreatment time in the study is relatively long and determination of the optimum conditions related to the pretreatment time have not been made. Therefore, in this study the effect of PA pretreatment time on EFB content, especially to the removal of lignin, will be considered.

PA pretreatment time varied between 1, 3, 5, 7 and 9 hours, while AP pretreatment time varied between 4, 6, 8 and 10 hours. In order to enhance the EFB delignification process, PA and AP pretreatment were combined with physical treatment using ultrasound. The results of EFB content after the sequential PA-AP pretreatment assisted with ultrasound are presented in Figure 2.

The results in Figure 2(d) show that after 3 hours of PA pretreatment, followed by 10 hours of AP pretreatment (E18), the hemicellulose, cellulose and lignin content were 14.13 %, 77.27 % and 8.6 % respectively. These are considered to be the best conditions for the delignification process, according to the criteria of high cellulose and hemicellulose content, and low lignin content. This is because the low lignin and high cellulose content will be preferable for achieving high sugar concentration in the enzymatic hydrolysis process, since the existence of lignin will prevent the enzymes from accessing cellulose and hemicellulose (Hendriks & Zeeman, 2009). Moreover, the overall pretreatment time is also reduced when compared to the previous study by Palamae et al. (2017).

According to the results, a reduction in pretreatment time has been achieved compared to previous research, with PA pretreatment for 9 hours, followed by AP pretreatment for 12 hours, yielding 2.8% lignin content (Palamae et al., 2017). This is because the combination of PA and AP pretreatment with ultrasound can help the destruction of the cell wall structure (Kumakura & Kaetsu, 1983). In this way, the delignification process can be improved. Besides, ultrasonic pretreatment will improve the specific surface area, reduce the degree of polymerization, and increase the biodegradability of lignocellulosic biomass (Mason & Peters, 1991). Therefore, the pretreatment time can be reduced with the assistance of ultrasound.

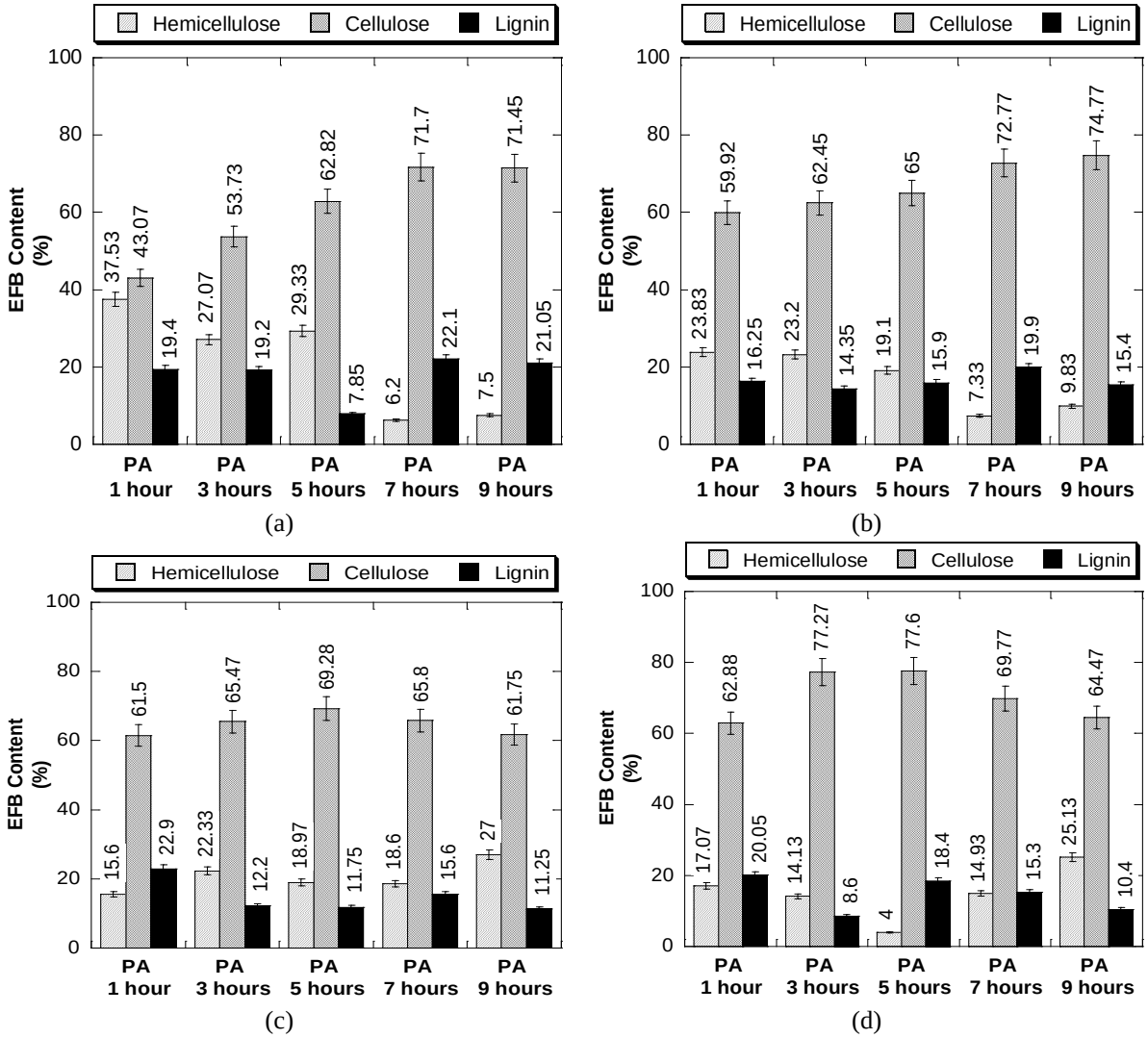


Figure 2 Effect of pretreatment time on EFB content: (a) 4 hours; (b) 6 hours; (c) 8 hours; (d) 10 hours of AP pretreatment

3.4. Comparison of Results from the Previous Study

A comparison of the results from the previous study by Palamae et al. (2017) is presented in the Table 2.

Table 2 Comparison of untreated and pretreated EFB content

Pretreatment Condition	Composition (% w/w)			Ref.
	Hemicellulose	Cellulose	Lignin	
None	36.60	28.30	35.10	(Palamae et al., 2017)
PA (35°C, 9 h) AP (20°C, 12 h)*	11.20	81.90	2.80	(Palamae et al., 2017)
None	37.67	34.83	27.50	This study
PA (35°C, 3 h) AP (35°C, 10 h)**	14.13	77.27	8.60	This study

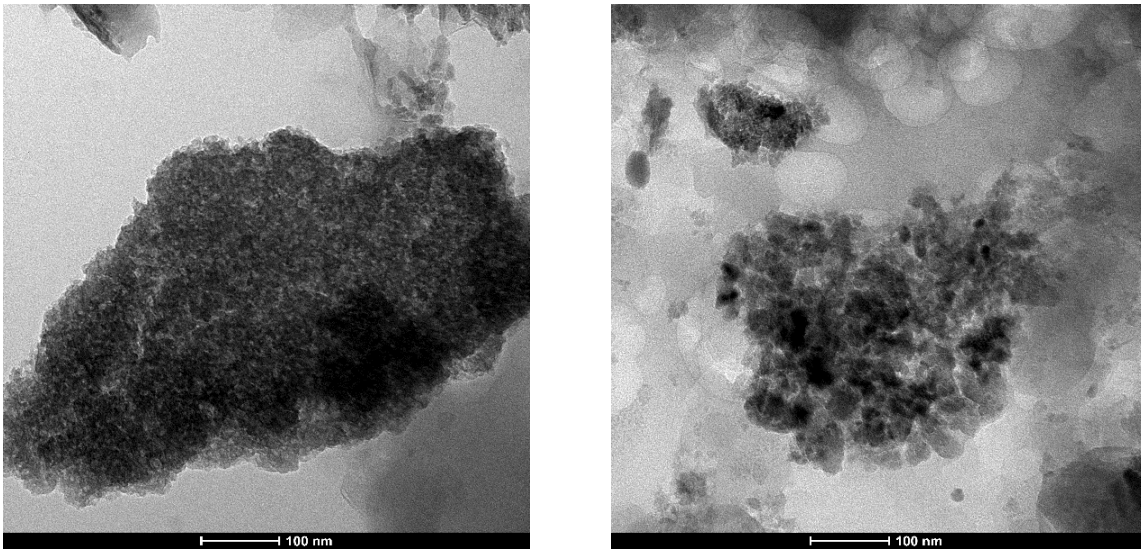
*without ultrasound-assistance; **with ultrasound-assistance

Based on the table, the results of this study show 68.73% lignin removal compared to the composition of untreated EFB. On the other hand, the cellulose content is 77.27%, which is an increase of 121.85% relative to the composition of untreated EFB; however, the loss of hemicellulose is 62.49%. Compared with the previous study, after the sequential PA and AP pretreatment, the lignin content removed was 92.02%, while the cellulose content increased by

189.4% and that of hemicellulose decreased by 69.39%, again relative to the composition of untreated EFB (Palamae et al., 2017).

3.5. Morphology Analysis

To ensure a thorough experiment, TEM analysis was conducted to further understand the effect of pretreatment on the morphology of EFB. The morphology of untreated and pretreated EFB is shown in Figure 3.



(a) (b)

Figure 3 Morphology of: (a) untreated EFB; (b) pretreated EFB (E18)

Based on the results of the TEM imaging, there is a visual difference between the untreated and pretreated EFB (E18: 3 hours PA pretreatment and 10 hours AP pretreatment). The reduced contrast of the cell walls by pretreatment is a result of lignin removal, while the decreased staining density indicates reduced lignin content in the cell wall following the pretreatment process. Lignin enriched regions are black to dark gray in color, while hemicellulose and cellulose enriched regions are lighter gray. The disruption of the biomass structure is caused by the ultrasound. This imaging observation result signifies the effect of ultrasonic assistance on the PA and AP pretreatment.

4. CONCLUSION

Delignification of EFB has been successfully achieved in this study. The best delignification was obtained by pretreatment using PA for 3 hours, followed by AP pretreatment for 10 hours and assisted by ultrasound. Hemicellulose, cellulose and lignin content of 14.13%, 77.27% and 8.6%, respectively was achieved. After pretreatment, lignin content was reduced by 68.37%, while that of cellulose increased by 121.82%, relative to the composition of untreated EFB. Besides, the overall pretreatment time was also reduced if compared to the previous study by Palamae et al. (2017).

5. ACKNOWLEDGEMENT

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