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Simultaneous Pretreatment Process on the Isolation of Cellulose Mycrocrystalline from Oil Palm Empty Fruit Bunches

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Abstract

This research was aimed to evaluate the isolation of cellulose derived from empty fruit bunches (EFB), especially to increase the purity of cellulose content by subsequent pretreatment process involving delignification using NaOH followed by bleaching process using hydrogen peroxide (H_2O_2). The result showed that the cellulose content of the raw EFB content at 37.6% ± 0.3 w/w increased to 89.2% ± 0.4 w/w after the simultaneous pretreatment process. Moreover, after the simultaneous of bleaching process using H_2O_2, the lignin content can be reduced up to 3.1% ± 0.1 w/w which similar to cellulose standard (2.7% ± w/w). The utilization of simultaneous pretreatment process can also reduce the hemicellulose content from 23.9% ± 0.3 w/w (raw EFB) to 7.7% ± 0.2 w/w even lower than the cellulose standard (13.1% w/w). FTIR spectrum showed that the peak absorption of cellulose increased significantly upon the simultaneous process of delignification-bleaching. Morphological changes of the raw EFB compared to pre-treated EFB as a note by SEM analysis showed the significant transformation from the larger size and tightly bundles of micro-fibril cellulose into smaller size and individually separated micro-fibrils. Further through the simultaneous process exhibited yield at 80-85%, high crystallinity (70-80%), and good thermal stability in terms of T_{onset} at about 230-290 °C. From these results, it can be concluded that the subsequent pretreatment process involving NaOH delignification and H_2O_2 bleaching processes more likely offer a high selectivity of cellulose microcrystalline from lignocellulosic biomass material by simultaneous reduction of the hemicellulose and lignin content without disrupting the structure of cellulose.

Keywords: *bleaching; cellulose; delignified; microcrystalline cellulose; oil palm empty fruit bunches*

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INTRODUCTION

Cellulose is the most abundant renewable organic material produced in nature. Cellulose can be obtained from various resources, such as plants, animals, bacteria, and algae (Amira *et al.,* 2013). Abundantly available cellulose mostly derived from lignocellulose resources. The lignocellulosic structure consist of three main structure which is cellulose, hemicellulose, and lignin. Cellulose is the main component of the cell wall that gives strength to plant (Zhao, Zhang, and Liu 2012). It consists of long carbon chains of polysaccharide (Dutra *et al.,* 2017). Hemicellulose is a branched polysaccharide containing sugar monomers with shorter chains and various conformations. It exists together with cellulose and lignin in the plant cell wall (Rezende *et al.,* 2011). On the other hand, lignin is a complex polymer consists of three phenylpropane units which are cross-linked together by alkyl-aryl, alkyl-alkyl, aryl-aryl ether bonds, and carbon-carbon bonds (Buranov and Mazza 2008), (Sills and Gossett 2012), (Shimizu *et al.,* 2012).

Very recently, cellulose fibers have been widely used in various fields, including as a thermal insulator (Septevani *et al.,* 2017), cosmetic and medical industries (Ramli *et al.,* 2015), transparent optical products (Xu 2016), (Zhang *et al.,* 2013), and various engineering products. For commercial purposes, the cellulose fibers are required to have high cellulose purity and crystallinity. To achieve high purity of cellulose derived from lignocellulose, a pretreatment is required to distort the lignocellulosic structure of the fiber and thus isolating cellulose from other components of lignin and hemicellulose. During pretreatment, lignin and hemicellulose will be removed from cellulose compound; and thus increase the crystallinity of cellulose; increase the porisity of the lignocellulosic materials as well as simultaneous surface modification (Zhao, Zhang, and Liu 2012), (Septevani *et al.,* 2017), (Sudiyani *et al.,* 2016). The pretreatment of lignocellulose can be classified into several categories; which are physical, chemical, biological or a combination of all these methods (Amira *et al.,* 2013), (Kumar *et al.,* 2009). Caustic or alkaline treatment using base aqueous solutions such as sodium hydroxide (NaOH) is one of the most used pretreatments of lignocellulosic material (Kumar *et al.,* 2009). NaOH helps in removing waxes, hemicellulose, pectin, and some lignin monomers (Xu 2016). This treatment disturbs the hydrogen bonding in cellulose and hemicellulose, breaking the ester linkages that crosslink lignin and xylan, and deprotonation of phenolic groups (Buranov and Mazza 2008).

To achieve the high purity of isolated cellulose, additional treatment after alkaline treatment is necessary. Ching *et al.,* (2014) have reported that alkali treatment was only be able to remove hemicellulose and lignin partially. Highly content of cellulose can only be obtained by further chemical treatment such as bleaching process. From the results , it is reported that the percentage of cellulose after bleaching process increased from 65 % (after alkaline process) to 80%. Bleaching process using chlorinated chemicals (chlorine and chlorine dioxide) can produce non-biodegradable chlorolignin as well as other toxic substances, although chlorine and its derivatives are effective and inexpensive bleaching agents (Roberto 2008). Because of the environmental issues, a more eco-friendly bleaching agent which are chlorine-free, is needed, including oxygen-containing oxidative chemicals (such as molecular oxygen, ozone, and hydrogen peroxide) (Dutra *et al.,* 2017). Among these chemicals hydrogen peroxide (H_2O_2) is known as a strong oxidizing agent that is environmentally friendly because hydrogen peroxide decomposes into hydrogen and oxygen and result in almost no residues (Bensah and Mensah 2013).

During the bleaching process, H_2O_2 decolorize the fiber by removing lignin, hemicellulose and surface impurities (Dutra *et al.,* 2017). H_2O_2 is always considered as a base even though it is a weak acid (pH ~6.2) and often is used as a bleaching agent in the pulp and paper industry. It is often being used on the pretreatment of various lignocellulose resources such as oil palm empty fruit bunch fiber (EFB) (Lani *et al.,* 2014), kenaf (Bast 2009), rice straw (Cheng *et al.,* 2010), banana (Abraham *et al.,* 2011), bagasse (Rezende *et al.,* 2011), and many others. Different treatments cause various effects to the properties in term of the degree of impurities and chemical composition, the thermal stability of the fibril (Zhao, Zhang, and Liu 2012).

EFB, solid waste from the palm oil industry, at which approximately 76.9 million tonnes of EFB is produced in 2012 causing serious environmental problems (Chemistry *et al.,* 2015). Although EFB has been intensively studied for decades those are still limited to give comprehensive studies about the structure-properties relationship on the obtained isolated cellulose from subsequent pretreatment process. Generally, EFB contain 45.0% cellulose, 32.8% hemicellulose and 20.5% lignin (Ramli *et al.,* 2015). In this study, a simultaneous pretreatment process including alkaline and hydrogen peroxide of EFB lignocellulose is conducted to achieve high selectivity of cellulose microcrystalline form by simultaneous reduction of the hemicellulose and lignin content without disrupting the structure of cellulose. Structure-properties relationship of the obtained isolated cellulose as a function of different types of treatment is thoroughly investigated in terms of morphological, chemical structure as well as crystallinity, and thermal properties. The mechanism of the subsequent pretreatment process is also well investigated.

MATERIALS AND METHOD

Materials

Oil Palm Empty Fruit Bunch (EFB) from PT Perkebunan Nusantara I (PT. PN 1), Medan, North

Sumatera. NaOH from Merck, MgO from Merck and H_2O_2 35% form technical grade chemicals.

Delignification with the chemical explosion method

The pretreatment process is begun by cutting, chopping, forming coarse powder from EFB at up to 30-40 mesh in size and drying with a maximum moisture content of 10% (w/w). Furthermore, delignification was carried out to remove the lignin content by adding 2.5% NaOH (w/w) solution to the stirred reactor at 150 °C and 4 bar pressure for 30 minutes. The treated EFB was then washed with water to a neutral pH to produce delignified EFB (D-eMC). Component analysis for cellulose, hemicellulose, and lignin were carried out before and after the delignification process to determine the effectiveness of the delignification process.

Bleaching

10 grams of delignified EFB, weighed and put into a glass beaker, then added 1 Liter of H_2O_2 and MgO 2% w/w. The mixture was then heated while stirring at 80 °C for 1 hour. The treated D-eMC cake is then filtered to separate from the filtrate and then washed with water until neutral (pH 7) to finally produce bleaching microcrystalline cellulose (B-eMC). The bleaching process is repeated for 1x, 2x, and 3x. The samples were then tested for cellulose, hemicellulose and lignin components. The results were characterized by various techniques, such as FTIR (Fourier Transform Infra-Red), SEM (Scanning Electron Microscopy, XRD (X-Ray Diffraction Technique), and TGA (Thermogravimetric Analysis).

Fourier Transform Infra-Red (FTIR) Spectroscopy

Fourier Transform Infra-Red (FTIR) spectroscopy studies were performed on an FTIR-Shimadzu type IR Prestige 21 to determine changes in functional groups that may have been caused by the treatments. Before the analysis, a mixture of 5.0 mg of dried fibers was ground and mixed with 200 mg of KBr. The results were analyzed in transmittance mode within range of 4000-500 cm^{-1}.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to observe the microstructure and the surface morphology of treated and untreated EFB. The instrument was a JEOL SEM with an acceleration voltage of 0.5 KV and magnification 200 times. The samples were coated with gold to provide about 200 Å gold layer thickness using a vacuum sputter coater.

X-Ray Scattering analysis

To investigate the crystallinity index of treated cellulose *X-ray scattering analysis was conducted.* The fibers (70 mg) were cut and pressed into a disk using a cylindrical steel mold (Ø= 1.3 cm). Ni-filtered CuKα radiation (l = 0.1542 nm) was generated at 40 kV and 35 mA using a PANalytical AERIS. The X-ray diffractograms were recorded form 5 to 60° 2^{θ} (Bragg angle).

Thermogravimetry

The thermogravimetric analysis was carried out on a LINSEIS STA PT 1600 under constant nitrogen flow (50 mL/min), from 25 to 800°C, at a heating rate of 10°C/min. Approximately 10 mg of each sample were used.

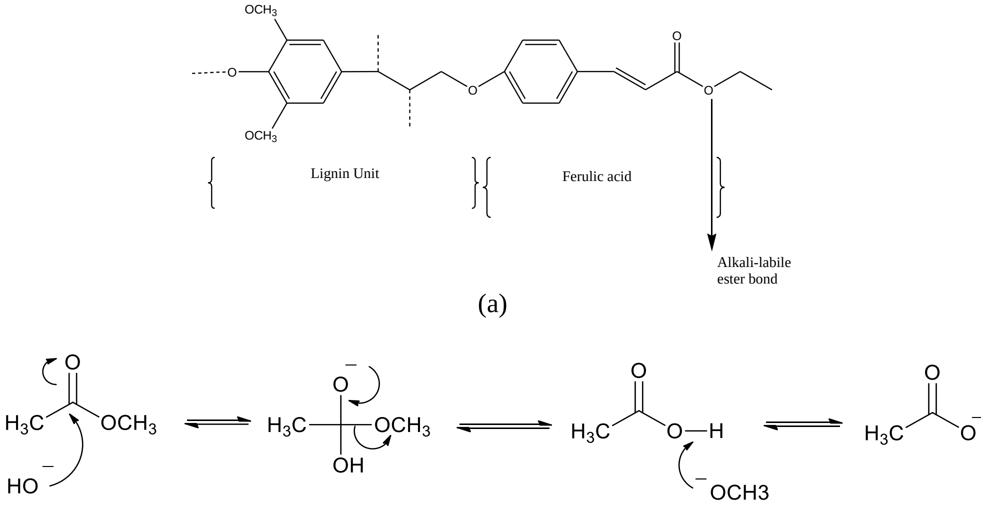
RESULTS AND DISCUSSION

Delignification with the chemical explosion method

The results of the delignification process indicate that improvement of cellulose purity from 37.6% ± 0.3 to 73.0% ± 0.2. This increase coincides with a decrease in lignin levels from 38.5% ± 0.0 to 12.1% ± 0.5, and hemicellulose from 23.9% ± 0.3 to 14.9% ± 0.2 (see Table 1). This pretreatment process using an alkaline solution (NaOH) can dissolve the components of lignin and some components of hemicellulose and increase accessibility on the lignocellulose surface so that it can increase the amount of cellulose content (Buranov and Mazza 2008).

The mechanism of sodium hydroxide pretreatment in EFB as shown in Figure 1. The ferulic acid as a linkage between the lignin and hemicellulose fractions is prone to the degradation reaction during NaOH pretreatment (Buranov and Mazza 2008) as shown in Figure 1 (a). The ester bond between the ferulic acid and the carbohydrate is easy to be degraded upon alkali reaction in the presence of hydroxide ion (dissociated from NaOH). Figure 1 (b) showed the mechanism of alkaline pretreatment, where the hydroxide ion attacks the carbon of ester bond (step 1), either between the lignin and carbohydrate or even between two lignin components or two carbohydrate components. Tetrahedral intermediate is formed (step 2) but it quickly collapses when a negatively charged oxygen atom expels an alkoxide ($-OCH_3$) from the carboxylic acid (step 3). In a very fast reaction, the resulting alkoxide acts as a base, deprotonating the carboxylic acid (step 4) (Buranov and Mazza 2008). The following result is the irreversible hydrolysis of the ester bond, weakening the structural integrity of the lignocellulose. Hemicellulose, a branched and rather irregular structure tends to be the most sensitive among the other three lignocellulose fractions to changes during pretreatment conditions (Abraham *et al.,* 2011), (Rezende *et al.,* 2011), (Alemdar and Sain 2008). In dilute alkaline pretreatment conditions, hemicellulose remains mostly intact with cellulose fraction, however, some studies have shown that hemicellulose can be solubilized in NaOH (Ching and Ng 2014). Also, the chemical changes during the alkaline pretreatment of lignocellulose include: (i) the saponification of the ester bonds that link hemicellulose to other lignocellulosic components;(ii) removal of acetyl and uronic acid substitutions on hemicellulose, and (iii) the formation of salts both in solution and incorporated into the lignocellulose (O

2009; Zhao, Zhang, and Liu 2012). Alkali treatment of the fiber will lead to the swelling of the fiber and subsequent increase in surface area and greater accessibility to the cellulose fraction, which increase of percentage crystallinity of the fiber (Abraham *et al.*, 2011).



(a)

(b)

Figure 1. Figure of the structural of lignin changes associated with alkaline pretreatment. Adapted from Buranov et.al (2008)

Bleaching with Hydrogen Peroxide

The basic principle in the process of manipulation and conversion of lignocellulose material into high-quality paper products is the selectivity of removal of lignin and hemicellulose without changing the structure of cellulose. Because lignin in the paper manufacturing process will reduce the brightness so that it must be removed optimally. While hemicellulose in high content will reduce the mechanical properties of the paper due to a decrease in resistance of individual fibers. The presence of hemicellulose also decreases optical properties (transparency) because of its opacity (Roberto 2008). The use of H_2O_2 in the bleaching process will increase the brightness and whiteness level of EFB. The physical differences in brightness and whiteness are shown in Table 2. Significant Decrease in the lignin content after the multistage bleaching process was observed from 12.1 % ± 0.5 in D-eMC into 3.1% ± 0.1 (after three repeated bleaching process) which was almost close to lignin content on the standard sample at 2.7%. This bleaching process not only lowered the lignin levels but also decreased the hemicellulose content to 7.7% ± 0.2, which was surprisingly, even lower than standard cellulose. Cellulose purity after repeating the bleaching process achieves up to 89.2% ± 0.4 even exceeding the cellulose standard (84.2%). This shows that the multi-stage H_2O_2 bleaching process is a promising method to simultaneously reduce the levels of hemicellulose and lignin and significantly increase the cellulose content. The cellulose yield from this treatment was 85.2% ± 0.2 (w/w) on a dry weight basis. The cellulose yield was higher than that reported studies by Ching *et al.*, (2014) at only approximately 58.5%. This simultaneous pretreatment using an alkaline-H_2O_2 solution, was more efficient than other methods because it could remove higher lignin content, while the other methods act mainly in the removal of hemicellulose (Ching and Ng 2014), (Dutra *et al.*, 2017).

Table 1. The result of the simultaneous pretreatment

Process	**Physical Appearance**	**Components Analysis** **Celullose (% w/w)**	**Hemicellulose (% w/w)**	**Lignin (% w/w)**
raw EFB		37.6 ± 0.3	23.9 ± 0.3	38.5± 0.0

Process	**Physical Appearance**	**Components Analysis**		
		Celullose (% w/w)	**Hemicellulose (% w/w)**	**Lignin (% w/w)**
D-eMC		73.0 ± 0.2	14.9 ± 0.2	12.1 ± 0.5
B-eMC 1x		79.2 ± 0.0	9.7 ± 0.1	11.1 ± 0.1
B-eMC 2x		81.5 ± 2.1	7.2 ± 0.0	11.3 ± 2.1
B-eMC 3x		89.2 ± 0.4	7.7 ± 0.2	3.1 ± 0.1
Standard Cellulose (alfa-cellulose from Sigma Aldrich)		84.2	13.1	2.7

Morphology of raw EFB, D-eMC, and B-eMC

The morphological changes of EFB fibers before and after pretreatment (delignification) were observed by Scanning Electron Microscope (SEM) (refer to Figure 2). Figure 2(a) shows fiber bundles consisting of microfibrils of raw EFB before pretreatment. Figure 2(b) is the D-eMC after alkaline pretreatment, where the fiber bundles are started to be separated into individual micro-sized fibrils caused by delignification (Alemdar and Sain 2008; Rezende *et al.*, 2011) because of the decreasing component of hemicellulose and lignin from EFB structure.

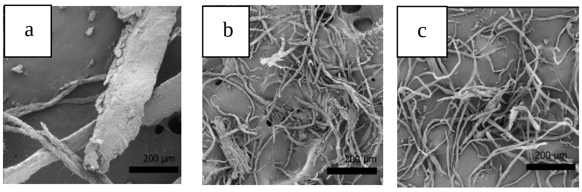


Figure 2. Scanning electron micrographs of the (a) raw EFB; (b) D-eMC; (c) B-eMC

Figure 2(c) shows the increasing number of individual microfibrils due to significant reduction of lignin and hemicellulose content after simultaneously alkaline and bleaching. This supports the previous statement that the bleaching process can further reduce lignin and hemicellulose compared to the alkaline pretreatment [22]. The average diamaters of the microcrystal after alkaline treatment (D-eMC), were 159 ± 60 µm in length, 14 ± 9.5 µm diameter, and 10 of aspect ratio. Furthermore, aftre the bleaching process, the dimension of N-eMC was 118 ± 51 µm in length, 7.7 ± 3.2 µm in diameter, and 15.2 aspect ratio, respectively. It is clear that simultaneous pretreatment resulted in the significant transformation from the larger size and tightly bundles of micro-fibril cellulose into smaller size and individually separated micro-fibrils. The mechanism of separation the lignocellulose biomass EFB can be described as an illustration in Figure 3.

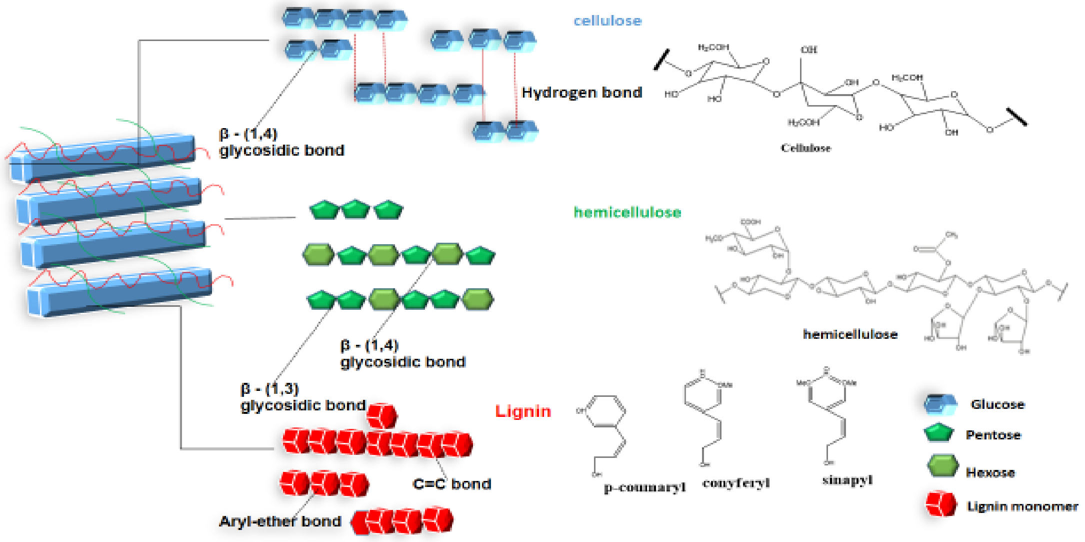


Figure 3. Illustration of separation the fibril bundles of lignocellulose from EFB

Chemical structure of raw EFB, D-eMC, and B-eMC

FTIR-ATR spectrum of the raw material of EFB, D-eMC and B-eMC are represented in Figure 4. A peak observed at the region 3600-3200 cm^{-1} indicating hydroxyl (O-H) group in phenolic and aliphatic structure (Alemdar and Sain 2008). As shown in Figure 4, all samples of both EFB raw materials and treated EFB (D-eMC and B-eMC) showed-OH groups stretching vibration at 3417 cm^{-1}, and vibration OH group bending at wavelength 1321 cm^{-1}. FTIR spectra for all samples showed a peak around 898 cm^{-1} which is attributed to the presence of β-glycosidic linkage between glucose units in cellulose (Alemdar and Sain 2008; Buranov and Mazza 2008; O 2009; Septevani *et al.*, 2017). The most significant difference from the FTIR spectrum is a significant reduction in absorption intensity from EFB raw material compared to pretreatment process both D-eMC and B-eMC at 2920 cm^{-1} and 2850 cm^{-1}. O Eronen *et al.*, (2009) and Harun *et al.*, (2013) showed that reduction in the second peak intensity between 2920 cm^{-1} and 2850 cm^{-1} was derived from C-H stretching vibration of lignin and hemicellulose which diminished after delignification and bleaching process. It can also be seen in Figure 4 that a significant reduction in absorption intensity was also found at the peak between 1614 cm^{-1}. According to Ngadi and Lani *et al.*, (2014), the peak of 1600 cm^{-1} in the EFB spectrum is the vibration of the C=C benzene chain bond on lignin. Besides, the peak at 1700 cm^{-1} in the spectrum of raw material EFB is also likely to show C=C stretching vibration in aromatic ring carbonyl (O 2009). Furthermore, the peak at 1724-1737 cm^{-1} in the raw material of the EFB is likely to be the absorption of carbonyl in uronic acids or acetyl in hemicellulose and dismissed its intensity after alkaline bleaching and delignification. From the above description, shows that the use of H_2O_2 as a bleaching agent can produce high selectivity which can simultaneously reduce the levels of hemicellulose and lignin without damaging the cellulose structure.

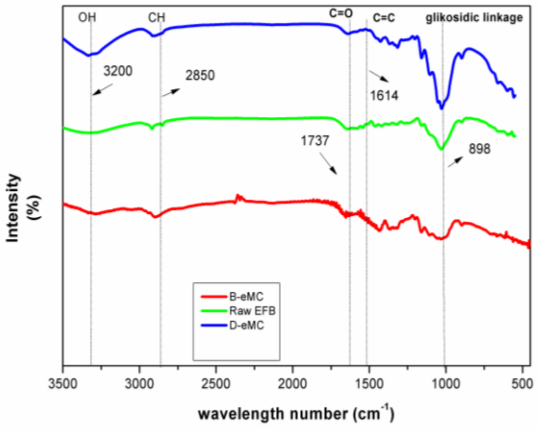


Figure 4. FTIR spectra of raw EFB; D-eMC; and B-eMC.

X-Ray Diffraction (XRD)

X-Ray diffraction (XRD) analysis was conducted to investigate the change in the crystallinity (see figure 5). Table 2 shows the calculated crystallinity index (CrI) of the samples. Lignocellulose fiber consists of amorphous and crystalline regions that were contributed by the constituents in the fiber (Bast 2009). The crystallinity index (CrI) of the samples was calculated using the Segal method in the following equation:

$$CrI,\% = \frac{I_{002} - I_{AM}}{I_{002}} \qquad (1)$$

Where I_{002} is the maximum intensity of the lattice diffraction at about $2\Theta \cong 22.4°$ and I_{AM} is the intensity attributed to the amorphous portion of the sample at $2\Theta \cong 18.6$ °. From Figure 5, all samples showed similar XRD patterns with significant peaks at 2Θ of 22.6° contributed by the crystalline regions of cellulose type I (Properties 2014). The sharper peak around 22.6° for D-eMB and B-eMC were observed compared to raw-EFB which was attributed to the gradual increase in the crystallinity index from 48% of the raw EFB to 68% after delignification and subsequent increase to 74% after bleaching. The increase in crystallinity was also attributed to the removal of the amorphous structure of hemicellulose and lignin during the treatment (Properties 2014) as supported by the reduction in the percentage of hemicellulose and lignin (see Table 2). However, XRD diffractograms of B-EMC and D-eMC showed similar diffraction pattern indicating that this further bleaching treatment has not made any significant changes in the crystal structure of the cellulose (Properties 2014).

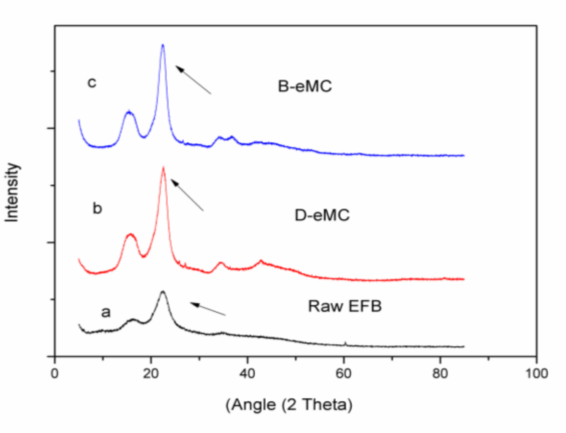


Figure 5. X-Ray Diffraction patterns for (a) Raw EFB; (b) D-eMC, and (c) B-eMC

Table 2. The crystallinity index of the raw EFB, D-eMC, and B-eMC

Sample	Raw EFB	D-eMC	B-eMC
Crystallinity, %	48	68	74

Thermogravimetric Analysis

The thermal stability of raw EFB, D-eMC and B-eMC are presented in Figure 6. The total weight loss was 56% for raw EFB, 68% for D-eMC, and 73% B-eMC. The weight loss of the samples is related to the thermal degradation of hemicellulose, cellulose, and lignin (Deepa *et al.*, 2011). The temperature region ranging from 250-300°C on the raw EFB was mainly related to the decomposition of extractives and hemicelluloses (Bast 2009). At this temperature range, the cleavage of glycosidic linkages of cellulose may occur as reported by Deepa *et al.*, (2011). Major weight loss for raw EFB occurred around 237°C, contributed by the degradation of cellulose. The weight loss of lignin occurs above 330°C (Deepa *et al.*, 2011). After alkali treatment, there is a shift in the major decomposition temperature from 237°C to 283°C. The decomposition of cellulose for D-eMC was slightly shifted to 283°C compared to raw EFB. This may be influenced by the arrangement of the molecular structure of the cellulose. Higher crystallinity means more heat is required to experience thermal degradation (Ouajai and Shanks 2005). B-eMC showed a significant weight loss degradation at a temperature around 297°C indicated the degradation ofcrystalline cellulose. In the study of Brebu *et al.*, (2010), lignin thermally decomposed over a broader temperature range of 200 – 500 °C due to different thermal stabilities of various oxygen functional groups from its structure. Thus its scissoring occurred at different temperatures (Brebu and Vasile 2010). It is clear that the degradation temperature of raw EFB is lower than that alkali treatment because in the raw EFB cellulose is organized into the bundle of fibrils, which are surrounded by a matrix of lignin, hemicellulose, and pectins. And alkali treatment can separates of the fiber bundle into smaller fibers (microfibril). The bleached fiber shows an increase in the weight loss temperature, which proves the ability of the developed cellulose to sustain the heat deformation temperatures (Properties 2014) confirming the higher thermal stability of microcrystalline cellulose from EFB. Therefore, it can be concluded from these results that the produced microcrystalline cellulose exhibits enhanced thermal properties than the untreated EFB.

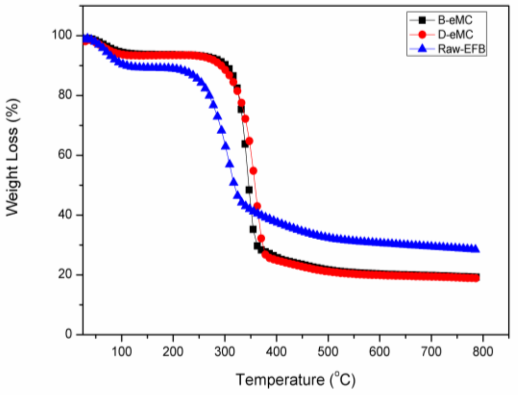


Figure 6. The thermogravimetric curves for (a) Raw EFB; (b) D-eMC; and (c) B-eMC.

CONCLUSION

Simultaneous pretreatment of EFB involving alkaline-delignification and H_2O_2-bleaching successfully resulted in increasing cellulose content of EFB almost double from the raw EFB, from 37.6% ± 0.3 to 89.2% ± 0.4. The use of these pretreatments also significantly reduced the lignin and hemicellulose content to 3.1% ± 0.1 and 7.7 % ± 0.2, respectively, that were lower than commercial cellulose standard. Based on the FTIR spectrum results, the intensity of vibration peak associated with cellulose groups increased significantly after the sequential delignification-bleaching process, whereas hemicellulose and lignin decreased in intensity. SEM analysis shows that alkaline delignification and bleaching process causes the significant transformation from the larger size and tightly bundles of micro-fibril cellulose into smaller size and individually separated micro-fibrils. Thermal study reveals that the thermal stability of cellulose microcrystal increased upon treatment. The results of this study indicate that the delignification process combined by using H_2O_2 as a bleaching agent provides high selectivity to simultaneously reduce the levels of hemicellulose, and lignin without damaging the structure of cellulose and increase the crystallinity and thermal stability.

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REFERENCES

Abraham, E *et al.*, (2011), Extraction of Nanocellulose Fibrils from Lignocellulosic Fibres : A Novel Approach., *Carbohydrate Polymers*, 86: 1468–1475.

Alemdar, Ayse, and Mohini Sain., (2008), Isolation and Characterization of Nanofibers from Agricultural Residues – Wheat Straw and Soy Hulls, *Bioresource Technology*, 99: 1664–1671.

Amira, Nor *et al.*, (2013), Cellulase Production from Treated Oil Palm Empty Fruit Bunch Degradation by Locally Isolated *Thermobifida Fusca*, *Bioresources*, 8: 676–687.

Bast, Kenaf., (2009), “Chemical Composition, Crystallinity, and Thermal Degradation of Bleached and Unbleached Kenaf Bast (*Hibiscus cannabinus)* Pulp and Nanofibers, *Bioresources*, 4: 626–639.

Bensah, Edem Cudjoe, and Moses Mensah., (2013), Chemical Pretreatment Methods for the Production of Cellulosic Ethanol : Technologies and Innovations, *Int. Journal of Chem. Eng*, 2013: 1-21.

Brebu, Mihai, and Cornelia Vasile., (2010), THERMAL DEGRADATION OF LIGNIN – A REVIEW, *Cellulose Chem. Technol*., 44(9): 353–363.

Buranov, Anvar U, and G Mazza., (2008), Lignin in Straw of Herbaceous Crops, *Industrial Crops and Products* 28: 237–59.

Chemistry, Procedia *et al.*, (2015), Potential of Oil Palm Empty Fruit Bunch (EFB) as Media for Oyster Mushroom, *Pleurotus ostreatu*s Culvitation, *Procedia Chemistry*, 16: 427–431.

Cheng, Yu-shen *et al.*, (2010), Evaluation of High Solids Alkaline Pretreatment of Rice Straw, *Appl. Biochem Biotechnol*, 162 : 1768–1784.

Ching, Yern Chee, and Tuck Sean Ng., (2014), Effect of Preparation Conditions on Cellulose from Oil, *Bioresource* 9(4): 6373–85.

Deepa, B *et al.*, (2011), Structure , Morphology and Thermal Characteristics of Banana Nano Fibers Obtained by Steam Explosion, *Bioresource Technology* 102: 1988–1997.

Dutra, E.D., Santos, F.A., Alencar, B.R.A. *et al.*, (2017), Alkaline hydrogen peroxide pretreatment of lignocellulosic biomass: status and perspectives, *Biomass Conv. Bioref*, 8: 225–234

Kumar, Parveen, Diane M Barrett, Michael J Delwiche, and Pieter Stroeve., (2009), Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production, *Ind. Eng. Chem. Res*, 48 : 3713–3729.

Lani, N S, N Ngadi, A Johari, and M Jusoh., (2014), Isolation , Characterization , and Application of Nanocellulose from Oil Palm Empty Fruit Bunch Fiber as Nanocomposites, *Journal of Nanomaterials*, 2014, 1-9

O, Paula Eronen Æ Monika., (2009), Effect of Alkaline Treatment on Cellulose Supramolecular Structure Studied with Combined Confocal Raman Spectroscopy and Atomic Force Microscopy, *Cellulose* 16 : 167–178.

Ouajai, S, and R A Shanks., (2005), Composition , Structure and Thermal Degradation of Hemp Cellulose after Chemical Treatments, *Polymer Degradation and Stability* 89: 327–335.

Properties, Thermal., (2014), Native Cellulose: Structure, Characterization and Thermal Properties, *Materials*, 7: 6105–6119.

Ramli, Ridzuan, Norhafzan Junadi, Mohammad D H Beg, and Rosli M Yunus., (2015), Microcrystalline Cellulose (MCC) From Oil Palm Empty Fruit Bunch (EFB) Fiber via Simultaneous Ultrasonic and Alkali Treatment, *Int. Journal of Chem. Molecular, Nuclear, Materilas and Metallurgical Engineering*, Vol. 9(1): 8–11.

Rezende, Camila Alves, Marisa Aparecida De Lima, Priscila Maziero, and Eduardo Ribeiro., (2011), Chemical and Morphological Characterization of Sugarcane Bagasse Submitted to a Delignification Process for Enhanced Enzymatic Digestibility, *Biotechnology for Biofuels*, 4:54.

Roberto, C. *et al.*, (2008), Hydrogen Peroxide Bleaching of Cellulose Pulps Obtained from Brewer ' s Spent Grain, *Cellulose*, 15 : 641–649.

Septevani, Athanasia A, David A C Evans, Pratheep K Annamalai, and Darren J Martin., (2017), Industrial Crops & Products The Use of Cellulose Nanocrystals to Enhance the Thermal Insulation Properties and Sustainability of Rigid Polyurethane Foam, *Industrial Crops & Products* 107(May): 114–21.

Shimizu, Satoko, Tomoya Yokoyama, Takuya Akiyama, and Yuji Matsumoto., (2012), "Reactivity of Lignin with Di Ff Erent Composition of Aromatic Syringyl/Guaiacyl Structures and Erythro / Threo Side Chain Structures in β - O -4 Type during Alkaline Deligni Fi Cation: As a Basis for the Di Ff Erent Degradability of Hardwood and Softwood, *Journal of Agricultural and Food Chemistry*, 60 : 6471–6476.

Sills, Deborah L, and James M Gossett., (2012), Using FTIR Spectroscopy to Model Alkaline Pretreatment and Enzymatic Saccharification of Six Lignocellulosic Biomasses, *Biotechonolgy and Bioengineering*, 109(4): 894–903.

Sudiyani, Yanni, Eka Triwahyuni, Dian Burhani, and Joko Waluyo., (2016), Alkaline Pretreatment of Sweet Sorghum Bagasse for Bioethanol Production, *Int. Journ. of renewable energy Dev.*, 5(2): 113–18.

Xuezhu Xu, Jian Zhou, Long Jiang, Gilles Lubineau, Tienkhee Ng, Boon S. Ooi, Hsien-Yu Liao, Chao Shen, Long Chen and J. Y. Zhu., (2016), Highy transparent, Low-haze, Hybrid Cellulose Nanopaper as Electrodes for Flexible electronics, *Nanoscale*, 8(24): 12294-12306

Zhang, Yu Rong, Xiu Li Wang, Guo Ming Zhao, and Yu Zhong Wang., (2013), Influence of Oxidized Starch on the Properties of Thermoplastic Starch, *Carbohydrate Polymers* 96(1): 358–364.

Zhao, X., Zhang, L. and Liu, D. (2012), Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuels, Bioprod. Bioref.*, 6: 465-482.