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Mechanical Production of Cellulose Nanofibrils (CNF) from bleached kawayan tinik (*Bambusa blumeana* Schult. F) pulp: Effect of holocellulose preparation and number of passes in the supermass colloider

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Abstract

The study determined the effects of holocellulose preparation and number of passes in the mechanical production of CNF from bleached Kawayan Tinik pulp. Pretreatment of bamboo included removal of branches and outer skin, cutting into rings, chipping, soaking in water, air-drying, and hammer milling. The Kraft Pulping and the one-step hypochlorite bleaching were used for pulp liberation and bleaching, respectively. A portion of the bleached pulp underwent the sodium chlorite treatment to isolate the holocellulose by removing extractives, residual lignin, and some hemicelluloses. The amount of acid-insoluble lignin present in the raw bamboo, Kraft pulp, bleached pulp, and holocellulose was determined. The bleached pulp and holocellulose were separately made to pass for a predetermined number of cycles in the supermass colloider to produce CNF. The yields were calculated, with ultimate values of 73.52% (200 passes) and 66.02% (300 passes) for the bleached pulp and holocellulose preparation, respectively. Optical microscopy was done to monitor the changes in the morphological characteristics of the CNF during the initial passes, while Scanning Electron Microscopy showed the nanosize dimensions of the final product with an average diameter of 58.35 nm and an average length of 2,169.26 nm. Dynamic Light Scattering (DLS) showed a homogeneous particle size distribution. In addition, the functional groups present in the CNF from bleached pulp and holocellulose were analyzed using Fourier Transform Infrared (FTIR) Spectroscopy and showed that the CNF samples contain peaks for O-H, C-H, and C-O-C stretching, but lacks groups related to lignin. X-ray Diffraction Analysis showed that the crystallinity of the CNF increased to 71% compared to the 60% from literature. Statistical analysis showed that holocellulose preparation and number of passes in the supermass colloider had significant effects on the CNF yield, length, width, and aspect ratio. The holocellulose which was made to pass the colloider for 200 times gave the highest yield and the morphological characteristics closest to reported literature values. For bleached pulps, the number of passes in the supermass colloider had significant effect on the CNF length and width.

I. INTRODUCTION

The demand for better alternatives to non-renewable resources is continuously increasing in the Philippines. Likewise, the research and production of sustainable resources are greatly growing which basically aim to create non-petroleum based, biodegradable, low carbon footprint, and minimal environmental and health impact. Hemp, linen, cotton, and wood which are natural cellulosic materials have been utilized for many years as reflected by the wide industry of forest products, papers, and other related prod-

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ucts on a global scale [1, 2].

Nanocellulose was coined to refer to cellulose that is structured in a nanoscale. It can be further specified with respect to its production process such as CNF, Bacterial Nanocellulose (BNC), and Cellulose Nanocrystals (CNC) using mechanical, biological, and chemical processes, respectively. It is known to have unique properties making it suitable for a number of useful industrial applications. In addition, nanocellulose is highly versatile and is different from other nanomaterials since it is derived from wood pulp, is nontoxic, and relatively cheaper compared to other raw materials [3]. According to [4], the current distribution of MFC applications are 38% in composite materials, 18% in nonwoven and adsorbent webs, 16% in paper and board, 13% in food products, 8% in paper and board coatings, 3% in cosmetics and toiletry, and 4% in the subfield of filter materials.

According to [1], the nanocellulose can improve the rheology or the study of flow and deformation of materials under applied forces of different media like polymer melts, particle mixtures, and liquids. In this way, the industries dealing with paints, adhesives, textiles, drugs, cement, and even food can improve their production processes. Moreover, production of lightweight, transparent, stable, flexible, resilient, and vigorous films can improve the packaging, selective filtration, and structural aspects of different areas. In addition, [5] reported that nanocellulose may also be used in the production of moulded composites for the creation of durable and light raw materials in vehicle production. On the other hand, transparent composites may also be created for the information and communication industry because of its excellent thermal stability and particle size. Furthermore, because of the increasing demand in environmental-friendly construction materials, processed nanocellulose such as aerogels and foams may be utilized in the building industry for their sound and thermal insulation characteristics.

Bamboo, which is one of the flowering perennial evergreen plants of the family Poaceae, is a readily available source of cellulose consisting of effective hardness, hygroscopicity, and high crystallinity. Likewise, it is a good feed for the production of cellulose nanocrystals and cellulose nanofibrils [6]. Furthermore, from 2007 to 2010, the global trade of bamboo and bamboo products averaged to $1.49B. The Philippines has the 6th place when it comes to exporting bamboo and rattan products [7]. Moreover, the 2009 Philippines Forestry Outlook Study of the Forest Management Bureau which indicates that there is an alarming decline in the bamboo supply in the Philippines and other ASEAN countries. This is a consequence of the sole effort of the private sector to invest in the bamboo industry with minimal to no help from the respective national governments. Likewise, there is a need to convert raw bamboo materials to other useful substances which could increase its demand, hence the supply as well. By using nanotechnoloy, the exploration of atoms and molecules have been used to improve the quality of life [8]. As such, one of its goals is to transform raw materials like cellulose to yield nanosize materials and use it without changing its chemical composition.

In recent studies, acid-catalyzed cellulose hydrolysis is used to form CNC for its almost-pure product brought by its complex and heterogeneous reaction [9]. But due to its low yield, other cost-effective, energy-efficient, and high-yielding methods shall be utilized. One of the possible alternative methods is the use of the Masuko Sangyo Friction Supermass Colloider to produce CNF. It produces ultra-fine particles with a paste-like structure. With the help of the colloider, steps such as the degradation of holocellulose to hemicellulose and cellulose and the application of the acid hydrolysis will not be needed [10]. According to [11], the term CNC is used when acid hydrolysis is employed, while the term CNF is used for mechanical nanocellulose production such as that from the friction supermass colloider.

Nanocellulose has various physical, morphological, and chemical properties that enable it to be a great starting material for other industrial applications. The applications of nanocellulose include bioplastic formation, fiber-reinforced composites, an additive for textile products, optical films, magnetic films, cosmetic products, rigidity-booster for paper products, and filtration membranes [4, 12]. [5], another use of polypyrrole with microfibrillated cellulose is the production of electronically conductive high-surface area nanocomposite substance through direct chemical polymerization method. In addition, in 2010, Bandyopadhyay-Ghosh and her colleagues, utilized nanocellulose in the manufacture of mechanical reinforcements of low-thickness polymer electrolytes in the production of a lithium battery. Likewise, [13], pointed out the other applications of nanocellulose in the increasing scientific modernity of our time. These include the fact that there is an inversely proportional relationship in the quantity of the nanocellulose present and the critical percolation threshold of the nanocomposite, while it is directly proportional to the stiffness of the continuous network of nanoparticles in the polymer matrix because of homogeneity.

Obtaining the optimal parameters of an operation is necessary to obtain the maximum yield of the desired product under the most efficient circumstances such as cost, energy,

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and manpower. Although process optimization for acid hydrolysis has been done in previous studies, there is still no parametric study on the effect of holocellulose preparation and number of passes using a friction supermass colloider on the characteristics of the cellulose nanofibrils produced from bleached Kawayan Tinik (*Bambusa blumeana* Schult.F.) pulps.

The general objective of the study was to conduct a parametric study on the effect of holocellulose preparation and number of passes on the characteristics of the cellulose nanofibrils from Kawayan Tinik bamboo. The specific objectives were to: determine the amount of residual lignin in the intermediate products of the nanocellulose production; identify the effect of holocellulose preparation as a preparatory step in the production of cellulose nanofibril from Kawayan Tinik bamboo using a friction supermass colloider; study the effect of the number of passes in the friction supermass colloider on the characteristics of the cellulose nanofibrils produced; evaluate the difference in the functional groups in the conversion of Kawayan Tinik fiber to cellulose nanofibrils; determine the morphological characteristics of the cellulose nanofibrils produced; determine the particle size distribution; and evaluate the level of crystallinity of the CNF.

II. MATERIALS AND METHOD

A. Raw Material

The pretreatment consisted of the bamboo collection, pole preparation, ring cutting, skinning, manual chipping, starch removal, air-drying, and hammer milling. The bamboo samples were obtained from the Bambusetum found in the Los Banos Experimental Station in the Mt. Makiling Forest Reserve under the Ecosystem Research and Development Bureau of the Department of Environment and Natural Resources (ERDB-DENR), Los Banos, Laguna. The harvested bamboo individuals were of three years of age. They were trimmed to about eight feet in length for ease of transportation to the DFPPS-CFNR Wood Chemistry Laboratory for further preparation. Only the middle parts of the bamboo culms were used.

Furthermore, the bamboo chips were fed to the hammer mill to reduce the size to milled form. Dirt and unmilled bamboo chips on the milled bamboo were manually checked and removed to prevent discrepancies in computations and possible source of errors. The weight and moisture content of the grounded bamboo were determined.

B. Obtaining CNF

The milled bamboo underwent pulping, bleaching, and holocellulose preparation using the Kraft pulping method, one-step hypochlorite bleaching method, and Jayme-Wise chlorite method, respectively. For Kraft pulping, 500 g of milled bamboo, at oven-dry basis, was prepared. Because of pressure cooker capacity limitations, two batches weighing 250 g each were prepared. Using a sulfidity value of 15% and an active alkali value of 18%, 49.35 g of NaOH and 8.49 g of Na_2S were mixed in 2,500 g of water. A water density of 1 g/cm^3 was assumed for calculation purposes. The moisture content of the milled bamboo was incorporated in the water added. The cooking liquor and the milled bamboo were placed in a desiccator covered with cheesecloth before placing in a pressure cooker. A liquid to wood ratio of 10:1 was utilized. It was heated until the maximum temperature of 120^{o}C was achieved where the temperature change was monitored every five minutes. An additional 3 hours was needed to complete the digestion. After the cooking time, the black liquor and pulped bamboo were removed from the pressure cooker. The pulp obtained was transferred in a clean cheesecloth and washed with distilled water to remove the remaining black liquor. The two batches of pulped bamboo were disintegrated in a commercial blender for 15 minutes for defibrillation and homogenization of the samples. The disintegrated pulps were filtered through a cheesecloth to remove the excess water before being squeezed under flowing distilled water.

For the one-step hypochlorite bleaching, the Zonrox® bleaching agent was used. Zonrox® contains 5.25% sodium hypochlorite. Further assuming that a Kappa Number of 35 and a pulp consistency of 3% exist, 3667.0 mL of Zonrox® and 3,436.2 mL of diluting water were mixed in a pail. Then, the 1230.5 g (oven-dry weight basis) of Kraft pulp was added to the pail for the 250 g requirement. The sodium hypochlorite was allowed to react with the pulp for 60 minutes at 29^{o}C with stirring every five minutes. After the prescribed reaction time, the bleached pulp was removed from the pail and filtered through a cheesecloth. The excess bleaching agent was washed and the pulp was pressed under flowing distilled water.

For the holocellulose preparation, the Jayme-Wise chlorite method was used. Because of glassware capacity constraint, two batches were used to prepare the holocellulose. For each set-up, a 1-L Erlenmeyer flask was used. A 15 g (oven-dry basis) bleached pulp was placed in each flask. A 225 mL stock solution A, consisting of 60 mL acetic acid (CH_3COOH), 1.3 g sodium hydroxide (NaOH), and 2 L of dis-

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tilled water, was added to each flask. Heating at 75°C using a hot water bath was done. A 22.5 mL of 20% (w/v) sodium chlorite ($NaClO_2$) solution was added to each of the heated flask, each covered with inverted 125-mL Erlenmeyer flasks. Next, 22.5 mL of 20% (w/v) NaClO2 solution was added after 0.5, 1.0, 1.75, and 2.5 hours have elapsed. Swirling of the flasks after every addition of sodium chlorite was done to aid in the reaction. An additional 0.75 hours was needed to complete the digestion. Afterwards, the flasks were removed from the hot water bath. The mixture inside the flasks was transferred to a Buchner funnel with a filter. For washing, a 450 mL chilled distilled water was used for each flask. Suction filtration was used to remove most of the fluid. Additional washings on the holocellulose were done using a total of 4,500 mL 1% (v/v) acetic acid and 900 mL methanol for holocellulose recovery. The excess reagents were removed using suction filtration. The holocellulose obtained was air-dried for 36 hours to reach equilibrium moisture content.

C. Determination of the Effect of Holocellulose Preparation

The mixture to be passed in the supermass colloider was prepared by suspending 20 g (oven-dry basis) holocellulose in 2 L of distilled water in a pail. After obtaining the holocellulose that passed through 150 times through the supermass colloider, about 1500 mL was collected in a plastic container. The large portion separated was essential to prevent too much mixture being passed in the equipment. Then, the passing was continued for an additional 50 passes to reach the maximum value of 200. Moreover, the same procedure was done on the bleached pulps.

1) Determination of the effect of the number of passes: A 20 g of the bleached pulps, oven dry basis, was suspended in 2 L of distilled water in a pail. It was passed for 150 passes in the colloider. After which, about 300 mL of the suspension was collected in plastic bottles. The passages through the colloider continued for 200, 250, and 300 times wherein the products were collected in containers as well.

2) CNF treatments: The samples were centrifuged for 15 minutes at 4000 rpm with two washings. The supernatant was discarded. Then, the samples were transferred to 25 mL beakers for a one-hour pre-sonication period. After that, about 20 mL of the sonicated CNF samples were transferred to cellulose acetate membrane having 25 mm width for dialysis. The cellulose acetate membranes were placed in a 1000-mL beaker with distilled water. Magnetic stirrers were used to agitate the water for 12 hours. The water was replaced every hour. After dialyzing, the samples were transferred to 100-mL beakers. Post-sonication for 30 minutes were done for homogenization. The CNF samples were placed in 250-mL Erlenmeyer flasks. Aluminum foil was used as cover. The flasks were placed in the freeze for about 16 hours. After freezing, the aluminum foils were punctured. The Erlenmeyer flasks were arranged in the freeze-dryer for two days to remove the moisture content. Finally, the freeze-dried cellulose nanofibrils were transferred to moisture-free vials.

D. Characterization of CNF

1) Lignin content: In determining the lignin content of the bamboo, intermediate products, and the CNF, the Klason Lignin Determination (KLD) procedure which primarily uses 72% H_2SO_2 was used.

2) Yields of CNF: The yields of cellulose nanofibrils were determined by determining the mass of suspension obtained from the colloider and the initial oven-dry feed and by determining the mass of the freeze-dried CNF relative to the volume of dialyzed samples.

3) Optical microscopy: The measurement of the length and width of thirty samples were measured using an Optika Microscope unit. The means and standard deviation were determined using MS Excel. The samples that underwent optical microscopy are the Kraft pulp, bleached pulp, holocellulose, and the cellulose nanofibrils taken at assumed number of passes.

4) Infrared (IR) spectroscopy: FTIR Spectroscopy analysis was used to determine the function groups present in the conversion of Kawayan Tinik bamboo to CNF. The holocellulose which passed for 200 times in the supermass colloider was centrifuged, dialyzed, and sonicated. Then, it underwent freeze-drying for two days until the moisture was removed. The freeze-dried CNF were collected in vials and were sent to the Industrial Technology Development Institute – Material Science Division of the Department of Science and Technology in Bicutan, Taguig for analysis. The FTIR Spectroscopy analysis was done on the holocellulose which passed 200 times in the colloider and on the bleached pulps which passed the colloider for 300 times.

5) Morphology: Scanning Electron Microscope (SEM) was used in determining the morphology of the cellulose fibers. The control was set at accelerating voltage of about 10 kV at various magnification. To avoid overcharging, the samples were freeze-dried and plated with gold palladium because of its organic nature. This was done by placing the sample in the conductive carbon tape. After applying air from the blower, the residues left on the tape were analyzed in the scanning electron microscope. The analysis was done on

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the holocellulose sample which passed for 200 times in the supermass colloider.

6) Crystallinity: To obtain information on the degree of crystallinity of the CNF, the X-ray Diffraction (XRD) Analysis was used. The holocellulose which passed for 200 times in the supermass colloider was centrifuged, dialyzed, and sonicated. Then, it underwent freeze-drying for two days until the moisture was removed. The size of the sample was reduced into powdered form using the Wiley Mill before being placed in the equipment.

7) Particle size distribution: The DLS was used to analyze the particle size distribution of the CNF in the suspension. To do this, the collected suspension from the colloider was centrifuged, sonicated, and dialyzed before being analyzed. The obtained suspension was placed in cuvettes. Bubble formation on the cuvette walls was avoided by tapping or tilting the cuvette [14]. The DLS was used on the holocellulose which passed for 200 times in the colloider and in the bleached pulps which passed for 300 times. The refractive index of the cellulose nanofibrils was assumed to be 1.469 at 632.8 nm [15].

III. RESULTS AND DISCUSSION

A. Pretreatment of Kawayan Tinik Bamboo

The preparation of the Kawayan Tinik sample into a more suitable form is necessary for ease in handling, improve mixing, improve dissolution rate, promote effective drying, increase surface area, and improve rate of absorption [16]. In addition, the moisture contents of the feed and intermediate products were determined because they are vital in the chemical utilization of wood and non-wood forest products. Likewise, the moisture content of the sample was taken into consideration in the calculation of reagents to be used in the conversion of bamboo to cellulose nanofibrils as well as in the determination of the yield.

B. Production of CNF

1) Kraft pulping: The liberation of the pulps or the lignocellulosic fibrous material in the bamboo was done using Kraft pulping. This chemical pulping method was performed in cooking the milled bamboo fibers using sodium hydroxide (NaOH) and sodium sulfide (Na_2S). The maximum cooking temperature for the pulping process was 120°C. However, the cooking temperatures at 130°C and below only have a small impact on the totality of the cooking process [17]. The common liquid-to-wood ratio used is 4:1 [17]; however, since a cooking temperature of 120°C and a pressure cooker was used in place of an actual digester, it was modified to 10:1 to ensure maximum reaction. This adjustment was also necessary to fully submerge the milled bamboo to prevent product burning and overcooking. Moreover, the active alkali and sulfidity were kept relatively low to obtain a higher yield and lower viscosity and residual alkali [18]. Likewise, the sulfide is needed to hasten the cleavage of phenolic units by attacking the ether links. The destruction of the carbon-oxygen bond is one of the most important reactions in the chemical pulping process [19]. The 18% active alkali gives the lowest bamboo Kappa number [18], while the sulfidity value of 15% results to the optimum delignification rate [20]. The computed yield of the Kraft pulp for this study is 57.93%.

2) Hypochlorite bleaching: Commonly used bleaching chemical used are hydrogen peroxide (H_2O_2), calcium hypochlorite ($Ca(OCl)_2$), and sodium hypochlorite (NaClO). However, hydrogen peroxide and calcium hypochlorite causes pulp discoloration or pulp darkening in the subsequent nanocellulose treatments [21, 22]. Hence, sodium hypochlorite is the most suitable available bleaching material to use. In the pulp bleaching done, a one-step bleaching technique was applied using sodium hypochlorite under room temperature (29°C) for one hour. The sodium hypochlorite results to cellulose degradation because it is a non-specific bleaching material [20]. Thus, one hour was set as the limit to prevent the unwanted cellulose degradation. An increase in the optical brightness of the pulp was observed after the sodium hypochlorite bleaching process. However, some portions still show pulp discoloration. This means that residual lignin was not totally removed. The yield of the bleached pulp was 99.99%. The losses can be attributed to the removal of organochlorine compounds in the washings.

3) Jayme-wise chlorite method: The holocellulose was prepared using the Jayme-Wise Chlorite Method. Holocellulose is the overall carbohydrate component in plants which is basically a mixture of cellulose and hemicellulose. The holocellulose was prepared by digesting the bleached pulp in a 20% (w/v) sodium chlorite solution. It was washed using cold distilled water, 1% (v/v) acetic acid, and methanol. Suction filtration was employed to maximize recovery. It is white in color and fibrous in appearance. Because of the sodium chlorite and subsequent washings, the ash-forming components, extractives, and residual lignin were removed. The yield of the holocellulose was calculated and was found to be 76.58%.

4) Grinding using the friction supermass colloider: The friction supermass colloider was used to reduce the size of the holocellulose into the nanosize region. By continuously passing the sample in the equipment, the grinding mecha-

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nism decreases the length and width of the fibers. Between the length and width, the length was used as the parameter to validate the morphology of the fiber as it approaches the nanosize region. The cellulose nanofibril length is about 2,000 nm [11]. The plot of fiber length vs. holocellulose made using MS Excel. From the plot, it was evident that the fibril length decreases with the increase in the number of passes in the supermass colloider. The change in length was large at the first 50 passes. The polynomial equation obtained for representing the plot is given by:

$$L = 0.5725N^2 – 168.47N + 13892 \quad (1)$$

Where:
L is the fibril length (nm)
N is the number of passes in the supermass colloider
The number of passes needed to achieve the required length was determined and was found to be between the 170th and 180th passes. Assuming the 175th pass as the centerpoint, the minimum and maximum value for the 2^k factorial design were the 150th and 200th passes. The plot of fiber length vs. bleached pulps without holocellulose preparation was made using MS Excel. The trend also showed that the length of the fibril from bleached pulp decreases with an increase in the number of passes. It was seen that a large change was seen at the first 150 passes. The polynomial equation representing the plot is given by:

$$L = 14.512N^2 – 6582.6N + 720012 \quad (2)$$

5) Centrifugation, dialysis, sonication, and freeze-drying: After the passing in the supermass colloider, the suspension obtained from different passes was placed in containers separately. The centrifugation was done for solubilisation and soluble material elimination. It also increases the concentration of the CNF produced. Sonication was employed for one hour to prevent agglomeration. Following this, the samples were placed in cellulose acetate membranes for dialysis. It is essential for the removal of ion traces caused by various treatments done. Sonication was applied for 30 minutes to homogenize the samples. Finally, freeze-drying was done to remove the moisture in the samples.

C. Characterization of CNF

1) Klason lignin determination: The lignin, which is a complex organic compound in plants, is the reason for the dark pigmentation of plants that also contributes to its structural integrity. In experiments wherein the cellulose is extracted, the presence of lignin is unwanted. As a result, bleaching techniques are needed to control or remove the lignin in plant.
The extent of lignin removal in various stages of the bamboo conversion to CNF was measured by comparing the amount of lignin left in the crucibles after treatment with 72% sulfuric acid and boiling water (Table 1). For the bleached pulps and the holocellulose, less than 1% lignin was found. Hence, the presence of lignin is negligible and the additional bleaching for the production of the cellulose nanofibrils from the bleached pulps and the holocellulose is no longer needed.

TABLE 1
LIGNIN CONTENTS OF EXTRACTIVE-FREE BAMBOO, KRAFT PULP, BLEACHED PULP, AND HOLOCELLULOSE

Material	Lignin Content[a] (%)
Extractive-Free Bamboo	27.87
Kraft Pulp	11.86
Bleached Pulp	0.89
Holocellulose	0.11

[a] Values are means of replicates.

2) Yield of CNF: The samples containing holocellulose that was passed in the colloider for 200 times and the samples containing bleached pulps that was passed in the colloider for 300 times gave the highest yields at 66.02% and 73.52%, respectively. This signifies that the amount recovered for the other samples were not high enough. Using statistical analysis, it was seen that both the holocellulose preparation and number of passes were significant in the CNF yield. The production of nanocellulose increased with the application of the friction supermass colloider [23]. [24] also confirmed that the yield of cellulose nanofibrils was affected by the number of passes because of the mechanical treatment intensity.
3) Optical microscopy: The fiber length and width of the Kraft pulps, bleached pulps, holocellulose, and CNF samples were determined using an Optika Optical Microscope. The aspect ratio and degree of polymerization were calculated. The length, width, and aspect ratio of the holocellulose with

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200 passes are 1366.67 nm, 109.77 nm, and 13.20, respectively. On the other hand, for the bleached pulps with 300 passes, the length, width, and aspect ratio were averaged at 3086.67 nm, 102.40 nm, and 36.19, respectively.

Optical microscopy showed the decrease in length and width of the fibers as chemical treatment is applied and as the number of passes in the supermass colloider increases. According to [23], microfibrillar cellulose are produced by applying the grinding mechanism in the bamboo. By doing so, smaller and finer particles are obtained. Application of chemical techniques may then be applied to further change the structure of the nanocellulose material. Moreover, it is evident that the bundling, aggregation, and overlapping of cellulose nanofibrils occur. In order to properly measure the CNF, [25] suggested that only the well-dispersed samples whose both ends could be identified were to be measured.

The surface of the Kraft pulps appears smooth. This is caused by the still abundant amount of lignin and other extractives in the sample. Comparing the Kraft pulps, bleached pulps, and the holocellulose, the hypochlorite bleaching on the bleached pulps and the application of sodium chlorite on the holocellulose resulted to rough fibers.

Using statistical analysis, it was seen that for CNF length, width, and aspect ratio, the holocellulose preparation and number of passes in the supermass colloider were significant parameters.

The formation of entangled networks of fibers appeared. A similar case was observed by [26] in the production of cellulose nanofibrils. The entanglement issue was explained by [27] as a result of the high aspect ratio of cellulose microfibrils and cellulose nanofibrils. Moreover, large bundles of fibers and fiber aggregates were seen. These can be attributed to the incomplete grinding of the supermass colloider. A possible solution for these problems was the increment in the clearance of the grinding stones to shorten their distances. This would allow maximum interaction between the grinding material and the feed.

Furthermore, to fully determine the effect of number of passes solely in the bleached pulps, the length, width, and aspect ratio of the cellulose nanofibril produced at 150, 200, 250, and 300 passes were determined. The design of experiment done was Completely Randomized Design (CRD). The CRD is useful for data obtained from experiments that are being classified according to only one treatment. Pairwise Mean Comparison with Scheffe Test was done to determine the presence of significant difference among the means of the different number of passes.

From the measurements using optical microscopy, it was seen that the CNF lengths from the 150, 200, 250, and 300 passes significantly differed from each other. Moreover, for the width, that of 150 and 200 passes did not significantly differ. The same can be said for the 250 passes and 300 passes. Likewise, the CNF widths for 150 and 200 passes significantly differed from the 250 and 300 passes. Furthermore, for the aspect ratio, determination of the significance in difference was difficult because of lack of trend in the means. Nevertheless, the CNF aspect ratio from the 150 passes significantly differed from the 200, 250, and 300 passes.

[28], discussed that the use of optical microscopy for the measurement of cellulose nanofibril length and width may not be suitable for very small fibrillated cellulose because of limited resolution. However, considering the financial cost of other microscopy techniques, optical microscopy may still be used.

4) Fourier transform infrared spectroscopy: The high densities of hydroxyl groups on the surfaces of cellulose nanocrystals and cellulose nanofibrils result to sharper stretching peaks. The CNC peak at 3336.0 and the CNF sample peaks at 3340.6 correspond to the intermolecular hydrogen bonding. It can be seen that the peaks for OH stretching vibrations, CH_2 bending, CH stretching vibration, and C-O-C stretching are present both CNF samples. As a result of alkali treatment in the CNF samples, an increase in –OH concentration occur as seen in the increased intensity of the peaks compared to that of the raw bamboo and Kraft pulps. Moreover, the peak at 1604 cm^{-1} corresponds to the aromatic C-C in plane symmetrical stretching vibration of aromatic rings in lignin. This is only observed in the raw bamboo and not in the chemically treated fibers and cellulose nanofibrils. This may be attributed to the removal of lignin and hemicellulose. It is notable to say that the spectra of the cellulose nanofibril is similar to that of the cellulose without lignin. In addition, the C-O-C pyranose ring stretching vibration is corresponded by the 1102-1104 peaks (Figure 1). This phenomena is observed for the treated bamboo and the cellulose nanofibrils but not in the raw bamboo. This is supported by the fact that the cellulose has cellobiose units consisting of oxygen connected only to carbons.

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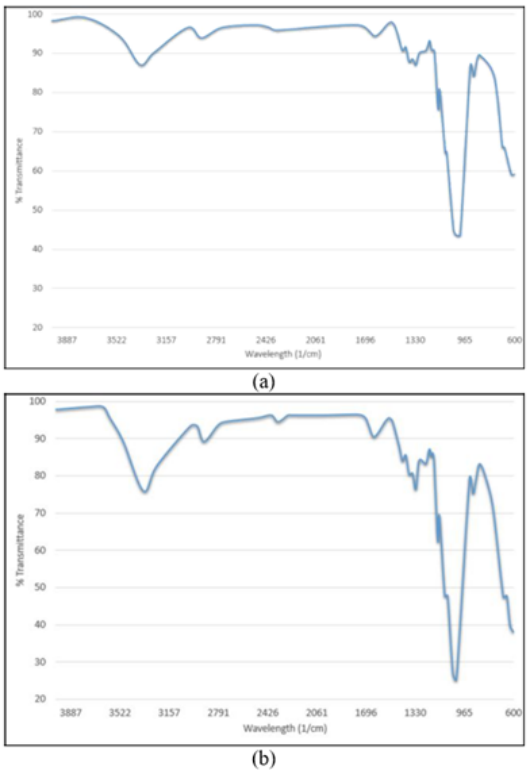

Fig. 1. Infrared spectra of (a) holocellulose with 200 passes and (b) bleached pulps with 300 passes obtained using FTIR Spectroscopy

According to the Thermo Galactic Library, as provided by ITDI-STD, the holocellulose with 200 passes and the bleached pulps with 300 passes has a 96.62% and 96.28%, respectively, similarity with methyl cellulose. Nevertheless, the backbone was still cellulose.

5) DLS: The samples were subjected DLS to determine the particle size distribution and the average particle size. The average particle size of the holocellulose with 200 passes and bleached pulps with 300 passes are 47.2 µm and 34.2 µm, respectively (Figure 2). The length is the average size of the microcellulose under the highest peak in the DLS particle size distribution graph. The cumulative percentage of particles under the peak being observed was represented as intensity. All of the graphs generated showed that there existed a narrow range among the fibril lengths.

Estimating from the graphs, a range of 10 µm and 200 µm was obtained. Although the average particle sizes gathered were far from the expected outcome, the DLS revealed that relatively homogeneous particles were produced from the supermass colloider.

The large particle size obtained can be attributed to the entanglement of the cellulose nanofibril because of its large aspect ratio. This was seen in the images produced using Optical Microscopy and SEM. The thread-like feature of CNF made the untangling difficult even with the application of sonication. Nevertheless, [26], viewed the entanglement of cellulose nanofibrils as an important aspect in the formation of strong polymers as reinforcing agents.

Methods on the prevention of tangling must be established to avoid errors in the characterization. However, for the holocellulose with 200 passes, there were sightings of CNF with 8.85 µm length (0.01% intensity). Likewise, for the bleached pulps with 300 passes, CNF with 5.54 µm length were observed (0.01% intensity).

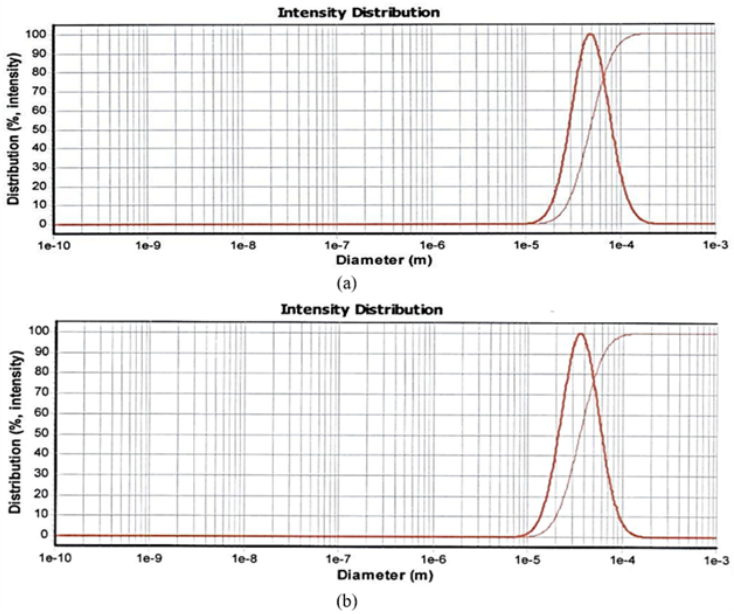

Fig. 2. Size distribution of the (a) holocellulose with 200 passes and (b) bleached pulps with 300 passes measured using DLS

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[24] suggested the use of the cellulose nanofibril supernatant upon centrifugation at low rpm as the material for DLS analysis. However, the sensitivity and selectivity of the testing equipment must be taken into consideration if such is to be done. According to [29], it is essential to determine the size and size distribution of cellulose nanofibrils. However, a sufficient and reliable validation mechanism is yet to be established because of the high aspect ratio of CNF. As a result of the lack of homogeneity and large difference in the average particle size observed in the results of DLS and that of Optical Microscopy and SEM, relating the corresponding results was not possible.

6) SEM: The surface morphology of the cellulose nanofibril was investigated using SEM which captures real-time photographs of the microstructure of the CNF at high magnifications. The SEM has higher scanning area compared to other microscopy techniques even though it only provides two-dimensional images. Because of the limitations of the Optical Microscopy, SEM is needed to produce photorealistic images for comparative visual analysis and morphological investigation relative to surrounding materials. From the SEM images obtained, fibers having large lengths and widths can be seen. This may be attributed to the incomplete grinding in the supermass colloider. Moreover, it can be seen in the figure that many fibers tangle to one another making an appearance of a large chunk of material. This observation can be related with the readings of the Dynamic Light Scattering which showed the presence of large particle sizes. In addition, SEM is a microscopy technique that requires minimal sample. This may result to insufficiency to represent the entire CNF population.

A mean value of 2169.26 nm and 58.35 nm were obtained for the length and width, respectively, leading to an aspect ratio of 39.6882 (Figure 3). Although the length and width obtained from the holocellulose with 200 passes were larger than that of the target value, the similar values for the aspect ratio corresponds to the same two-dimensional shape of the cellulose nanofibrils obtained relative to the literature. Furthermore, results showed that the passing in the supermass colloider had a major effect in the fibrillating of the cellulose fibers into cellulose nanofibrils having a homogenous size distribution. In addition, the mean fiber length and its intensity are greatly influenced by the chemical and mechanical pretreatment [28].

Comparing the SEM images of the cellulose nanofibril from Kawayan Tinik and that of the original grounded Kawayan Tinik from literature, the original bamboo had fibrils of several hundred microns. Also, the smooth surface of the bamboo fibers resulted from the presence of pectin, waxes, and lignin. Likewise, the removal of lignin, hemicellulose, and other extractives was evident in the cellulose nanofibril and the individual microcellulose fibers emerged from the original fiber bundles.

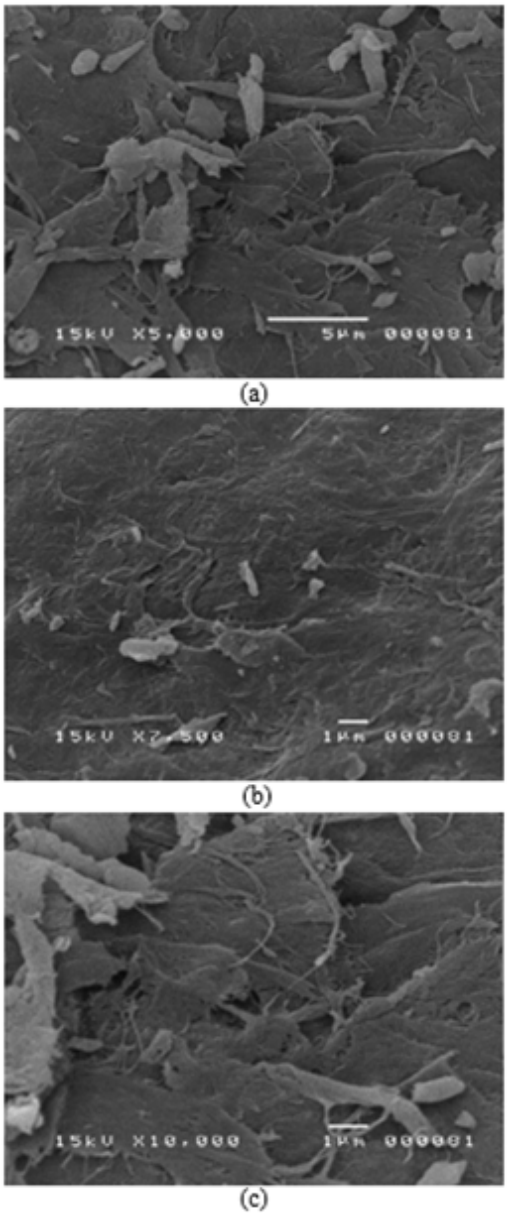

Fig. 3. Holocellulose with 200 passes viewed using SEM at 15kV (a) 5000X, (b) 7500X, and (c) 10000X.

7) XRD analysis: The XRD Analysis was done in the ITDI-MSD. The XRD graph of the cellulose nanofibril from holocellulose which was passed for 200 times in the supermass colloider was produced. The three strongest peaks detected were located at 2θ = 22.18, 15.81, and 20.24 with d values of 4.00 $\mathring{A}$, 5.60 $\mathring{A}$, and 4.38 $\mathring{A}$, respectively (Figure 4).

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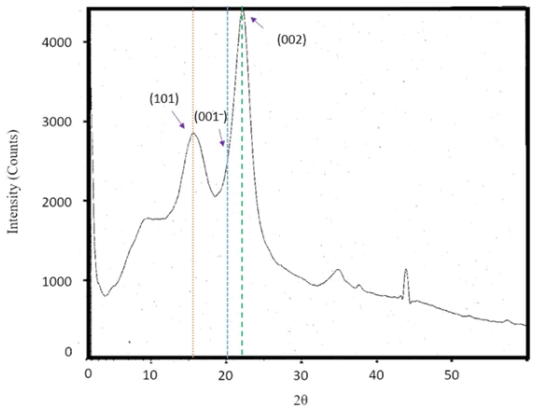

Fig. 4. XRD results of cellulose nanofibril from holocellulose with 200 passes in the supermass colloider

The degree of crystallinity of the cellulose nanofibril sample was determined:

$$\%\ Crys = \frac{(Imax - Imin)}{Imax} \times 100 \qquad (3)$$

Where:

%Crys is the degree of crystallinity (%)

I_{max} is the height of the peak at (002) configuration above the base line

I_{min} is the height of the amorphous diffraction

The degree of crystallinity of the cellulose nanofibril from the holocellulose with 200 passes was calculated to be 71%. From [11], the percent crystallinity of raw bamboo, cellulose nanofibril, and cellulose nanocrystals were 60%, 68%, and 73%, respectively. The degree of crystallinity of CNF was lower than that of the CNC literature value because the hydronium ions in the acid hydrolysis enter the amorphous regions of the cellulose which result to hydrolytic cleavaging of the glycosidic bonds that releases the crystallites. Nevertheless, the percent crystallinity of the CNF obtained in the experiment can be attributed to the high number of passes in the supermass colloider since the mechanical treatment resulted to the attrition of the amorphous regions in the holocellulose.

IV. SUMMARY AND CONCLUSION

CNF were produced from Kawayan Tinik (*Bambusa blumeana* Schult.f) pulps. Kraft pulping using NaOH and Na2S was employed to free the bamboo pulps. The bleached pulps were obtained by using the household bleaching agent Zonrox® which consists of 5.25% sodium hypochlorite. Moreover, the holocellulose was obtained by using 20% $NaClO_2$ on the bleached pulps. The bleached pulps and the holocellulose were subjected to mechanical grinding using the Masuko Sangyo Friction Supermass colloider to produce cellulose nanofibrils. The two were subjected to the same number of passes (150 and 200). The effects of the different combinations of the holocellulose preparation and number of passes in the supermass colloider on the yield were determined. The same was also done to identify the effects on the morphological characteristics of the cellulose nanofibrils. Likewise, the effect of the number of passes solely in the bleached pulps was determined by increasing the number of passes (250, 300) to see if doing so will compensate the absence of holocellulose preparation.

The presence of lignin in the raw bamboo and in the intermediate products were determined using KLD. The transformations of Kawayan Tinik from raw to nanofibril were characterized using FTIR Spectroscopy. The length and width of the CNF were determined using Optical Microscopy and SEM. In addition, the estimated particle size distribution of the CNF was determined using DLS.

Using KLD, it was found that the chemical treatment of Kawayan Tinik resulted to significant decrease in its lignin content. The bleached pulps and the holocellulose have 0.89% and 0.11% lignin content, respectively, making the lignin present in the samples negligible. As a result, further determination of the lignin content of the CNF produced was not necessary.

The different combinations of holocellulose preparation and number of passes in the supermass colloider had significant effects in the CNF yield. The treatment that uses holocellulose preparation and 200 passes is the most suitable combination for the production of CNF from the holocellulose with a yield of 66.0223%. However, using only the bleached pulps and increasing the number of passes to 300 would result to a yield of 73.5228%. Likewise, using optical microscopy, it was found that the holocellulose with 200 passes gave the shortest length at 1,367 nm. The width and aspect ratio was difficult to determine using optical microscopy because of the limitations in the resolution. Nevertheless, the bleached pulps with 300 passes showed an aspect ratio of 36.2 which is nearest to the set point (40). The result of the DLS was different from what was expected. Because of the high aspect ratio of cellulose nanofibril, entanglement of the individual fibers with other fibers occur. This phenomenon causes bundling and overlapping of CNF resulting to an incorrect analysis of particle size and particle size distribution. Nonetheless, this entanglement is desired for polymer production.

Using two-level factorial, it was found that the holocellulose preparation and number of passes in the supermass colloider has significant effects in the yield, length, width, and aspect ratio of cellulose nanofibril production. Furthermore, using the Completely Randomized Design with

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Pairwise Mean Comparison and Scheffe Test, it was seen that the number of passes has significant effect on the CNF length. For the width, the outcome from 150 and 200 passes were not significantly different and so are those from 250 and 300 passes. However, the CNF width from 150 and 200 and that of 250 and 300 passes are significantly different. Furthermore, a conclusion for the aspect ratio was difficult due to lack of trend in the means.

Using FTIR Spectroscopy, the backbone of the CNF samples were proven to be cellulose. The presence of vital functional groups such as OH stretching vibrations. C-H bending, C-H stretching, and C-O-C stretching were corresponded from the peaks observed. An increase in the hydroxyl concentration compared to raw bamboo was seen as a result of alkali treatments done. Moreover, the peaks corresponding to the presence of lignin were not observed for the CNF samples as a result of the delignification processes.

Using XRD Analysis, the degree of crystallinity of the cellulose nanofibril was determined which was 71%. The value was higher than the raw bamboo crystallinity (60%), but lower than that of cellulose nanocrystals (73%). The mechanical treatment of the holocellulose contributes to the attrition of the amorphous region which increases the crystalline portion of the CNF.

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