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Extraction and characterization of cellulose nanofibers from yellow thatching grass (*Hyparrhenia filipendula*) straws via acid hydrolysis

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Abstract

Purpose

A variety of lignocellulosic raw materials have been previously reported for the production of cellulose and cellulose derivatives, but little research effort has been dedicated to producing cellulose from *Hyparrhenia filipendula*. In this study, cellulose nanofibers (CNFs) were extracted from *Hyparrhenia filipendula* waste straws via sulphuric acid hydrolysis.

Methods

The straws were pretreated with a combination of physiochemical processes and hydrolyzed using sulphuric acid at three different concentrations (1 M, 3 M and 5.6 M) for 2 hours at 80 °C. The properties of the CNFs was checked by Fourier Transform Infrared spectroscopy (FTIR) for surface chemistry, X-ray diffraction (XRD) for crystallinity, Scanning Electron microscopy and Transmission electron microscopy (TEM) for morphology. A high-performance liquid chromatograph (HPLC) was used to quantify the amount of biopolymers in the CNFs.

Results

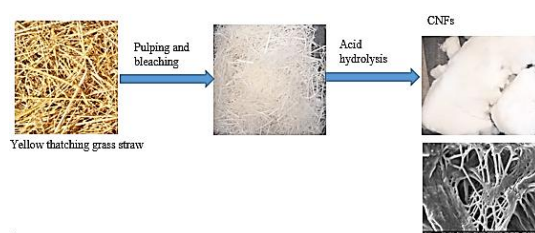
The results show that CNFs, denoted as *CNF 1*, *CNF 3*, and *CNF 5.6* for 1 M, 3 M, and 5.6 M sulphuric acid, respectively, were successfully extracted at the various concentrations of sulphuric acid. The cellulose content of *CNF1*, *CNF3*, and *CNF5.6* determined by HPLC analysis were 85%, 77 % and 78 % respectively. Also, the hemicellulose content in *CNF 1*, *CNF 3*, and *CNF 5.6* was 10 %, 15 %, and 12 % respectively, showing a high carbohydrate content of the CNFs. The FTIR spectra confirm the absence of characteristic peaks for lignin in the CNFs. The XRD analysis reveals presence of characteristic cellulose I_β peaks at 2θ of 18°, 26°, and 40° with the crystallinity of 78%, 74 % and 73% for *CNF1*, *CNF3* and *CNF5.6*, respectively. Moreover, SEM analysis shows the deposition of lignin polycondensate on the surface of *CNF 1*, *CNF 3*, and *CNF 5.6* while the bleached sample has a smooth and glossy appearance. The TEM analysis shows long unbranched nano-sized fibers for *CNF 1* and shorter fibrous network of fibers for *CNF 3*, and *CNF 5.6*. The average diameter of the fibers, measured with ImageJ software is 40 nm for *CNF 1*, 57 nm for *CNF 3*, and 92 nm for *CNF 5.6*.

Conclusion

CNFs were successfully produced from *Hyparrhenia filipendula* and reported for the first time in open literature. In view of the structure and properties of the produced CNFs, they are a potential material for value-added applications such as polymer matrices, films, and membranes, thus enabling efficient utilization of agricultural waste.

Keywords: Cellulose nanofibers, Acid hydrolysis; Yellow thatching grass; Lignocellulosic waste.

Graphical Abstract



Statement of novelty

This research was undertaken to promote the utilization of non-conventional biomass for high-value products like cellulose. *Hyparrhenia filipendula* is a grass that is commonly found in the cattle corridor of Uganda. The grass is mainly used for roofing houses, and grazing cattle in its early stages of growth. It is common practice to burn

mature plants, posing fire and health- related hazards. Grasslands also cover approximately 21 % of the total area in Uganda, but they have not been reported as a source of raw materials for value-added products. The extraction of nanocellulose from *Hyparrhenia filipendula* is expected to stimulate entrepreneurship in production of nanocellulose. Additionally, farmers will generate income from a plant, previously considered a weed and discarded.

1 Introduction

Cellulose fibers are natural biopolymers found abundantly on earth [1, 2]. Cellulose fibers can be extracted from various natural fibers such as roselle, date palm, hemp, bamboo, kenaf, sisal, cotton and agricultural residues such as bagasse, palm oil residue, soybean hulls, banana fibers, potato tubers, sugar beets, wheat, flax and rice straw [1, 3-6]. Cellulose is a renewable, biocompatible, biodegradable, semi-crystalline, low density, non-toxic material and can be sourced at low cost [1]. Cellulose nanofibers (CNFs) are cellulosic fibers with at least one dimension less than 100 nm. They possess excellent properties such as high strength and toughness, low thermal expansion, high aspect ratio and good biocompatibility [3, 4]. CNFs have shown potential applications in many fields such as water purification, bio packaging, electromagnetic interference shielding, biomedical scaffolds, tissue engineering, sensors, optically transparent functional materials, and nanocomposites [3, 4, 7].

Yellow thatching grass (*Hyparrhenia filipendula*) is a perennial grass widely distributed in East and Southern Africa [8]. The grass is mainly used for roofing houses, traditional medicine, mulching, and grazing cattle in its early stages of growth [8, 9]. It is common practice in these regions to burn mature plants, posing fire and health-related hazards. Yellow thatching grass also grows naturally in degraded soils, producing large amount of above-ground biomass [8]. The potential of the grass as a source of CNFs has not been previously reported in literature. The findings of this study have potential positive impact such as new economic opportunities for farmers, creation of small and medium-scale processing facilities for high-value cellulose and sustainable utilization of lignocellulosic waste, eliminating the risks associated with burning these waste materials.

CNFs have been previously isolated from non-woody plants through mechanical processes such as high-pressure homogenization, micro fluidization, grinding, high-speed blending, and high-intensity ultrasonication [4]. However, these mechanical processes are associated with a high energy input, necessitating enzyme, alkali, and acid pretreatments to remove non-cellulosic materials and facilitate subsequent mechanical processes [2,4]. Alkaline pretreatment is considered a viable strategy for industrial application as the process offers mild reaction conditions with low pressure and temperature for the disruption of intricate polymeric biomass. Sodium hydroxide (NaOH) is the most selective alkaline reagent for lignin and water-soluble hemicellulose removal in lignocellulosic biomass [10, 11]. Furthermore, acid hydrolysis using inorganic acids such as sulphuric acid, hydrochloric acid, hydrobromic acid and organic acids like citric acid is used to isolate high crystalline CNFs. Sulphuric acid is the most commonly used acid for hydrolysis because the CNFs extracted are stable in colloid due to the esterification of the hydroxyl groups by sulphate ions [12]. The main factors that affect the properties of the nanocellulose obtained are the reaction time, temperature and acid concentration [12]. Additionally, a comprehensive review of pre-treatment and extraction of nanocellulose from lignocellulosic biomass is provided by other authors [5, 10-14].

Against this background, this study was aimed at extracting CNFs from yellow thatching grass via a combination of pretreatment techniques and acid hydrolysis. In addition, the extracted CNFs were characterized and quantified using standard scientific methods.

2 Materials and Methods

2.1 Materials

The *Hyparrhenia filipendula* grass was obtained from Palabek Kal, Lamwo district, Uganda in January 2021 and sun-dried for two weeks. The dried blades and sheaths were removed to expose the culm that was used in subsequent processes. The straws were manually removed from the culm using a knife, shredded in a blender and milled using a PM 100 ball mill (Retsch, Germany) for 10 minutes, with 10 mm diameter stainless steel grinding balls at 300 rpm. The straws were then passed through a 425 μm sieve and stored in an air tight container to prevent degradation of the straws. The extractives were removed using a Soxhlet extractor at 110 °C for 4 h with toluene/ethanol (2:1 v/v). The extractive-free straws were then washed with de-ionized water, and dried at 80 °C for 1 h.

The reagents, Glacial acetic acid (Sigma-Aldrich, South Africa, 99.7 %), Sodium hydroxide (Sigma-Aldrich, South Africa, 98 %), Toluene (ACE chemicals, South Africa, 99.5%), Ethanol (ACE chemicals, South Africa, 95%), Sodium chlorite (Sigma-Aldrich, South Africa, 80%), and Sulphuric acid (Glassworld, & Chemical Suppliers CC, Maraisburg, South Africa, 99.8 %) were used without further purification.

2.2 Pulping and bleaching

The pulping of the extractive-free straws was carried out using 10 wt % NaOH with a fiber /liquid ratio of 1:15(w/v) at 100 °C for 4 h in a Parr reactor. The pulp was removed after 4 h and washed several times with deionized water until a neutral pH was obtained. Then, the pulp was dried in the oven at 105 °C for 1 h to remove water and stored in an air-tight container at room temperature for further analysis. The pulp was bleached using acidified Sodium Chlorite (200 mL de-ionized water, 4 g of fiber, 2.4 g Sodium Chlorite and 0.6 mL Acetic acid) at 95 °C for 6 h in a Parr reactor. The bleached fibers were also washed with de-ionized water until a neutral pH was obtained and dried at 105 °C for 1 h. Bleaching was a critical step in the CNF isolation, otherwise the fibers turned black during acid hydrolysis.

2.3 Acid hydrolysis

The bleached fibers were treated with three different concentrations of Sulphuric acid (1 M, 3 M and 5.6 M) using a fiber/acid ratio of 1:20 (w/v) at 80 °C for 2 h in a Parr reactor. The samples hydrolyzed with 1M, 3M, and 5.6 M sulphuric acid are denoted as *CNF 1*, *CNF 3*, and *CNF 5.6* respectively. The CNFs were washed with de-ionized water until a neutral pH was obtained. Then, the fibers were suspended in de-ionized water, homogenized using a Diahann Scientific Homogenizer (HG-15D) at 4000 rpm for 60 s, and centrifuged at 2000 rpm for 300 s using a Hettich Zentrifugen Rotofix 46 H centrifuge. The sample was then refrigerated at - 40 °C for 12 h and freeze-dried using a Drawell DW-18ND freeze drier for 72 h. The dried sample was stored in an air-tight container for further analysis.

2.4 Quantification of carbohydrates and lignin

A high-performance liquid chromatograph (Agilent 1260 Infinity, Germany) and UV-visible Spectrophotometer (Hitachi U-3900 Spectrophotometer) was used to determine the carbohydrates and lignin in the sample using the procedure described by Sluiter et al. [15]. All measurements were done in triplicate.

2.5 Physicochemical Characterization of samples

The morphology and elemental composition was examined using a transmission electron microscope (JEOL JEM 2100F) and Zeiss Ultra plus FEG Scanning electron microscope equipped with an electron dispersive spectroscope (EDS). The samples were coated with carbon using a Quorum Q150T ES sputter coater to enhance image resolution. Additionally, the energy dispersive X-ray (EDX) compositions were measured at 10 sites for all the samples. The trace elements in the sample were analysed with a Thermo Fisher ARL Perform'X sequential XRF spectrometer with Uniquant software. The thermal properties of the samples was determined by the ASTM E1131-08 standard procedure using a thermo gravimetric analyzer (HITACHI STA7300), with a flow of nitrogen of 10 mL/min, and heating rate of 10 °C/min. Functional groups in the samples were determined by Fourier Transform Infrared spectroscopy (FT-IR) in the medium infrared region of 4000 – 400 cm⁻¹ with a Bruker Alpha FTIR spectroscope. X-ray diffraction (XRD) analysis of the samples was conducted on a PANalytical X'Pert Pro powder diffractometer with Fe filtered Co-K α radiation ($\lambda=1.789\text{\AA}$). The crystallinity index, I_C , was obtained from Equation 1[16].

$$I_C = \frac{I_{002} - I_{am}}{I_{002}} \quad (1)$$

Where I_{002} is the diffraction intensity close to $2\theta = 22^\circ - 24^\circ$ and represents a crystalline and amorphous component of the material; I_{am} is the diffraction intensity close to $2\theta = 16^\circ - 18^\circ$ and refers to amorphous component of cellulosic fibers.

3 Results and discussion

3.1 Compositional analysis

The chemical composition of the samples is shown in Table I. The primary component of lignocellulose materials is cellulose. The typical cellulose content in perennial biomass species is approximately 20 - 40 % [17]. The cellulose content of yellow thatching grass straws (30 %) is much lower than similar species like *Miscanthus spp.* (45 - 44 %), switch grass (40 %), reed canary grass (39 %), rice straw (35 %), and millet straw (41 %) [17-20]. It is likely that the composition differences result from the different growing conditions of the plants, different stages of growth and, in some cases, the composition of the entire stem is reported by the authors. The lignin and extractive components of the yellow thatching grass are also comparable to the values reported by other authors for similar species [17]. The limitation of this study is that the extractives were measured on a weight basis and the results in Table I also confirm that the extractable material was not fully removed. The failure to remove extractable material results in higher lignin values as unhydrolyzed carbohydrates condense with acid-insoluble lignin, thus accounting for the higher lignin content in the dewaxed sample [21].

The lignin removal increased with each pre-treatment stage. The highest lignin removal was achieved for *CNF1* (98 %), *CNF 5.6* (97 %) and *CNF3* (96 %). The highest hemicellulose removal was also observed for *CNF1* (59 %), followed by *CNF 5.6* (50 %) and *CNF3* (38 %). This is in line with the common trend of observation due to the decrease of amorphous components like lignin and hemicellulose that easily absorb chemicals, whereas the compactness of the crystalline regions makes it difficult for chemical penetration [22, 23]. The cellulose content increased significantly after acid hydrolysis. The highest cellulose of 85 % is observed for *CNF1*, followed by 78 % for *CNF 5.6* and 77 % for *CNF 3*. The reduced cellulose yield with increased concentration of sulphuric acid is attributed to the unavoidable degradation of cellulose chains into water-soluble sugar oligomers under strong acidity of sulfuric acid [24].

Table I: Chemical composition of *Hyparrhenia filipendula*

Sample	Composition %				
	Cellulose	Hemicellulose	Lignin	Acid insoluble Ash	Others
Untreated	29.64 ± 0.81	23.94 ± 0.46	20.70 ± 7.07	10.67 ± 0.00	15.05 ± 0.00
Dewaxed	29.83 ± 1.02	23.45 ± 0.32	22.63 ± 5.19	7.47 ± 0.00	16.62 ± 0.00
Pulped	56.17 ± 7.14	13.22 ± 1.82	12.17 ± 2.36	3.90 ± 0.00	14.54 ± 0.00
Bleached	62.63 ± 5.98	13.93 ± 1.08	0.26 ± 0.00	-	-
<i>CNF1</i>	85.38 ± 1.92	9.92 ± 0.24	0.48 ± 0.00	-	-
<i>CNF 3</i>	76.65 ± 0.17	14.96 ± 1.71	0.74 ± 0.00	-	-
<i>CNF5.6</i>	77.84 ± 5.37	11.96 ± 1.29	0.66 ± 0.00	-	-

3.2 Physicochemical characterization

3.2.1 Morphology

The morphology of the samples is shown in Figure I (Supplementary material). The micrographs for the raw and dewaxed samples show an intact fiber with some fractures due to the presence of lignin and mechanical size reduction. After alkali pre-treatment, the serrated surface of the cellulose bundles is exposed due to the chemical reduction of hemicellulose and dissociation of lignin [25]. The cellulose fibrils can be clearly seen in the micrographs for *CNF1* and *CNF 3* in Figure 2(e) and Figure 2(f) respectively. Additionally, characteristic spherical lignin structure is observed on the surface of *CNF5.6* in Figure 2(g). The lignin is re-deposited as droplets on the surface of cell walls when the melting temperature of the lignin is exceeded during acid hydrolysis [26]. Additionally, *CNF5.6* had a yellowish hue that was not noticeable with *CNF1* or *CNF 3*.

The TEM images in Figure II (Supplementary material) confirm the isolation of *CNFs* via Sulphuric acid hydrolysis. The TEM images shows long unbranched nano-sized fibers for *CNF 1* in Figure 2 (d) and shorter

fibrous network of fibers for *CNF 3*, and *CNF 5.6* in Figure 2 (e) and Figure 2 (f) respectively. The diameters of the fibers were measured using Image J software. The average diameter of the fibers is 40 nm for *CNF 1*, 57 nm for *CNF 3*, and 92 nm for *CNF 5.6*. The diameters of the CNFs are higher than those previously reported CNFs from grasses but comparable to woody biomass; 50-70 nm from eucalyptus [27]; Napier fiber (16.10 nm-167.6 nm)[28]; Jute (15-25nm)[22]; marram grass (4-5 nm)[29] and Australian desert grass (16-32.2 nm)[25].

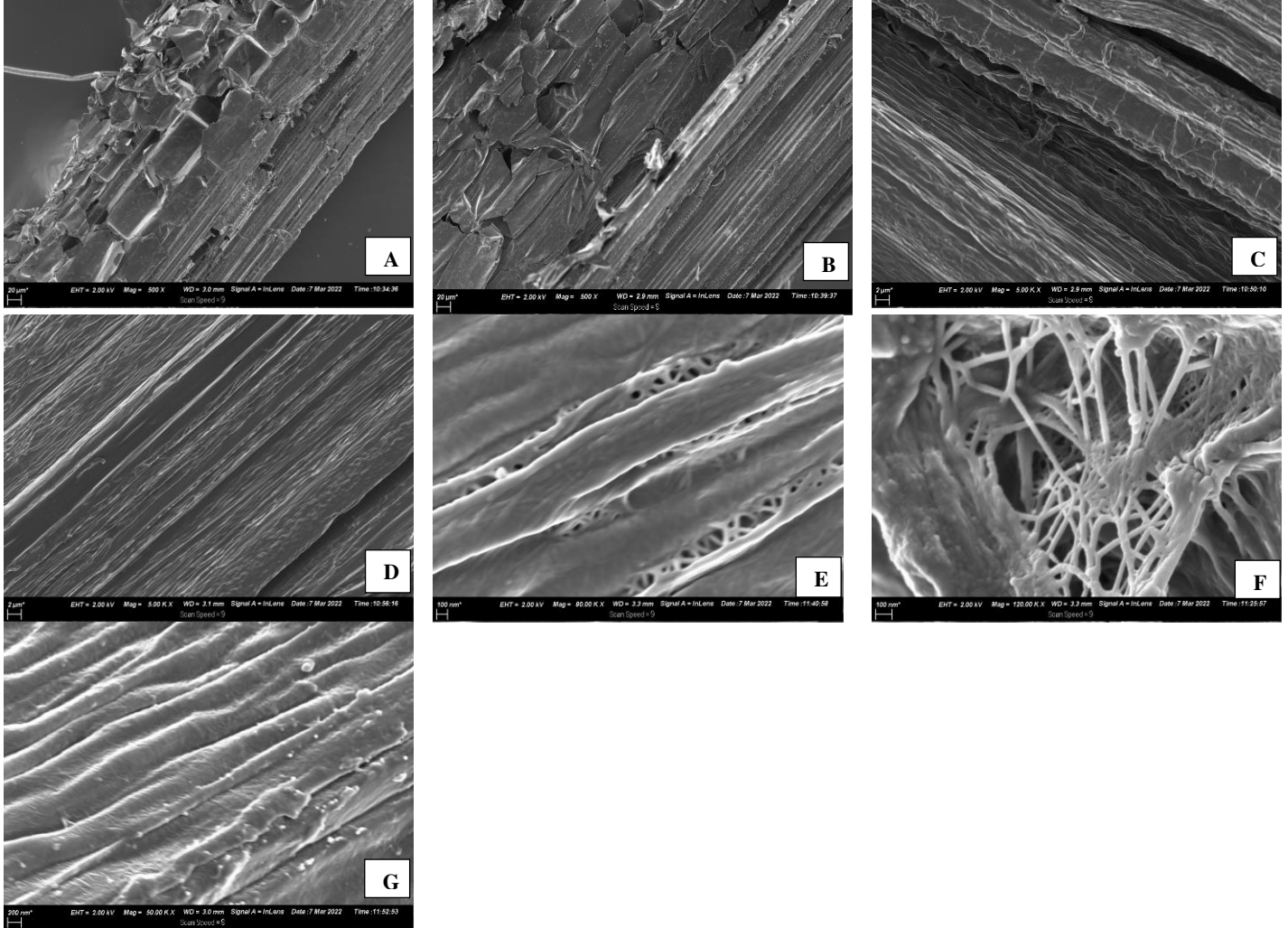


Figure I: SEM images for yellow thatching grass; (a) for untreated sample ;(b) for dewaxed sample; (c) for pulped sample; (d) for bleached sample; (e) for *CNF1*; (f) for *CNF 3* and (g) for *CNF 5.6* respectively.

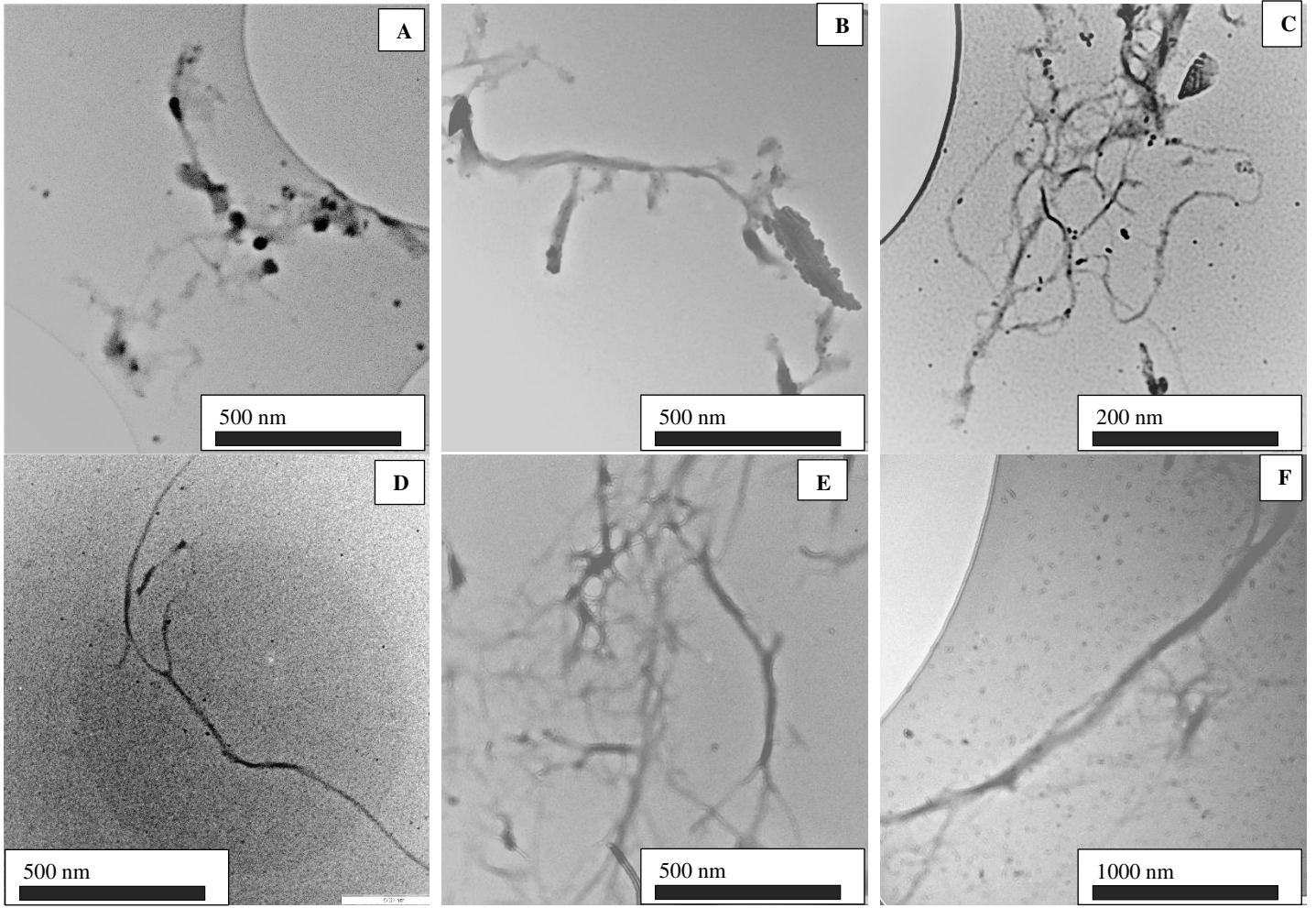


Figure II: TEM images of yellow thatching grass; a (Raw sample); b (De-waxed); c (Bleached); d (CNF1); e (CNF 3); f (CNF 5.6) respectively.

3.2.2 Analysis of major and trace elements in *Hyparrhenia filipendula*

The normalized analysis of the major and trace elements in the yellow thatching grass is shown in Table II. The dominant elements are Silicon (Si), followed by Potassium (K), and Phosphorus (P). Silicon is a key structural element in grasses as it enhances the strength of the plant and prevents lodging and shading of leaves[30]. The major element in similar grass species like eri grass and barley, sorghum and wheat straws is also Silicon [31-33].

Table II: Major and trace elements in yellow thatching grass straws

Element	Wt %
Si	2.23
Al	<0.01
Mg	0.10
Na	<0.01
P	0.14
Fe	0.01
K	0.75
Ca	0.07
Zr	<0.01
Cr	<0.01
S	0.02
Cl	0.10
Ba	0.09
I	0.04
Cs	0.05
W	0.02
Cellulose rest%	96.35

TOTAL	99.96
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3.2.3 Elemental composition

The elemental composition of the samples is shown in Table III. The prominent peaks for all the samples were Oxygen and Silicon. The silicon content decreases due to the elimination of non-cellulosic components during pulping, bleaching and acid hydrolysis [34]. The elemental carbon content is not reported because the samples were coated with carbon. Further still, the Oxygen peaks detected in the samples most likely indicate the presence of C-O, C-C, O-C-O, C=O, O-C=O, and C-OH groups[35] on the surface of the samples. However, the higher oxygen content of the *CNFs* points to the presence of more carboxyl groups than hydroxyl groups on the surface of the *CNFs*[35]. The other trace elements in the sample; P, K, Ca, Na, were reduced with successive treatment as expected. However, the increase in the concentration of trace elements like Chlorine (Cl) and Na in the processed samples is attributed to the introduction of these elements from the reagents used to process the sample. Nevertheless, there is an increase in Sulphur content of the *CNFs*, which could have been due to inadequate washing of the *CNFs*, since there is no absorbance band at 1380 cm⁻¹ due to S = O stretching in the FTIR spectra [28].

Table III: Elemental analysis of samples

Sample	Element, wt %							
	O	Si	P	Cl	K	Ca	S	Na
Untreated	75.31 ± 9.17	16.42 ± 9.00	1.79 ± 2.47	0.22 ± 0.28	3.60 ± 1.12	2.32 ± 3.63	-	-
Dewaxed	81.81 ± 3.75	9.49 ± 4.20	0.93 ± 0.43	0.56 ± 0.27	5.81 ± 3.68	1.16 ± 0.95	-	-
Pulped	83.15 ± 6.45	2.08 ± 1.86	-	-	-	12.55 ± 5.19	-	1.47 ± 1.70
Bleached	93.76 ± 4.18	3.70 ± 4.89	-	2.54 ± 1.73	-	-	-	-
CNF1	93.15 ± 3.13	1.84 ± 0.74	0.02 ± 0.07	2.36 ± 1.29	0.40 ± 0.62	1.64 ± 3.18	0.39 ± 0.43	-
CNF 3	91.72 ± 4.08	0.68 ± 0.95	-	3.67 ± 1.87	-	-	3.93 ± 1.65	-
CNF5.6	87.10 ± 6.32	0.20 ± 0.30	-	-	0.15 ± 0.29	0.97 ± 0.54	11.58 ± 6.12	-

3.2.4 Surface Chemistry of the *CNFs*

The FTIR spectra of the samples shown in Figure III is typical for lignocellulosic biomass. The broad band between 3650-3000 cm⁻¹ is attributed to O-H stretching vibrations from intra and intermolecular hydrogen bonding. It also describes the hydrophilic nature of cellulose and *CNF* [36]. The band at 2900 cm⁻¹, denotes the symmetrical and asymmetrical stretching vibrations of the C-H bonds present in cellulose chains[36]. The peaks in the range of 1350–1250 cm⁻¹ are attributed to the presence of chemical groups of the hemicelluloses[37]. The peaks at 1190, 1070 and 890 cm⁻¹ are associated with the stretching and rocking vibrations of the C-O, C-H and CH₂ groups of cellulose[37]. Nonetheless, there are notable differences in the FTIR spectra between the samples. The absence of the characteristic absorption peak at 1730 cm⁻¹ (C= O stretching of the acetyl and urate groups of hemicellulose, or the ester bond of carboxyl groups in lignin to fragrant acid and ferulic acid) in the *CNFs* shows that lignin and hemicellulose were significantly removed in the *CNFs*[38]. Additionally a shift in the peak at 1629 cm⁻¹ in the raw sample to 1642 cm⁻¹ for *CNF1*, *CNF3* and *CNF 5.6* indicates a conversion cellulose I to cellulose II during treatment, as reported by other authors [38]. The FTIR spectra also showed a peak at 1510 cm⁻¹, characteristic of lignin, only for the raw and pulped sample, confirming significant delignification of the *CNFs* [37]. A peak at 1730 cm⁻¹, corresponding to hemicellulose is only present in the spectrum for the raw sample, indicating significant removal of hemicellulose with treatment.

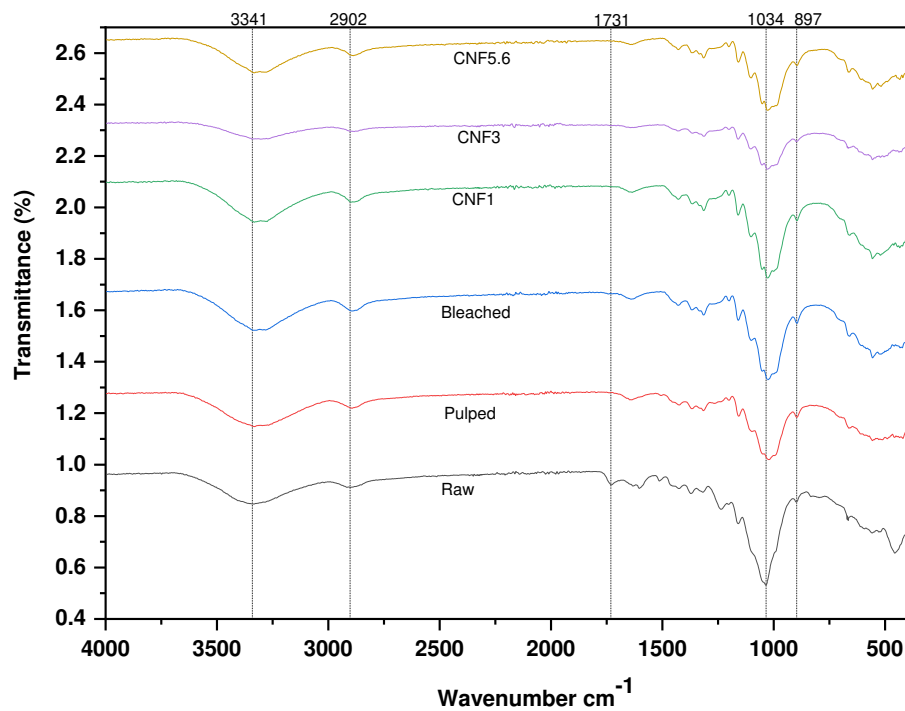


Figure III: FTIR Spectra of raw, bleached, pulped and acid-hydrolyzed samples

3.2.3 Crystallinity of CNFs

The XRD patterns of the CNF samples are shown in Figure IV. The XRD peaks of the CNFs are observed at $2\theta = 18^\circ$ (110), 26° (200), and 40° (004) for cellulose I. The crystallinity of *CNF1*, *CNF3* and *CNF5.6* is 78%, 74 % and 73% for respectively, hence the proportion of crystalline to amorphous regions in the cellulose was increased during hydrolysis[38]. The crystallinity of CNFs from yellow thatching grass is higher than the crystallinity of CNFs obtained from bagasse (63.82%), and eucalyptus (72.1 %), previously reported in literature[27, 38].

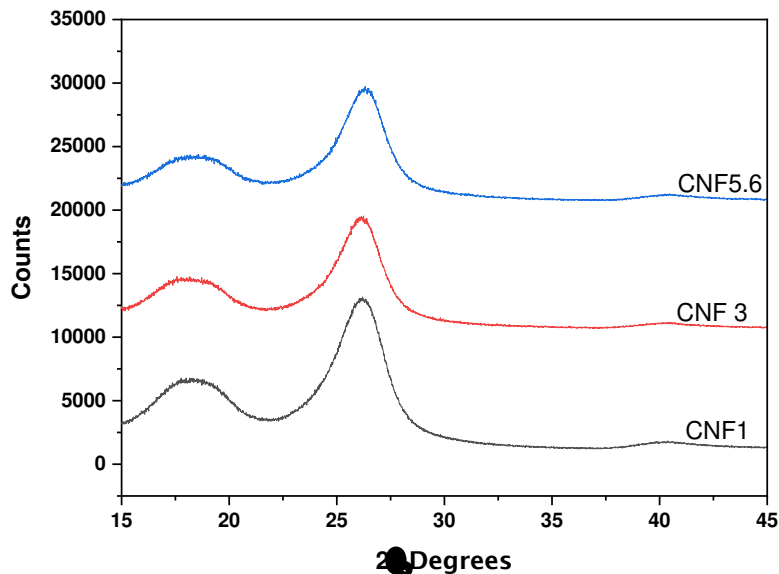


Figure IV: XRD patterns of acid-hydrolyzed CNF samples

3.2.4 Thermal stability

The TGA and DTG curves of the samples are shown in Figure V. The residual mass of the dewaxed sample is higher than that of the CNFs, attributed to its higher lignin content, confirming the results in Table 1[38]. Also, the bleached sample showed the highest peak decomposition temperature of 330°C , followed by dewaxed sample (327°C), *CNF1* (318°C), *CNF3* (317°C), pulped (316°C), and *CNF5.6* (309°C). The thermal stability of the CNFs is lower than the pre-treated samples, a finding contrary to what was expected, based on previously reported

literature[22, 38] . However, Kumari et al. also synthesized CNFs from lemongrass with a lower thermal stability than the pristine biomass. The authors attributed the lower decomposition temperature of the CNF to the decrease in molecular weight and crystallinity of the CNF[36]. The residual solids in the treated samples is due to the presence of small amounts of hemicellulose or lignin which withstood the extraction process[22].

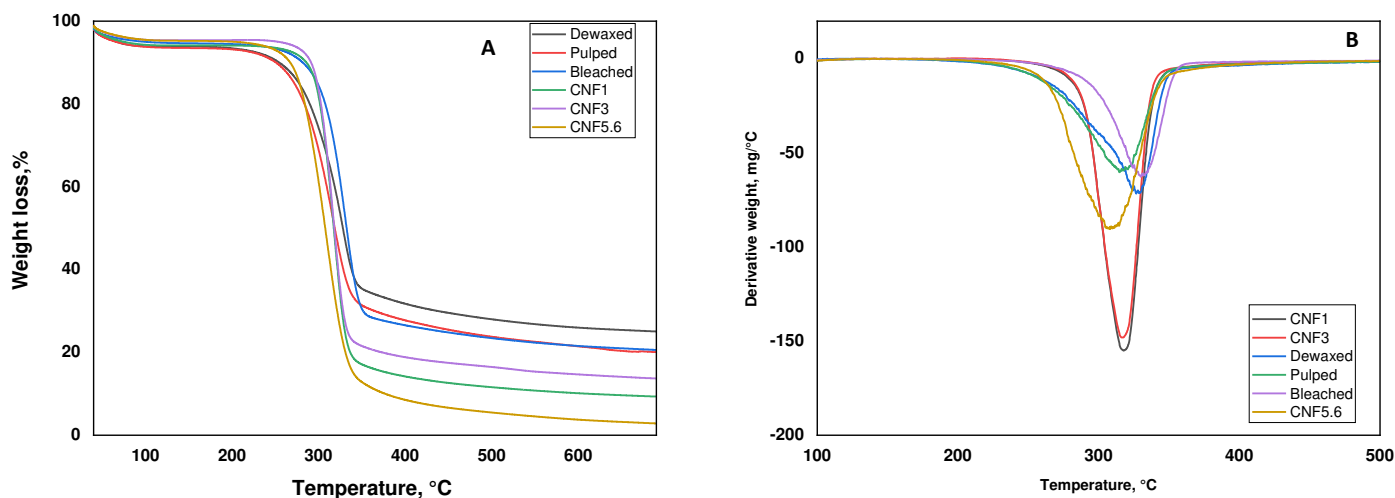


Figure V: (a) TGA and (b) DTG curves for samples

4. Conclusion and future perspectives

This study shows the potential of yellow thatching grass as a source of CNFs reported for the first time in literature. The CNFs were successfully extracted from biomass using Sulphuric acid and characterized by TEM, SEM, FTIR, TGA and HPLC. A high cellulose composition of 85 % was obtained for the sample hydrolyzed with 1 M H_2SO_4 . There was an observed carbohydrate loss with the samples hydrolyzed with higher concentrations of Sulphuric acid. However, other factors that affect acid hydrolysis like the time and temperature were not thoroughly investigated during this study. Future studies can focus on studying the effect of time, concentration of acid and temperature on the physicochemical properties of the CNFs. The pre-treatment of the straws was also time consuming, accompanied by a high consumption of reagents. In case yellow thatching grass is integrated in a bio-refinery, this leads to higher energy consumption and higher production costs. Thus, pulping and bleaching stages of the process need to be optimized in order to optimize the production of CNFs at industrial scale. The morphology of the CNFs also confirmed successful synthesis of nanofibrils with different concentrations of H_2SO_4 . The change in the surface chemistry and elemental composition of the samples during the treatment process also confirmed the successful extraction of cellulose from yellow thatching grass. The samples hydrolyzed with a higher concentration of H_2SO_4 had a lower crystallinity possibly due to the degradation of crystalline cellulose. The thermal analysis also revealed that *CNF1* was more thermally stable than *CNF3* and *CNF5.6*. The overall analysis of the results therefore shows that 1M H_2SO_4 is sufficient to hydrolyze yellow thatching grass. In view of the structure and properties of the produced CNFs, they are a potential material for value-added applications such as polymer matrices, films, and membranes, thus enabling efficient utilization of agricultural waste. However, up- scaling production of CNFs from yellow thatching grass requires an economic evaluation of the process and thorough planning to ensure sustainability and minimal adverse environmental impacts. Future work will investigate the application of the CNFs in new materials development, especially polymer matrices, films, and membranes.

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6 Statements and declarations

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6.2 Competing interests

The authors declare no conflict of interest.

6.3 Author contributions

All authors contributed to the successful completion of this study. Material preparation and analysis were performed by Ndwandwa Nolundi, Ayaa Fildah and Samuel Ayodele Iwarere. The first draft of the manuscript was written by Ayaa Fildah and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

6.4 Data availability

The datasets generated during and/or analysed during the study are not publicly available due to Copyright regulations by Makerere University and University of Pretoria, but are available from the corresponding author on reasonable request.