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Properties of paper incorporated with nanocellulose extracted using microbial hydrolysis assisted shear process

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Abstract. In the past two decades, nocellulose is widely as a renewable source for various industrial applications. Nanocellulose in the form of nanocrystalline (NCC) or nanofibrillated (NFC) has many applications, mainly in enhancing the mechanical strength of composite materials, or as precursors for supercapacitors, aerogel, hydrogel and membrane fabrication processes. In this study, microbial hydrolysis combined with shear mechanical treatment was used as an alternative method to produce nanocellulose. Commercial cellulase enzyme from *Trichoderma reesei* (ATCC 26921) was used to hydrolyse bleached soda cellulose from *Macaranga*, a tropical pioneer forest species. The enzymatic hydrolysis was followed by enzyme deactivation, purification, homogenization and sonication. Resulting nanocellose was added at 1%, 2.5%, 5% and 10% loading into 60±3 gsm laboratory handsheets prepared according to TAPPI T205 method. The handsheets were tested for physical, mechanical and optical properties based on TAPPI T220 method. Results, among others, showed that the addition of nanocellulose up to 10% can reduce the air permeability of paper by 50%. This indicates the potential applications in food and pharmaceutical packaging in which degradation due to bacterial activities can be reduced and product shelf life can be prolonged.

1. Introduction

Cellulose is one of the most abundant renewable materials which mainly comes from plants such as wood, cotton, sugar bagasse, jute fibers and a variety of other natural products including bacteria, algae, fungi, invertebrates, amoeba and even in tunicates [1]. Recently, nanocrystalline cellulose (NCC) production from lignocellulosic materials attracts more and more attention due to their promising properties such as high mechanical strength (modulus 100-140 GPa), low density (1.6 - 3 gcm^{-3}), high aspect ratio, high surface area, chemical tenability, modifiable surface properties due to their reactive hydroxyl (-OH) side group, environmental sustainability and low cos [2-3]. Hence, NCC has been widely tested for diversed applications (polymer nanocomposites towards high-value added materials, enzyme immobilization, drug delivery and biomedical applications). During the 10th Malaysia Plan, Forest Research Institute Malaysia has studied several tropical forest species such as Mahang for the application of high efficiency processing technology towards juvenile wood. Mahang

(*Macaranga* spp.) belongs to the family Rubiaceae. There are about 27 species of *Macaranga* in Malaysia and 280 worldwide. Studies conducted in Peninsular Malaysia indicated they are common pioneering species and amongst the first to successfully colonise open or disturbed areas. The species is distributed throughout Malaysia and is locally abundant on disturbed sites of virtually any soil type [4, 5]. Its long fibres and light coloured wood makes it a commercially potential source of timber for pulp, paper and wood composite boards. In the past, it has been used as match sticks. Compared to other Malaysian timbers, *Macaranga* fibres have a high length to diameter ratio (L/D) of 52.2 and cellulose content of 46.5% [6]. These two properties indicate that *Macaranga* is a potential candidate for pure cellulose production with good properties. Thus, as an extension of the previous research findings, *Macaranga* cellulose was further exploited as a raw material for nanocellulose production.

Incorporation of nanocellulose in biocomposite products such as paper has been known to improve strength and surface properties [7]. Adding nanocellulose in other polymer films such as polyvinyl acid (PVA) and styrene-butadiene latex was reported to improve the modulus of elasticity but no significant improvement of tensile strength was observed [8]. However, other researchers have observed improvement of tensile strength (up to 30%) and thermal properties upon addition of nanocellulose in PVA composite [9, 10]. For textile such as polyester, incorporating nanocellulose has been reported to improve breaking load, increase onset temperature and reduce air and water [11]. Although the nanocellulose extraction processes can sometimes be tedious and costly, the improvement or benefits gained when incorporated into products can compensate the cost mainly due to the fact that most of the time, the dosage level of nanocellulose is below 10%.

In this work, the physical, optical and mechanical properties of paper added with nanocellulose are evaluated and the potential applications of the paper produced are recommended.

2. Materials and method

Logs from *Macaranga tanarius* trees were obtained from FRIM campus in Kepong, Selangor, Malaysia. Chemicals used were sourced from local Malaysian suppliers. Cellulase enzyme from *Trichoderma reesei* (ATCC 26921) was purchased from Sigma Aldrich Malaysia. *Macaranga* logs were processed into sawn timbers at FRIM sawmill before being chipped and screened into various sizes and the portion between 2 and 2.5 cm width and 3 to 8 mm thick was taken for chemical pulping. Soda pulping using 25% (wt/wt) sodium hydroxide (NaOH) was carried out in a 16L rotary digester at 170°C for a total of 3.5 hours (1.5 hrs heating time) with 1:6 wood-to-liquor ratio. The resulting pulp had a Kappa number of 14. This pulp was next bleached using a 5-stage elemental chlorine free (ECF) DEDED process with 3% Sodium chlorite ($NaClO_2$) for D stages and 2% NaOH for E stages. All bleaching stages were carried out at 70°C with reaction time of 120 minutes for stage 1, 60 minutes for stages 2 and 4 and 90 minutes for stages 3 and 5.

For the extraction of nanocellulose, bleached cellulose was mixed with 0.05M sodium acetate buffer at 5% consistency in a flask. 2.5% cellulase enzyme in liquid form was added and the mixture was placed in a shaking water bath at 25°C, 130 rpm and for 72 hours. The hydrolysis process was terminated by immersing the flask in a water bath at temperature above 80°C for 10 minutes. The residual liquid was filtered and the cellulose was thoroughly washed and later homogenised for 2 hours at 10,000 rpm. The homogenised suspension was filtered and the supernatant collected was sonicated, filtered and freeze-dried. Details of the nanocellulose extraction process are presented elsewhere [12-14]. The properties of nanocellulose were determined using Transmission Electron Microscope (TEM), Atomic Force Microscope (AFM) and X-Ray Diffraction (XRD).

Standard laboratory handsheets of 60±3 g/m^2 were prepared according to TAPPI T205 sp-02 method using unbeaten *Macaranga* bleached soda pulp obtained earlier. Bleached pulp was first disintegrated with NCC (dosage of 1%, 2.5%, 5% and 10%) before alum was added at 2% dosage as a retention aid in the stock chest. The pH of the stock was maintained between 5.5 and 6.5. Six most uniform pieces were selected for testing based on TAPPI T220 sp-01 method. Individual tests were carried out according to methods listed in Table 1. For air permeance, Lorentzen-Wettre Bendtsen type Smoothness and Porosity tester model 6 was used and samples were tested based on ISO 5636-3 isostatic method.

Table 1. Test method for preparation and testing of laboratory handsheets.

Properties	Method
Handsheet preparation	TAPPI T205 sp-02 [15]
Physical Testing	TAPPI T220 sp-01 [16]
Specific volume (bulk)	MS ISO 534: 2007 [17]
Air permeance	ISO 5636-3 1992 [18]
Opacity	MS ISO 2471: 2010 [19]
Brightness	MS ISO 2470-1: 2010 [20]
Tensile strength	MS ISO 1924-2: 2010 [21]
Tear strength	MS ISO 1974: 1999 [22]
Folding endurance	ISO 5626: 1993 [23]

3. Results and discussion

The results of pulping and nanocellulose extraction process are summarised on Table 2. The pulping yield is relatively low (typical hardwood timbers have pulping yield in the range of 40-50%) possibly due to the high sodium hydroxide level of 25% (wt/wt). The initial alpha-celllulose content of Mahang is about 46.5% [6]. The yield of only 35.4% and kappa number of 14 indicate that some of the cellulose have been degraded during pulping. Another 10% weight was lost during bleaching. Furthermore, it was observed that the nanocellulose yield or recovery from the bleached cellulose is very high compared to other works using enzyme reported in the literature between 63-84% [24, 25]. However, based on the XRD result (Figure 1) the crystallinity is marginally low compared to nanocellulose extracted using acid hydrolysis, typically in the range of 65-85%. Our previous work using acid hydrolysis showed crystallinity of 79% for NCC from *Acacia mangium* [10]. Other researchers have reported the crystallinity of nanocellulose obtained using enzyme pre-treatment in the range of (45-68%) [24-26]. The crystallinity of enzyme treated nanocellulose followed by mechanical treatment have shown reduction mainly due to the mechanical or hydrodynamic forces applied to the cellulose [27-28]. Figure 1 shows the diffractogram of *Macaranga* nanocellulose with the most intense peak for the 200 crystal plane ($2\theta = 22.5$). The peaks observed in the diffractogram correspond to typical crystalline character of cellulose Iβ [29]. The crystallinity measured for Macaranga nanocellulose NCC was 0.54 estimated using the Segal formula. The TEM and AFM images of the nanocellulose extracted are shown in Figure 2 (a) and (b) respectively. From the analysis of AFM image, the resulting nanocellulose has an average width of 8.1±1.4 nm and average length of 146±0.2 nm. The resulting width result agrees with the findings of other researchers who used enzyme pre-treatment in nanocellulose extraction [30-31]. The aspect ratio of NCC obtained in this work is smaller than NCC obtained in our previous work (18 vs. 26) [10]. In papermaking terminology, aspect ratio is also known as felting power and higher values have a positive effect of strength on paper strength [32]. This can also be applied to other products such as thin films.

Table 2. Overall *Macaranga* nanocellulose production results [33]

Properties	
Pulping yield	35.4%
Kappa number	14
Bleaching yield	90%
Nanocellulose yield	90.2%
Yield from wood	27.6%

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Average nanocellulose length	146±0.2 nm
Average nanocellulose width	8.1±1.4 nm
Crystallinity	54%

The freeness of pulp stock at 3% consistency determined using TAPPI T 227 om-99 method is shown in Figure 3. The addition of 1 to 10% NCC showed less than 2.3% difference in the stock freeness. The specific volume or bulk of laboratory handsheets produced slightly decreased (by 13%) with higher addition of NCC (Figure 4). This could be explained by the fact that nanocellulose take less space and provide compact structure to the paper, thus reducing the volume while retaining the same mass. However, no significant changes were observed on the opacity and brightness of the paper (both change by less than 1%). These results are similar to works by Gonzalez [34]. Another researcher reported an increment of 2.8% and 3.2 % of opacity and brightness, respectively, with the addition of 10% to chemi mechanical pulp [35].

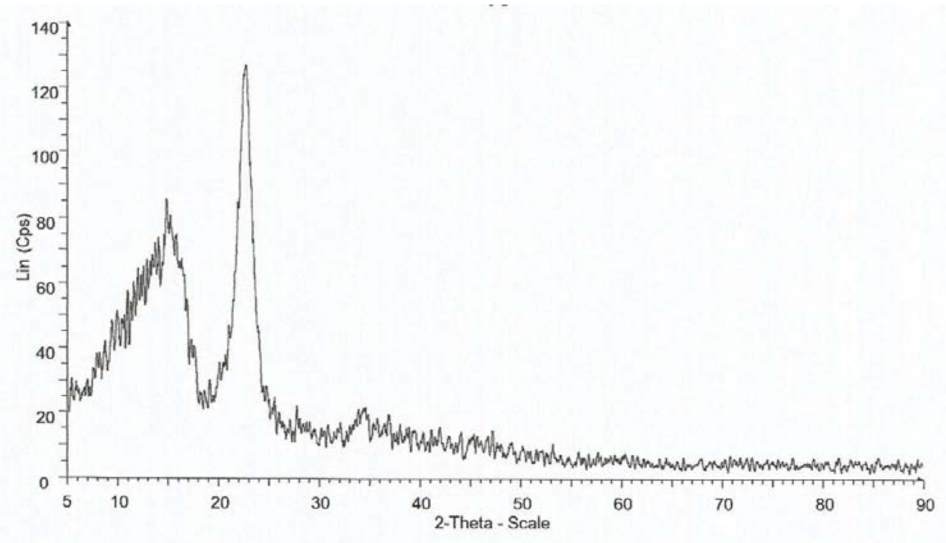
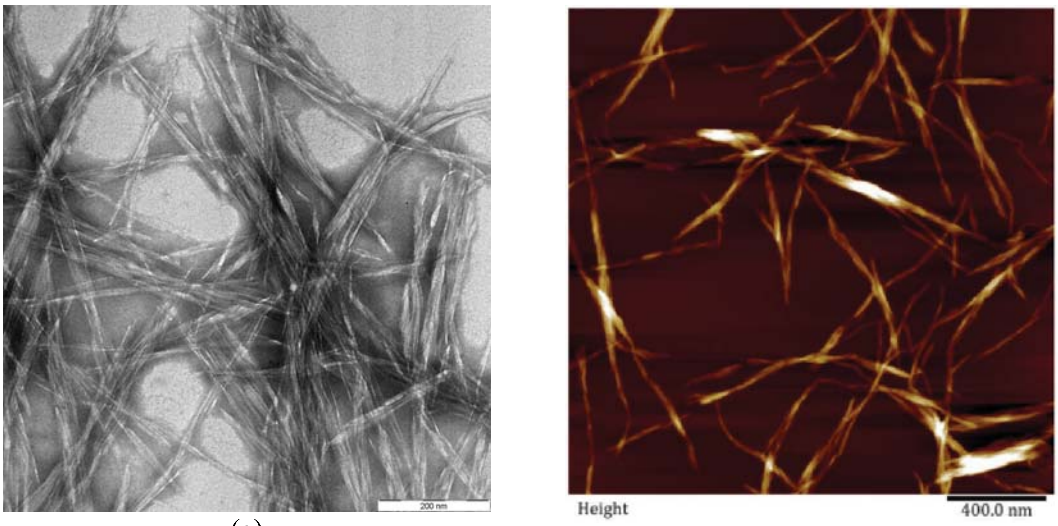


Figure 1. X-ray diffraction (XRD) diffractogram of nanocellulose.

All three strength tests conducted showed improvement of laboratory handsheets at higher NCC additions (Figure 5). The folding endurance improves by four-folds, tensile strength by 58% and tear strength by 35%. The enzymatic hydrolysis reaction cleaves the amorphous region of cellulose at the C-O-C bond on the cellulose chain, creating new chain ends with a new hydroxyl group on the surface of each as proposed by Schmidt [36]. Thus, each C-O-C bond broken will result in two additional hydroxyl groups. The presence of hydroxyl charge on the surface of nanocellulose is enhanced with higher dosage of nanocellulose addition. This factor, cumulatively increases the hydroxyl charge of the mixture which improves fibre bonding (intramolecular hydrogen bonds with neighboring glucose base unit) when the laboratory handsheets are dried.



(a) (b)

Figure 2. (a) TEM image of nanocellulose (b) AFM image of nanocellulose.

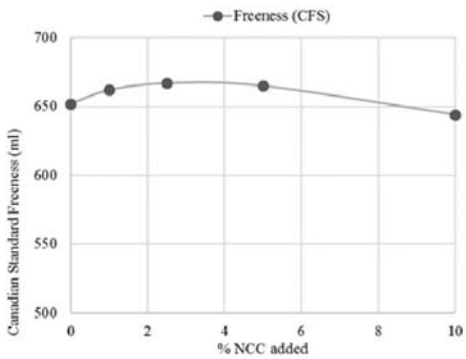


Figure 3. Canadian Standard Freeness of paper incorporated with nanocellulose.

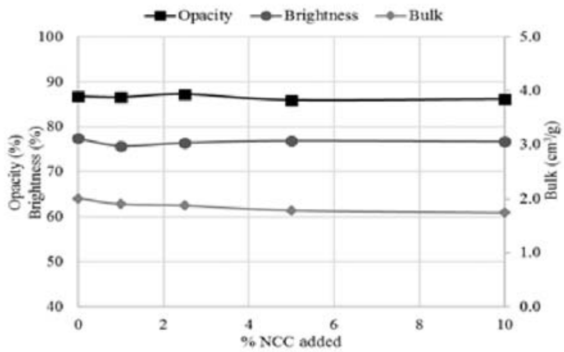


Figure 4. Bulk, opacity and brightness of paper incorporated with nanocellulose.

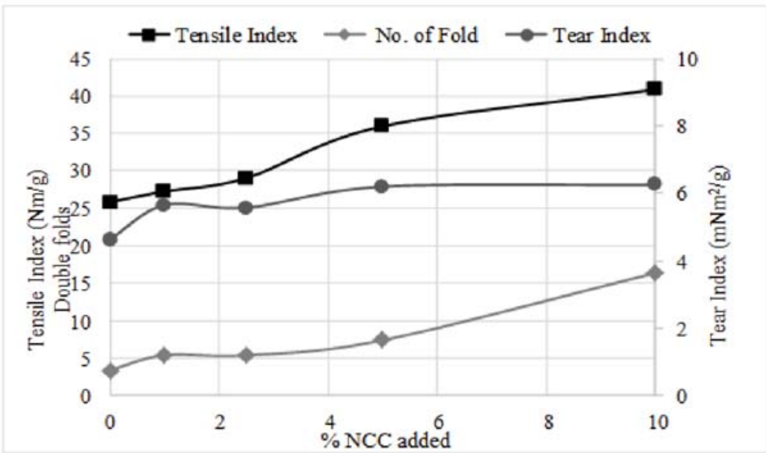


Figure 5. In this case simply justify the caption so that it is as the same width as the graphic.

The most interesting improvement of paper properties obtained in this study is the reduction of air permeance as shown in Figure 6. Increasing the dosage of nanocellulose to 10% reduce the air permeance by up to 53.7%. The conventional laboratory handsheet produced in this study has air permeability of 21.2 µm/Pa.s and this value was reduced to just 9.8 µm/Pa.s upon addition of 10% nanocellulose. The compact assembly of the cellulose/ nanocellulose in the paper provides longer routes for air molecules to diffuse through as explained by Nair et al. [37]. This result is very useful in packaging grade papers for food and pharmaceutical products as the reduction in air permeability will slow down microbial growth, thus lengthening the product shelf life.

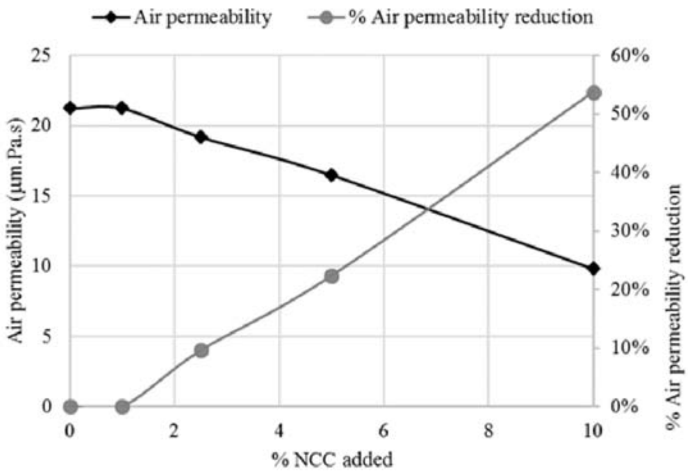


Figure 6. Air permeability of paper incorporated with nanocellulose.

4. Conclusions

This study shows that nanocellulose can successfully be extracted via microbial hydrolysis pretreatment followed by mechanical process. The nanocellulose recovery from the bleached cellulose obtained in this study is very high, exceeding 90%. The crystallinity is quite low compared to nanocellulose extracted using acid hydrolysis reported by others (54% vs. 80%). However, the observed crystallinity in this study is still in the range reported by other researchers (45-68%). Nanocellulose obtained in this study was added to paper and results showed that some of the paper properties are affected. The addition of up to 10% nanocellululose slightly reduces the specific volume and significantly improve tear strength, tensile strength and folding endurance. However, no significant changes were observed on the opacity and brightness of the paper. In terms of paper barrier properties, the reduction of air permeability up to 53.7% was observed upon addition of nanocellulose of 10%. This result is very useful in packaging grade papers for food and pharmaceutical products as the reduction in air permeability will slow down microbial growth, lengthening the product shelf life.

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