Preparation of cellulose nanocrystals from empty fruit bunch of palm oil by using phosphotungstic acid

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Abstract. Empty fruit bunch (EFB) of palm oil, an abundant agro-waste in Indonesia, was used as raw material of Cellulose Nanocrystals (CNCs) preparation. Instead of conventional acid mineral, phosphotungstic acid (H\textsubscript{3}PW\textsubscript{12}O\textsubscript{40}, HPW) was used to hydrolyze cellulose due to recycling ability and easy handling. Before hydrolysis process, dried EFB was treated by 3\% NaOH solution at 90\(^o\)C for 2 hours and then bleached using 2\% NaClO\textsubscript{2} solution at 80\(^o\)C for 3 hours to remove hemicellulose and lignin. Hydrolysis reaction parameters such as temperature, acid concentration, and reaction time were optimized with fixed solid-liquid ratio of 1:40. Response surface method was used for experimental design to determine the optimum condition of each parameter by using software Minitab. In this study, pulp from dried EFB produced 44.8\% yield of CNCs. Dynamic Light Scattering (DLS) analysis showed that most of CNCs equivalent diameter was 140 nm. Crystallinity index was observed at 73.3\% using X-ray Diffraction (XRD) analysis. Thus, a green established process for the preparation of CNCs was achieved.

1. Introduction

Palm Oil is a commodity mainstay of Indonesia which its development is quite rapid. This plantation plays an important role in the Indonesia economic sector with production reaching 33.4 million tons and accounted for 11\% of Indonesia’s export revenue. According to Directorate General of Estate Crops (2015), oil palm plantations have spread in 25 provinces, and the area reached 10.8 million ha in 2014\textsuperscript{[1]}. The increasing of plantation area will be followed by the escalating of oil palm production and volume of waste. Empty fruit bunch of palm oil (EFBPO) as the most abundant waste of oil palm production has not yet been widely utilized. This waste contains high cellulose content, which is 40-60\%\textsuperscript{[1,2]}, therefore, it is potential to be used as raw material for the manufacture of Cellulose Nano Crystals (CNCs).

CNCs is one of advanced material that is currently studied because of their amazing material properties, such as low density, high mechanical strength, high surface area, low toxicity, and other

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intriguing optical properties [3,4]. Hence, CNCs attracts researchers and industries to be applied in various fields, such as optical and electronic devices, catalyst support, wound dressing, paint and coating, food and cosmetic, enzyme immobility and controlled drug delivery [4-8].

The common method used for synthesizing CNCs is hydrolysis of cellulose by mineral acids, including sulfuric acid, hydrochloric acid, phosphoric acid, and their mixture [9-11]. Although this method is simple, CNCs produced through sulfuric acid hydrolysis are usually sensitive to temperature because of the presence of acidic moieties at their surface [12]. This condition will limit their applications in some areas. In addition, some issues still need to be addressed, such as large water consumption, serious equipment corrosion and great amount of waste produced [13,14].

Recently, many studies have been done to optimize the hydrolysis parameters, avoid the corrosion, and reduce the waste. In this study, solid acid is investigated to substitute the usage of strong liquid acids to overcome the limitation of CNCs produced by liquid acid. The main advantages of solid acid are the easy recovery of solid acid, less corrosion, high thermal stability, and relatively safe working environment [13,14]. However, the limited contact between solid acid and cellulose significantly increases the hydrolysis time. Previous studies showed that phosphotungstic acid (H₃PW₁₂O₄₀, HPW) has many Bronsted acid sites, so it can be used as an alternate catalyst of liquid acids for the hydrolysis of cellulose [15]. Hence, its usage is effective for the hydrolysis reaction. Therefore, it is expected that CNCs can be synthesized through hydrolysis using HPW by controlling the hydrolysis parameters.

2. Materials and Methods

2.1. Materials

EFBPO used as raw material was obtained from PTPN VIII located at Bogor, West Java, Indonesia. It was dried, ground and chipped into 40-80 mesh size.

2.2. Methods

2.2.1. Alkali pre-treatment. The alkali pre-treatment was completed by heating the mixture of EFB and NaOH solution (3-wt%) under mechanical stirring for 2 hours. The solid product was washed several times using distilled water. Neutral solid was dried in oven at 105°C for 8h.

2.2.2. Bleaching. The resulting solid from previous treatment was mixed with solution consisting of equal parts (v:v) of acetic acid buffer (27 g NaOH and 75 mL glacial acetic acid, diluted to 1 L of distilled water) and 2% aqueous sodium chlorite (pH of mixed solution is 3.6-4) [16]. The mixture was heated at temperature of 80°C for 3 h under continuous stirring. Bleached solid (EFB pulp) was filtered and washed using deionized water until neutral pH was reached. Drying of pulp was done in oven at 105°C for 8 h.

2.2.3. Acid Hydrolysis. One gram of pulp was placed in an erlenmeyer and HPW was added with concentration varied from 65-85%-wt. The mixture was heated at temperature 80°C-100°C under mechanical stirring for time range of 25-35 h. The reaction was stopped by rapid cooling in chilled water bath until reached room temperature. The resulting solution was then extracted with excess diethyl ether in separating funnel. Three layers were formed. The lowest layer was the mixture of HPW and diethyl ether, the middle layer was consisted of CNCs, water, and degraded sugar and the upper layer was diethyl ether [17]. The lowest layer was distilled at 45°C to recover HPW and diethyl ether. The middle layer was centrifuged at 4000 rpm for 15 min at room temperature to and the supernatant was decanted. The decanted solid was mixed with deionized water and centrifuged three times. Near neutral pH solid was then mixed with ethanol and centrifuged to remove unreacted cellulose. CNCs was collected by further centrifugation with deionized water. Wet CNCs was dried in oven at 105°C for 8h to obtain dried CNCs. Yield of CNCS was calculates by equation (1).
2.2.4. Characterization. The CNCs produced was characterized using X-ray Diffraction (XRD), Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM). XRD pattern for CNCs was perform on Bruker D8 Discover Diffractometer (Bruker Co., USA) using Cu Kα radiation (λ=1.54 Å) at 40 kV and 30 mA. Diffactogram was collected in the range 2θ of 5-60°. Crystallinity Index (CI) was determined by peak deconvolution method. This method extracts individual crystalline peak by a curve-fitting process from diffraction intensity profiles [18]. PeakFit v4.12 trial version (SeaSolve Software, Inc. USA) was used to separate amorphous and crystalline contributions to the diffraction spectrum. CI is calculated from ration of the area of all crystalline peaks to the total area. DLS was used to analyze particle size distribution of CNCs. This analysis used common Delsa TM Nano Submicron particle analyzer (Beckman Coulter, Inc., USA) at fixed angle 165°. Morphology and size of CNCs were observed by TEM with a Hitachi HT-7700 transmission electron microscope (Hitachi High-Technologies Co., Japan) using an accelerated voltage of 80 kV. Diluted suspension of CNCs was deposited on an 20 nm carbon-coated grid.

2.2.5. Experimental Design. Response Surface Method (RSM) and Box-Behnken Design were used for experimental design to optimized CNCs yield (Y, %) with software MiniTab 16.2.4.0 (Minitab Inc., US/Canada/Mexico). The independent variables were HPW concentration (X1, g/mL), reaction time (X2, h), and reaction temperature (X3, °C). Yield data obtained from the experiment were analyzed using Multiple Regression Analysis and fitted to second-order polynomial model expressed by equation (2).

\[
Y = X_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \epsilon
\]  

(2)

where, \( \beta_0, \beta_i, \beta_{ii}, \) and \( \beta_{ij} \) are regression coefficients, \( X_i \) and \( X_j \) are independent variables, and \( \epsilon \) corresponds to the residue of the model. Test of statistical significance was applied to model and lack of significance using F and P-test from analysis of variance (ANOVA) at significant level (\( \alpha \)) of 0.05.

3. Results and Discussions

3.1. Model Fitting and Statistical Analysis

Design of experiment and yield of CNCs as response was shown in table 1. Second-order polynomial model that relates each parameters and the experimental yield was shown in equation 3. This regression model was then evaluated using analysis of variance (ANOVA). The result of ANOVA was shown in table 2.
Table 1. Box-Behnken Design and CNCs Yield as Response

| Coded Variable | Yield (%) |
|----------------|-----------|
| \( X_1 (\%) \) | \( X_2 (h) \) | \( X_3 (^\circ C) \) |
| -1 (65) | -1 (25) | 0 (90) | 64.47 |
| 1 (85) | -1 (25) | 0 (90) | 54.55 |
| -1 (65) | 1 (35) | 0 (90) | 50.71 |
| 1 (85) | 1 (35) | 0 (90) | 70.42 |
| -1 (65) | 0 (30) | -1 (80) | 53.34 |
| 1 (85) | 0 (30) | -1 (80) | 70.20 |
| -1 (65) | 0 (30) | 1 (100) | 61.92 |
| 1 (85) | 0 (30) | 1 (100) | 57.80 |
| 0 (75) | -1 (25) | -1 (80) | 50.90 |
| 0 (75) | 1 (35) | -1 (80) | 64.69 |
| 0 (75) | -1 (25) | 1 (100) | 55.57 |
| 0 (75) | 1 (35) | 1 (100) | 52.48 |
| 0 (75) | 0 (30) | 0 (90) | 44.05 |
| 0 (75) | 0 (30) | 0 (90) | 47.15 |
| 0 (75) | 0 (30) | 0 (90) | 45.04 |

Table 2. ANOVA for Response Surface Second-Order Polynomial Model

| Source | DF | Seq SS | Adj MS | F | P |
|--------|----|--------|--------|---|---|
| Model  | 9  | 994.34 | 110.482| 32.39 | 0.001|
| Linear | 3  | 100.09 | 33.364 | 9.78  | 0.016|
| \( X_1 \) | 1  | 63.45  | 63.45  | 18.6  | 0.008|
| \( X_2 \) | 1  | 20.51  | 20.51  | 6.01  | 0.058|
| \( X_3 \) | 1  | 16.13  | 16.13  | 4.73  | 0.082|
| Square | 3  | 493.49 | 164.945| 48.22 | 0   |
| \( X_1^2 \) | 1  | 303.4  | 352.051| 103.21| 0   |
| \( X_2^2 \) | 1  | 72.76  | 87.196 | 25.56 | 0.004|
| \( X_1 X_2 \) | 1  | 117.33 | 117.329| 34.4  | 0.002|
| Interaction | 3  | 400.76 | 133.586| 39.16 | 0.001|
| \( X_1 X_3 \) | 1  | 219.48 | 219.484| 64.35 | 0   |
| \( X_2 X_3 \) | 1  | 110.04 | 110.04 | 32.26 | 0.002|
| \( X_1^2 X_2 \) | 1  | 71.23  | 71.234 | 20.88 | 0.006|
| Residual | 5  | 17.06  | 3.411  |      |     |
| Lack of fit | 3  | 12.04  | 4.014  | 1.6  | 0.407|
| Pure error | 2  | 5.01   | 2.507  |      |     |
| Total     | 14 | 1011.39|        |      |     |

Figure 1. Contour plots as a function of (a) time and temperature at 75% HPW, (b) HPW concentration and temperature at 30 h, and (c) HPW concentration and time at 90°C

Figure 2. TEM image of CNCs sample, (a) rod-like and (b) spherical shape

Figure 3. XRD spectra of CNCs sample for parameters 75% HPW, 30 h, and 90°C
Y = 1191.02 - 12.07X1 - 11.6026X2 - 11.6737X3 + 0.0976X1^2 + 0.1943X2^2 + 0.115X3^2 + 0.1482X1X2 - 0.075X1X3 - 0.121X2X3

(3)

Determination coefficient of the model was close to 1 ($R^2 = 98.31\%$ and adjusted-$R^2 = 95.28\$). Calculated F-value of model was higher than tabulated F-value ($F_{0.05}=4.77$) and P-value was less than 0.05. Lack of fit was insignificant since the calculated F-value is less than tabulated F-value ($F_{0.05}=19.6$) and P-value is higher than 0.05. These results were indicated that the model equation was well fitted to the experimental data [19].

3.2. Optimization of Experimental Parameter
Contour plot (Figure 1) was constructed based on equation (3). These plots correlated the three independent variables and the response keeping one of the independent variables in its central point. According to equation (3), the higher importances on the response were three square terms and term of interaction between HPW concentration and reaction time because of its positive signal. The positive values in the model indicate that an increase of factor values tends to increase the response [20].

Three of the contour plots indicates that initially, yield decreased with increase of each factor. After yield reached its minimum point, yield increased with increase of factor. The decrease of yield was due to further hydrolysis of cellulose on crystalline region. This excessive hydrolysis happened may because of the too high level of reaction parameters. The increase of yield after minimum point was resulted by the formation of amorphous nanoflocks. A tendency of nanoflocks formation is higher when the reaction parameters was too high [13]. These amorphous nanoparticle was about the same size as CNCs that make them difficult to separate by centrifuging. Optimum yield based on equation (3) was 88.38\% under $%\text{-HPW}$, time, and temperature value of 85\%, 35h, and 80°C, respectively. The possibility of nanoflocks formation made this yield value not convincing. CNCs produced under high level reaction parameter had to be analized further by XRD analysis to make sure the present of nanoflocks in sample.

3.3. Characteristics of CNCs
Diffractogram of CNCs sample produce under middle level parameter value was shown in figure 3. Crystallinity Index of sample was 84.0\% and indicated that most of cellulose amorphous regions were hydrolyzed. Particle size distribution was observed in the range of 23-770 nm and mean value of 140 nm as equivalent diameter by DLS analysis. TEM image of CNCs sample was shown in figure 2. Two types of CNCs morphology were observed, rod-like and spherical. Most CNCs morphology was rod-like with length under 200 nm.

4. Conclusions
In this study, a green established process for the preparation of CNCs from was achieved using phosphotungstic acid hydrolysis with minimum 44.04\% yield. Characterization of sample show that the crystallinity and size of CNCs sample was meet the CNCs characteristic. Moreover, the recycling ability and less corrosivity of HPW made this the best among other acid hydrolysis method.

5. References
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