An eco-friendly preparation of cellulose nano crystals from oil palm empty fruit bunches

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Abstract. Cellulose Nanocrystals (CNCs) have attracted a lot of attention as one of the promising green nanomaterials because of their interesting properties such as low density, high mechanical properties, high surface area, high biocompatibility and low toxicity. However, sulphuric acids is generally used in conventional method to prepare CNCs which is highly corrosive and harmful to environment. Replacing sulphuric acid with Ammonium Persulfate (APS), which possess lower toxicity, are desirable from environment point of view. In this research, preparation of CNCs from Oil Palm Empty Fruit Bunches (OPEFB) as alternative raw materials and using APS is reported. Three kinds of method were carried out before applying APS solution: (i) without pre-treatment, (ii) with alkali pre-treatment and (iii) with alkali-chloride pre-treatment. White-colored sample with crystallinity reaching 78% (XRD result) and particle size ranging between 31-113 nm (DLS result) was obtained. Those results implied that there were potential existence of CNCs in the samples.

1. Introduction
In the last decades, Cellulose Nanocrystals (CNCs) have attracted a lot of attention as one of the promising green nanomaterials because of their interesting properties such as low density, high mechanical properties, high surface area, high biocompatibility and low toxicity [1]. Those superior properties make CNCs useful for many applications from various fields such as reinforcement of nanocomposites, plasticizer and strength enhancement of cement, films for optical and barrier properties, catalyst support, etc. So, due to its advantages and broad potential of application, many researchers have been studying CNCs extraction from various lignocellulosic source such as wood, cotton, bamboo, hemp, flax, kenaf etc. [2,3,4,5,6,7]. Some CNCs research groups or institutions have reached either a commercial pilot scale (kg/day) or even real industrial scale (ton/day)[8].

Common process used to prepare CNCs uses acid hydrolysis, which results in non-eco-friendly acid waste. Only one of at least five commercial scales of CNCs production does not use the acid hydrolysis method as reported by Reid et al. [8]. Some researchers try to develop new processes, which have less environmental damage risk by reducing dangerous waste in its process. The use of Ammonium Persulfate (APS) as reported by Leung et al. (2011) could be an interesting option to meet
better environmental impact [9]. APS is a chemical with high water solubility and low long-term toxicity, which results in free radical $\text{SO}_4^{2-}$ and hydrogen peroxide ($\text{H}_2\text{O}_2$) in high temperature and acidic condition. Those free radical and hydrogen peroxide can be used to remove the lignin and cut the cellulose to produce CNCs. In their research, Leung et al. succeeded to extract CNCs from flax, hemp, triticale, MCC, wood pulp, and bacterial cellulose [9]. Other researchers have also adopted this method to extract CNCs from other sources as reported by Hu (2014) and Mascheroni (2016) who studied the extraction of CNCs from bamboo and cotton, respectively [11,12]. This method offers simple process, easy to scale-up, and eco-friendly that could also decrease the waste treatment cost.

In this study, the CNCs was synthesized from solid waste containing lignocellulosic component from Oil Palm Empty Fruit Bunch (OPEFB), which is abundant amount of solid waste in palm oil mill. The empty fruit bunch may contain cellulose up to 45%, which is very potential for CNC production. Indonesia is well-known as the second largest of palm plantation area in the world, which has 11 million hectares of palm plantation area producing 33 million ton of palm fruit bunch during 2016 [12]. OPEFB is the largest solid waste of palm oil production in palm oil mill (approximately 23%-wt of palm fruit bunch), which has not been utilized well [13]. Some of researchers have used OPEFB as raw material for CNCs preparation such as Dasan (2015) and Fahma (2010) [14,15]. Herein, the feasibility study of the utilization of ammonium persulfate for producing CNCs from OPEFB is studied. In the present work, the synthesis of CNC from OPEFB as raw material for high-value product through an eco-friendly process is investigated.

2. Materials and Methods
2.1. Materials
Oil Palm Empty Fruit Bunches (OPEFB) used in this research was obtained from PT. Perkebunan Nusantara VIII, Cikasungka, Bogor, West Java, Indonesia. The dried empty fruit bunch was chopped in the form of short fiber. All chemicals used for one-step extraction of CNCs were purchased from Merck KgaA, EMD Millipore Co. with pro analysis grade. The demineralized water was used in all experiments.

2.2. Main Experiment: CNCs Preparation
Three kinds of pre-reacted materials were first prepared consisting of OPEFB without any treatments, OPEFB with alkali pre-treatment, and OPEFB with alkali-chloride pre-treatment. The experiment was carried out by reacting 3 grams of pre-reacted material with 1-2 molar of Ammonium Persulfate (APS) in a refluxed round flask at 70°C for 15 h [16]. The mixture of reaction products obtained after 15 h operation was filtered using vacuum filtration to remove remained APS in the mixture. Remained solid state sample was washed several times until its pH reached 7.

2.3. Alkali Pre-treatment
Three grams of OPEFB was added into 30 mL sodium hydroxide solution (4-wt%) and then vigorously agitated for 2 h at 90°C to purify the cellulose by removing hemicellulose and lignin. The solid was then filtered and washed several times using demineralized water until reached pH of 7.

2.4. Alkali-Chloride Pre-treatment
The alkali-chloride pre-treatment was performed by adding a buffer solution of aqueous chlorite (1.7-wt%), acetic acid, and demineralized water into erlenmeyer. The solution was heated at 90-100°C for 4 h under mechanical stirring. The resulting solution was cooled, filtered, and washed until pH neutral.

2.5. Composition Analysis
Composition analysis was carried out based on SNI 0492:2008, SNI 14-1304-1989, and SNI 0444:2009 for determining the contents of lignin, hemicellulose, and α-cellulose, respectively.

2.6. Crystallinity Measurement
X-Ray Diffraction method was used to determine the Crystallinity Index (CrI) of the sample. X-Ray Diffractometer Bruker D8 Discover with CuKα Radiation (wavelength = 1.54 Å) was used with 20
range of $5^\circ$-$80^\circ$ with 0.010 step size. The Crystallinity Index was determined using empirical method with following equation:

\[
CrI = \frac{l_{002}-l_{am}}{l_{002}} \times 100
\]

where $l_{002}$ and $l_{am}$ are the peak intensities of crystalline and amorphous materials, respectively [16].

2.7. Particle Size and Morphological Analysis
Prediction of particle size and its distribution was carried out by Dynamic Light Scattering (DLS).

3. Results and Discussion
3.1. Visual Observation
It was visually observed that the pre-treatment of OPEFB could reduce the lignin content, which was indicated by discoloration. Figure 1 shows discoloration of OPEFB after typical pre-treatment. The brighter pre-reacted materials, the lower its lignin content. Refer to Figure 1c, OPEFB using alkali-chloride pre-treatment showed that the lignin content could be removed significantly.

Figure 1. Three kinds of pre-reacted materials: (a) OPEFB without any pre-treatment, (b) OPEFB after alkali pre-treatment, and (c) OPEFB after alkali-chloride pre-treatment.

Figure 2. Mixture after reaction with 1 M of APS (a) and 2 M of APS (b) without any pre-treatment at 70°C for 15 hours.

Figure 3. Mixture after reaction with 1 M of APS with alkali pre-treatment at 70°C for 15 hours.

Figure 4. Mixture after reaction with 1 M of APS with alkali-chloride pre-treatment at 70°C for 15 hours.
Based on the experiment, dry OPEFB without any pre-treatment has lignin, α-cellulose and hemicellulose content of 17.45%, 36.68%, and 26.26%, respectively. The lignin content of dry OPEFB is high enough compared to lignocellulosic materials used by Leung et al. (2011) [9], who used flax, hemp, triticale, MCC, wood pulp, and bacterial cellulose as raw material for CNCs synthesis and succeeded to yield CNCs from those materials without any pre-treatment through oxidation by 1 M of APS.

In line with Leung et al. (2011) that the oxidation by using APS can produce CNCs from lignocellulosic materials with lignin content up to 20%. Although OPEFB has high lignin content, the experimental results show the existence of CNCs in the product mixture, which was indicated by white-colored mixture as increasing APS concentration and decreasing lignin content by pre-treatment. Figures 2, 3, and 4 shows the mixtures after reaction with APS.

In this work, it was also indicated that reaction between OPEFB without pre-treatment with 1 M of APS did not represent the white-colored mixture (Figure 2a), which was probably induced by high lignin content in the OPEFB. In addition, the extractives content existing in the OPEFB may reduce penetrability of reaction agent [17]. Unlike flax, hemp, triticale, MCC, bacterial cellulose, and water hyacinth, which have small number of extractives, OPEFB has higher extractives content. Therefore, different composition of lignin and extractives content may affect the reaction condition.

Empirically, the white-colored mixture was obtained after increasing APS concentration to 2 M (Figure 2b). Increasing the APS concentration was based due to fact that the OPEFB lignin content is more than twice than other lignocellulosic materials used by Leung et al. (2011). However, increasing APS concentration to 2 M might increase the chemical cost as twice. This implication must be considered for future scale-up processes.

Another alternative to produce CNCs from OPEFB through oxidation by APS was trying by reducing the lignin content, which could be achieved by conducting the pre-treatment for OPEFB. In this research, two kinds of pre-treatment were conducted, consisting of alkali pre-treatment and alkali-chloride pre-treatment. Alkali pre-treatment could loosen the lignocellulosic structure, break lignin-carbohydrate bonds, and increase surface area to ease the penetration of oxidizing agent [18]. When alkali pre-treated OPEFB was used as raw material, the white-colored mixture was obtained which indicate most of lignin have been removed. It may be caused by easier penetration of oxidizing agent due to pre-treatment process. While further delignification by chlorine results sample with least lignin which yield the most promising product when reacted with APS. Thus, decreasing lignin content could give more promising mixture to obtain CNCs.

![XRD curve of obtained samples following raw materials](image)

**Figure 5.** XRD curve of obtained samples following raw materials: (a) OPEFB without any pre-treatment (2M APS), (b) OPEFB after alkali pre-treatment (1M APS), and (c) OPEFB after alkali-chloride pre-treatment (1M APS).

| Sample | Crystallinity Index (%) |
|--------|-------------------------|
| a      | 68                      |
| b      | 75                      |
| c      | 78                      |
3.2. Crystallinity Measurement
All obtained samples have Crystallinity Index higher than 54%, which is the lower limit of Cellulose Nanocrystals criterion. The XRD Curve and the Crystallinity Index for three samples are showed in Figure 5.

Decreasing lignin content by applying pre-treatment of OPEFB could give higher crystallinity index than increasing the APS concentration. Hence, decreasing the lignin content might be more preferable to yield higher quality of CNCs. In addition, conducting pre-treatment using sodium hydroxide was more cost effective than the oxidative by APS with double amount of concentration, which was more expensive. Thus, decreasing lignin content may be the best choice for next research of CNCs preparation from OPEFB through oxidation by APS by considering the quality of sample and the production cost.

The lignin content can be decreased by conducting alkali and alkali-chloride pre-treatment. The data shows that there was no significant difference of the crystallinity index between sample from alkali and alkali-chloride pre-treated OPEFB. Nevertheless, those two kinds of pre-treatment had different implication in the CNCs preparation process. The alkali pre-treatment was a one-step pre-treatment, which was more practical, time saving, and cost effective than alkali-chloride pre-treatment.

3.3. Particle Size Determination and Morphological Analysis
The result of Dynamic Light Scattering (DLS) characterization shows that obtained samples have particle size ranging between 31 to 113 nm. Table 1 shows particle size distribution and its average size.

| No | Sample | Particle Size (D-90%) (nm) | Average Size (nm) |
|----|--------|---------------------------|------------------|
| 1  | Sample from OPEFB without pre-treatment (2M of APS) | 113 | 90 |
| 2  | Sample from OPEFB after alkali pre-treatment (1M of APS) | 101 | 85 |
| 3  | Sample from OPEFB after alkali-chloride pre-treatment (1M of APS) | 31 | 25 |

The particle size range of all samples was very close to the criterion of CNCs, which had length in the range of 100-250 nm and diameter of 5-70 nm [1]. This was a rough determination of particle size of samples, but those data represented very potential existence of CNCs in the samples.

4. Conclusions
The synthesis of CNCs from Oil Palm Empty Fruit Bunch (OPEFB) has been conducted by using Ammonium Persulfate (APS) as oxidation agent. The produced CNCs by using APS with crystallinity index higher than 54% can be achieved and the size distribution is also very close to criterion of CNCs. It was also found that the lignin content of pre-reacted materials has significant effect in CNCs preparation through oxidation with APS.

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6. References
[1] Brinchi L, Cotana F, Fortunati E, and Kenny J 2013 Carbohydr. Polym. 94 154-169
[2] Beck-Candanedo S, Roman M, Gray DG 2005 Biomacromolecules 6 1048–54
[3] Chang C P, Wang I C, Hung K J, Perng Y S 2010 Taiwan J. Fos. Sci. 25, 251-264
[4] Yu M, Yang R, Huang L, Cao X, Yang F and Liu D 2012 BioResources, 7
[5] Cao X, Chen Y, Chang P R, Stumborg M, and Huneault M A 2008 *J. Appl. Polym. Sci.* **109** 3804-3810
[6] Cao X, Dong H, and Li C M 2007 *Biomacromolecules*, **8** 899-904
[7] Zaini L H, Jonoobi M, Tahir P M and Karimi S 2013 *J. Biomater. Nanobiotechnol.* **4** 37-44
[8] Reid M, Villalobos M and Cranston, E 2016 *Langmuir* **33** 1583-1598
[9] Leung A C, Hrapovic S, Lam E, Liu Y, Male K B, Mahmoud K A and Luong J H 2010 *Small* **7** 302-305
[10] Hu Y, Tang L, Lu Q, Wang S, Chen X and Huang B 2014 *Cellulose* **21** 1611-1618
[11] Mascheroni E, Rampazzo R, Ortenzi M A, Piva G, Bonetti S and Piergiovanni L 2016 *Cellulose* **23** 779-793
[12] Direktorat Jendral Perkebunan 2015 *Statistik Perkebunan Sawit Indonesia 2014-2016 Kelapa Sawit*
[13] Darnoko Z, Poeloengan and Anas I 1992 *Buletin Penelitian Kelapa Sawit* **2**
[14] Fahma F, Iwamoto S, Hori N, Iwata T and Takemura A 2010 *Cellulose* **17** 977-985
[15] Dasan Y K, Bhat A H and Faiz A 2015 *AIP Conf. Proc.* **1669** 020058
[16] Majdanac L D, Poleti D, Teodorovic M J 1991 *Acta Polym.* **42** 351-357
[17] Rowe J W 2014 *Natural Products of Woody Plants Chemicals Extraneous to the Lignocellulosic Cell Wall* (Berlin : Springer)
[18] Zhao Y, Wang Y, Zhu J, Ragauskas A and Deng Y 2008 *Biotechnol. Bioeng.* **99** 1320-1328