Isolation and characterization of cellulose nanofibres from three common Nigerian grasses

KO Ejeta*, TO Azeez, AT Banigo, KI Nkuma-Udah and E Ajuogu
Department of Biomedical Technology, Federal University of Technology, PMB 1526, Owerri, Imo state, Nigeria.

*Correspondence author: koejta@yahoo.co.uk

Abstract
Nanocellulose may be in nanocrystal or nanofibre form which may be extracted from purified cellulose by different methods. These include methods like dual-opposite-spinneret electrospinning, mechanical methods, a combination of chemical and mechanical methods, cryocrushing and enzymatic approaches. Nitrocelluloses have been extracted from various plants but not much has been reported on the yield from grasses. In this study, cellulose nanofibres (CNFs) were extracted from three common grasses; Pennisetum purpureum, Cynodon dactylon and Axonopus compressus by formic acid hydrolysis with the micrograph of CNF for each grass obtained. The CNFs are web-like long fibrous structure with diameter ranging between 3 to 5 nm and yield of over 75%. The crystallinity index averaged 76% and the onset temperature of thermal decomposition was 199 °C. Thus, Pennisetum purpureum, Cynodon dactylon and Axonopus compressus are good eco-friendly sources of CNF for potential application as new source of nanofillers for reinforcement of nanocomposite films.

Keywords: Nanocellulose, yield, grasses, crystallinity, nanofiller

1 Introduction
Nanocellulose may be crystal called nanocrystal cellulose (CNC) or fibre called cellulose nanofibres (CNF) [1, 2]. Cellulose material provides a source for preparation of CNC or CNF [3]. Moreover, CNC are chiral or rod like and contains only crystalline domain, while CNF are web-shaped fibres which consists of both crystalline and amorphous domains [4, 5]. The promising nature of nanocellulose to improve material properties for many applications such as optical filters, sensor and reinforcement of polymer resins in composites have been motivating factors for research communities. Other factors that have steered tremendous
interest in isolation and characterization of nanocellulose include the aspect ratio, crystallinity index, surface area and mechanical properties which are very high and its variable ability to absorb and reflect visible light as well as low cost production [2, 6-8]. These properties make nanocellulose to be regarded as the new generation of nanomaterials for the development of high performance composite materials [7-9].

Isolation approaches of cellulose nanofibre and cellulose nanocrystal differs in many ways. For instance, cellulose nanocrystal is mostly isolated by acid using H₂SO₄, HCl, HBr or H₃PO₄ and enzyme hydrolysis whereas cellulose nanofibre is isolated by mechanical, chemomechanical and combination of enzyme and mechanical processes [10-14]. Reports from investigations have shown that the properties of nanocellulose depend on the source, pretreatment methods and experimental conditions [14-17]. The morphology of nanocellulose is usually studied by Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and polarized light scattering [18-21]. Studies on cellulose plant fibres for various numerous applications have generated great interest worldwide because they are sustainable, green and environmental friendly [6-10, 22]. In the present study, isolation and characterization of CNF from three Nigeria grasses; Elephant grass (*Pennisetum purpureum*), Bermuda grass (*Cynodon dactylon*) and carpet grass (*Axonopus compressus*) were investigated.

2. Materials and methods

2.1 Isolation of cellulose from the grass samples

Three grass samples used in this study were sun dried for 3 days, washed thoroughly with distilled water and oven dried at 60 °C for 24 h. Dried grass (10 g) was de-lignified using 300 ml of 1 M NaOH for 1 h and 30 min at 80 °C, then washed with distilled water and centrifuged. This was followed with addition of 150 ml of 5% NaOH for 20 min at 80 °C, and then washed with distilled water and centrifuged. The cellulose obtained was dried in an oven at 70 °C for 3 h and milled with mortar and pestle to obtain the powder.

2.2 Formic acid Hydrolysis of Cellulose to obtain CNF

The procedure described by Hamid *et al.* [20] was used for acid hydrolysis of isolated cellulose. Exactly 90 ml of 50% of formic acid was added to 3 g of cellulose powder in two neck flasks
followed by the addition of 1g FeCl₃ at 90 °C and in a rotator flask at 400 rpm for 4 h. The CNF was obtained, washed with distilled water, centrifuged 5 to 7 times at 1400 rpm for 30 min, dried in an oven at 70 °C for 3 h and powdered using mortar and pestle. The yield of CNF was determined using equation (1)

\[
Y (\text{in } \%) = \frac{M_1 - M_3}{M_2 - M_0} \times 100
\]  

(1)

2.3 Scanning Electron Microscopy and Transmission Electron Microscopy of extracted CNFs

Microstructure surface of extracted CNFs was determined by Scanning Electron Microscope (SEM) with model EVO MA10 (Carl Zeiss SMT Germany) at a set accelerated voltage of 15 kV after being coated with gold. Transmission Electron Microscope (TEM) with image analysis system model Image BioPlusVer 701 (Media Cybernetics Inc. USA) was used to determine the morphology of CNF and its diameter.

2.4 Crystallinity Index

Crystallinity index and crystal size of samples were determined using X-ray diffraction; X-ray diffractometer with measurement condition of 40 kV and current 30 mA. The range of scattering angle (2θ) was from 10-80° with the scan rate of 10°/min. The crystallinity index (CrI) was calculated using equation (2) and crystal size determined using equation (3).

\[
CrI (\%) = (1 - \frac{I_m}{I_{200}}) \times 100
\]  

(2)

Where \( I_m \) = intensity minimum between the 200 and 110 peaks and \( I_{200} \) = maximum peak intensity at lattice diffraction 200.

Or

\[
CrI (\%) = \left(\frac{I_{\text{crystalline}}}{I_{\text{total}}}\right) \times 100
\]

(3)

\[
\tau = \frac{K\lambda}{\beta \cos \theta}
\]

(3)

Where \( k \) = Scherer constant, \( \lambda \) = wavelength of X-ray radiation and \( \theta \) = Bragg angle and \( \beta \) is the line broadening at half the maximum intensity.
3. Results and Discussion

Figure 1 shows the micrograph of CNF obtained; they are long fibrous structure consisting of both crystalline and amorphous domain. They have average width of 3.5 nm and length ranged from 1.5 to 2.0 microns. The CrI and crystalline size increased up to 78.23% and 3.65 nm respectively. This result is in agreement with works reported in literature [4, 5].

Figure 1. TEM for CNFs obtained from (a) elephant (b) bermuda (c) carpet grass

Figure 2. SEM for CNFs obtained from (a) elephant (b) bermuda (c) carpet grass

The electron micrograph revealed that before ultrasonication, the cellulose filaments presented a diameter in the scale of tens of micrometres.
Table 1. Yield of cellulose and CNF from the grass Samples

| Samples | Yield (%) | Crystallinity (%) | Crystalline Size (nm) |
|---------|-----------|-------------------|----------------------|
| Cellulose | -         | 42.10             | 2.00                 |
| 1 (CNF) | 79.23     | 76.30             | 3.45                 |
| 2 (CNF) | 81.05     | 78.23             | 3.65                 |
| 3 (CNF) | 76.42     | 73.04             | 3.13                 |

Table 2. Chemical composition of lignocelluloses materials from grass samples

| Grass  | Cellulose (mm) | Hemicellulose | Lignin |
|--------|----------------|---------------|--------|
| Elephant | 45          | 28             | 20     |
| Bermuda | 43           | 25             | 16     |
| Carpet | 47           | 30             | 24     |

Table 1 and 2 show the yield of cellulose and CNF from the grass samples analyzed. The yield of CNF was over 75% for all samples of grasses, CrI and crystalline size increased up to 78% and 3.65 nm, respectively. The crystallinity index increased significantly from 42% in crude cellulose to 78% in CNF. The higher crystallinity index values indicate the removal of amorphous non-cellulosic compounds induced by the purification treatment (pulping and bleaching treatments) performed to purify cellulose stem.

The yield of cellulose obtained from cellulose nanofibres of elephant, bermuda and carpet grasses were found to be more than that of Walnut shell as reported by Zheng et al. [24] by 64.42, 56.93 and 71.53% [24]. This indicates that elephant, bermuda and carpet grasses possess low amorphous constituents (lignin, hemicelluloses, ash and extractive soluble materials) compared with Walnut shell. This corroborates morphology and network structure in which the more cellulose or crystalline cellulose, the less the irregular pore formation as shown in SEM.
and TEM (Figures 1 and 2). It can also be observed that the crystallinity index of cellulose nanofibre of elephant, bermuda and carpet grasses are greater than that of walnut fibre and SantaFe Kraft pulp mill as reported [23- 25]. Furthermore, crystalline size of cellulose nanofibre of used grasses were also more than that of Santa Fe Kraft pulp mill as reported [25].

4. Conclusion

Cellulose nanofibres (CNF) were successfully isolated from all the three samples of Nigerian grasses investigated using formic acid hydrolysis with yield of over 75%. Isolated CNF exhibited web-like long fibrous structure with diameter of 3 to 5 nm. The crystallinity index averaged 78% and the onset temperature of thermal decomposition was 199 °C. The nanocellulose isolated from the grass samples have high potential to be used as new source of cellulose based nanofillers for reinforcement of bionanocomposite films.

References

[1] Peng BL, Dhar N, Liu HL and Tam KC 2011 Chemistry and applications of nanocrystalline cellulose and its derivatives: a nanotechnology perspective. Can. J. Chem. Eng. 89 (5) pp 1191-206.

[2] Turbak AF, Snyder FW and Sandberg KR 1983 Microfibrillated cellulose, a new cellulose product: properties, uses, and commercial potential. J. Appl. Polym. Sci. Symp. 37, No. CONF-8205234.

[3] Turbak AF, Snyder FW and Sandberg KR 1984 Microfibrillated cellulose—a new composition of commercial significance. Atlanta, GA., USA. Chapter 16 pp 115-124.

[4] Herrick FW, Casebier RL, Hamilton JK and Sandberg KR 1983 Microfibrillated cellulose: morphology and accessibility. InJ. Appl. Polym. Sci.: Appl. Polym. Symp. 37, No. CONF-8205234.

[5] Berglund L 2005 Cellulose-based nanocomposites In: AK Mohanty, M Misra, and L Drzal (eds.). Natural fibers, biopolymers and biocomposites. Boca Raton, Florida: CRC Press, 807-832.

[6] He W, Jiang S, Zhang Q and Pan M 2013 Isolation and characterization of cellulose nanofibers from Bambusa rigida. BioResour. 8 (4) pp 5678-5689.
[7] Lavoine N, Desloges I, Dufresne A and Bras J 2012 Microfibrillated cellulose–Its barrier properties and applications in cellulonic materials: A review. *Carbohydr. Polym.* **90** (2) pp 735-764.

[8] Missoum K, Martoïa F, Belgacem MN and Bras J 2013 Effect of chemically modified nanofibrillated cellulose addition on the properties of fiber-based materials. *Ind. Crops Prod.* **48** pp 98-105.

[9] Effendi DB, Rosyid NH, Nandiyanto AB and Mudzakir A 2015 Sintesis Nanoselulosa. *Jurnal Integrasi Proses.* **5** (2). [http://dx.doi.org/10.36055/jip.v5i2.199](http://dx.doi.org/10.36055/jip.v5i2.199).

[10] Jonoobi M, Harun J, Mishra M and Oksman K 2009 Chemical composition, crystallinity and thermal degradation of bleached and unbleached kenaf bast (*Hibiscus cannabinus*) pulp and nanofiber. *BioResour.* **4** (2) pp 626-639.

[11] Chan HC, Chia CH, Zakaria S, Ahmad I and Dufresne A 2013 Production and characterisation of cellulose and nano-crystalline cellulose from kenaf core wood. *BioResour.* **8** (1) pp 785-794.

[12] Jonoobi M, Oladi R, Davoudpour Y, Oksman K, Dufresne A, Hamzeh Y and Davoodi R 2015 Different preparation methods and properties of nanostructured cellulose from various natural resources and residues: a review. *Cellulose* **22** (2) pp 935-969.

[13] Wulandari WT, Rochliadi A and Arcana IM 2016 Nanocellulose prepared by acid hydrolysis of isolated cellulose from sugarcane bagasse. *IOP Conf. Ser.: Mater. Sci. Eng.* **107** 012045. [https://doi.org/10.1088/1757-899X/107/1/012045](https://doi.org/10.1088/1757-899X/107/1/012045).

[14] Aprilia NS, Davoudpour Y, Zulqarnain W, Khalil HA, Hazwan CC, Hossain MS, Dungani R, Fizree HM, Zaidon A and Haafiz MM 2016 Physicochemical characterization of microcrystalline cellulose extracted from kenaf bast. *BioResour.* **11** (2) pp 3875-3889.

[15] Haafiz MM, Eichhorn SJ, Hassan A and Jawaid M 2013 Isolation and characterization of microcrystalline cellulose from oil palm biomass residue. *Carbohydr. Polym.* **93** (2) pp 628-634.

[16] Ahmad Z, Roziaizan NN, Rahman R, Mohamad AF and Ismail WI 2016 Isolation and characterization of microcrystalline cellulose (MCC) from rice husk (RH). *MATEC Web Conf.* **47** 05013. [https://doi.org/10.1051/matecconf/20164705013](https://doi.org/10.1051/matecconf/20164705013).
[17] Nuruddin M, Chowdhury A, Haque SA, Rahman M, Farhad S, Jahan MS and Quaiyyum A 2011 Extraction and characterization of cellulose microfibrils from agricultural wastes in an integrated biorefinery initiative. *Cellulose Chem. Technol.* **45** (5-6) pp 347-354.

[18] Siqueira G, Bras J and Dufresne A 2010 *Luffa cylindrica* as a lignocellulosic source of fiber, microfibrillated cellulose and cellulose nanocrystals. *BioResour.* **5** (2) pp 727-740.

[19] Wicaksono R, Syamsu K, Yuliasih I, Nasir M and Street K 2013 Cellulose nanofibers from cassava bagasse: Characterization and application on tapioca-film. *Chem. Mater. Res.* **3** (13) pp 79-87.

[20] Hamid SB, Chowdhury ZZ, Karim MZ and Ali ME 2016 Catalytic isolation and physicochemical properties of nanocrystalline cellulose (NCC) using HCl-FeCl₃ system combined with ultrasonication. *BioResour.* **11** (2) pp 3840-3855.

[21] Saurabh CK, Dungani R, Owolabi AF, Atiqah NS, Zaidon A, Aprilia NS, Sarker ZM and Khalil HA 2016 Effect of hydrolysis treatment on cellulose nanowhiskers from oil palm (*Elaeis guineensis*) fronds: Morphology, chemical, crystallinity, and thermal characteristics. *BioResour.* **11** (3) pp 6742-6755.

[22] Börjesson M and Westman G 2015 Crystalline nanocellulose—preparation, modification, and properties. Cellulose-fundamental aspects and current trends. 159-191. https://dx.doi.org/10.5772/61899.

[23] Zheng D, Zhang Y, Guo Y and Yue J 2019 Isolation and characterization of nanocellulose with a novel shape from walnut (*Juglans regia* L.) shell agricultural waste. *Polym.* **11** (7) 1130. https://doi.org/10.3390/polym11071130.

[24] Wang QQ, Zhu JY, Reiner RS, Verrill SP, Baxa U and McNeil SE 2012 Approaching zero cellulose loss in cellulose nanocrystal (CNC) production: recovery and characterization of cellulosic solid residues (CSR) and CNC. *Cellulose* **19** (6) pp 2033-2047.

[25] Aguayo MG, Fernández Pérez A, Reyes G, Oviedo C, Gacitúa W, Gonzalez R and Uyarte O 2018 Isolation and characterization of cellulose nanocrystals from rejected fibers originated in the kraft pulping process. *Polym.* **10** (10) 1145. https://doi.org/10.3390/polym10101145.