Journal of Bioresources 13 (2): 60 - 64, April – June 2025

JOURNAL OF BIORESOURCES

journal webpage: https://jbr.rgu.ac.in

ISSN: 2394-4315 (Print)
ISSN: 2582-2276 (Online)

RESEARCH ARTICLE

Extraction and structural characterisation of nanocrystalline cellulose from bamboo biomass

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Article No: JPJBR160; Received: 18.04.2025; Peer-reviewed: 25.05.2025; Accepted: 30.05.2025; Published: 30.06.2025
Doi: https://doi.org/10.5281/zenodo.16821860

Abstract

Nanocrystalline Cellulose (NCC) is a bio-based nanomaterial of growing interest due to its renewable origin, excellent mechanical strength, and modifiable surface chemistry. In this study, NCC was extracted from bamboo fibre via. controlled acid hydrolysis, followed by purification and ultrasonication. Bamboo, a fast-growing and sustainable resource, was selected for its high cellulose yield and widespread availability. The isolated NCC was characterised using SEM, TEM, EDX, FTIR, XRD, and TGA to assess its morphological, chemical, structural, and thermal properties. SEM and TEM revealed uniformly dispersed, rod-shaped nanocrystals, while EDX confirmed high elemental purity with dominant carbon and oxygen peaks. FTIR analysis verified the removal of non-cellulosic components, and XRD results indicated an increase in crystallinity from 57.73% in raw fibre to 68.32% in NCC. Thermogravimetric analysis showed enhanced thermal stability, with 50% mass loss occurring at 301.21 °C. These results demonstrate that bamboo-derived NCC possesses favourable characteristics for integration into advanced material systems. Its structural stability and thermal resilience support its use in bio-composites, packaging materials, and biomedical applications. Future research may focus on surface functionalization of NCC and optimisation of the extraction process for industrial-scale implementation.

Keywords: Nanocrystalline Cellulose; Bamboo Biomass; Ultrasonication; Crystallinity; Thermal stability.

1. Introduction

There is a growing awareness of environmental issues and the impact of synthetic chemicals on ecosystems. Natural products are often seen as more environmentally friendly due to their biodegradability and sustainable potential. In the recent decades, one of the most widely exploited natural resource is the lignocellulosic biomass (LCB). LCB is the key component in plant, bacteria, fungi, algae and tunicates. The three major components of LCB includes lignin and the polysaccharide cellulose and hemicellulose. Cellulose, which is a component of LCB biomass is considered as one of the most abundant products available in nature (Khandelwal and Windle, 2013). Structurally, the cellulose is regarded to have para-crystalline form with highly ordered crystalline region and interrupting areas of non-crystalline/ amorphous region. Treating the cellulose for removal of amorphous region by several processes like enzymatic, mechanical or chemical yield cellulose with only crystalline area. In fact, cellulose is a chain of hundreds to thousands of β (1 → 4) linked D-glucose units (Gupta et al., 2019) and many such parallel chains stack together to form nano-fibrillar structure and addition of further stacks give rise to the microfibrillar structure of cellulose. Cellulose nanocrystals (CNCs) are advanced materials obtained from cellulose by hydrolysis of amorphous region. CNC, sometime also referred as nanocrystalline cellulose (NCC) or cellulose whiskers have needle-like or rod-shaped structures. They typically have diameters ranging from 5 to 50 nm and lengths from 100 nm to 500 nm (Spagnuolo et al., 2022). NCC is gaining attention for its high strength, low density, biodegradability, and large surface area, making it suitable for applications in polymer composites, electronics, biomedical devices, and packaging (Cheng et al., 2023; Agate et al., 2018; Ahankari et al., 2021). It can be derived from various plant sources, including wood (Beck-Candanedo et al., 2005), bamboo (Yu et al., 2012), wheat straw (Bian et al., 2022), and kenaf (Sabaruddin and Paridah, 2018), as well as lesser-used plants like banana pseudostem and sago seed shell (Faradilla et al., 2016; Naduparambath et al., 2018). Agricultural residues such as sugarcane bagasse, rice husk, corncob, and coconut husk offer low-cost, eco-friendly feedstocks for NCC (Islam et al., 2018; Louis and Venkatachalam, 2020; Vu et al., 2024; Poornachandhra et al., 2023). Non-plant sources like bacteria and tunicates also produce nanocellulose with similar structural properties (Sacui et al., 2014; Dunlop et al., 2018). High-cellulose-content materials, such as cotton, flax, and hemp, are especially favoured for extraction (Elfaleh et al., 2023). To efficiently extract NCC, pretreatment steps like delignification, dewaxing, and mercerization are crucial, as they help expose and activate the cellulose fibres (Nagarajan et al., 2021). Common methods include acid hydrolysis, often with sulphuric acid, followed by ultrasonication, which produces NCC with high crystallinity. Mechanical techniques like high-pressure homogenization, cryo-crushing, microfluidization, and ultrasonication are also widely used (Wang et al., 2015; Ramesh et al., 2017; Campos et al., 2017; Ishak et al., 2020). Ongoing research is exploring newer approaches such as enzyme-assisted hydrolysis, electrospinning, and ionic liquids to improve yield, lower costs, and scale up production.

2. Material and method

2.1. Materials

Bamboo culm was obtained from Rainforest Research Institute, Jorhat, Assam. Chemicals used in the study like ethanol, toluene, sodium chlorite, acetic acid, sodium hydroxide, potassium hydroxide, hydrochloric acid, sulphuric acid was of analytical grade and purchased from Sigma Aldrich and Hi-media.

2.2. Methods

The bamboo culm obtained was cleaned with distilled water, air dried, chopped into pieces and then grinded into fine powder. The powder obtained was sieved in a mesh 60 having 250 µ pore size.

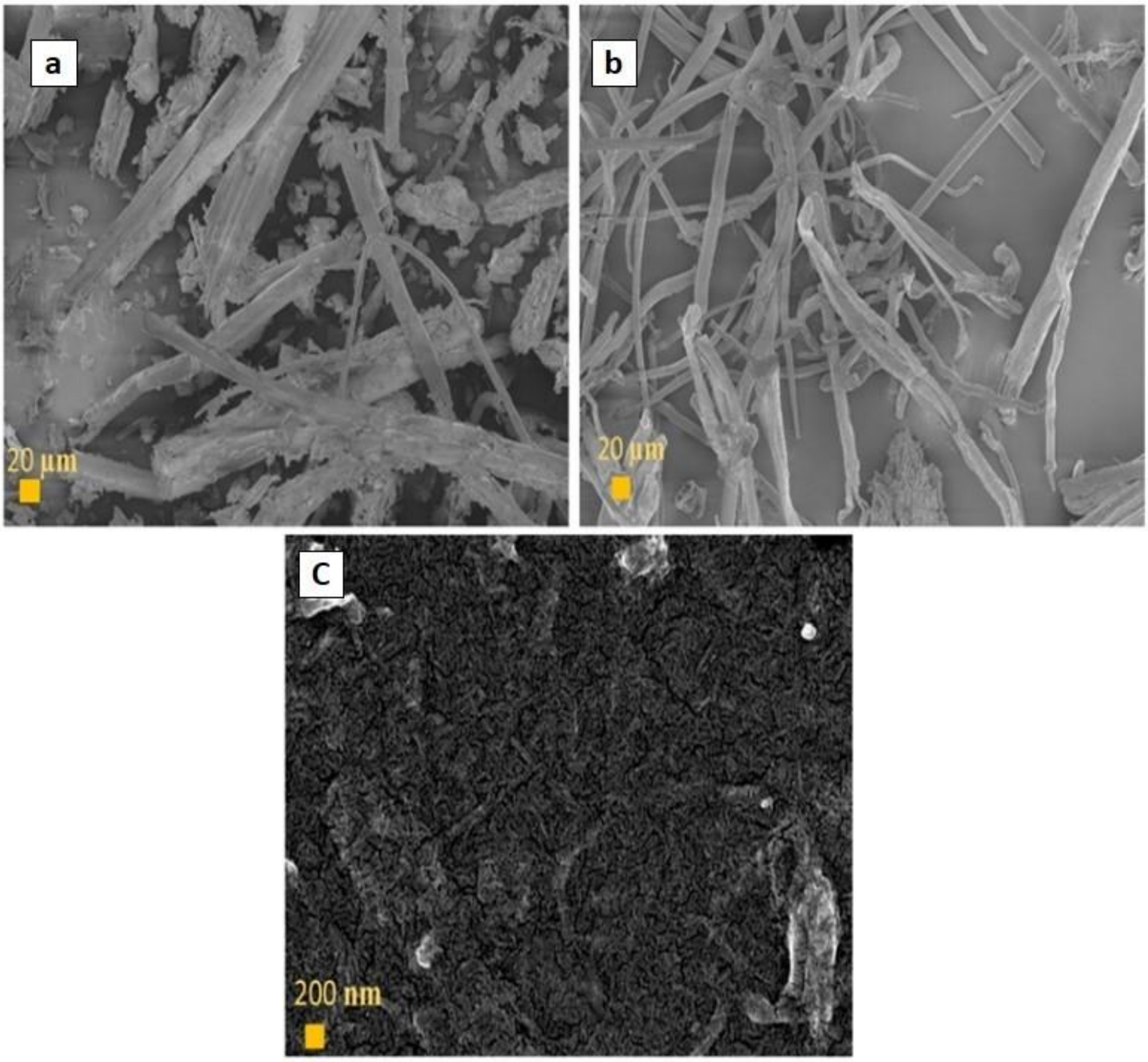

Figure 1. SEM micrograph, (a) Raw fiber of bamboo; (b) Alpha cellulose and (c) Isolated NCC sample

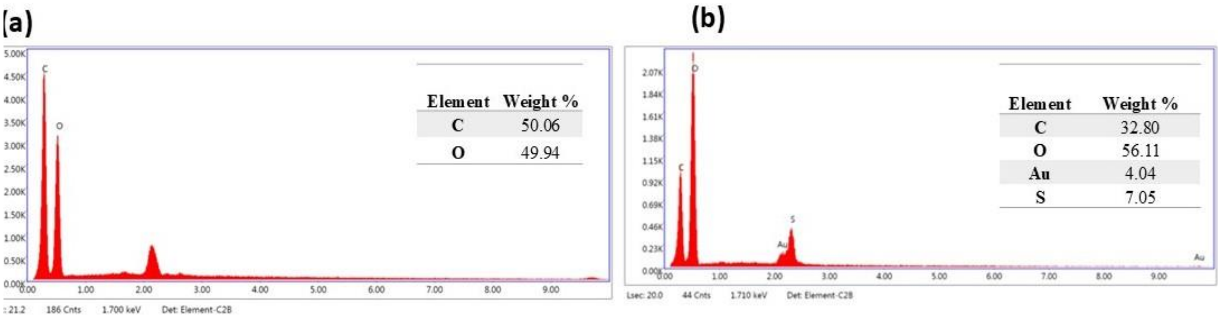

Figure 2. EDX analysis- (a) Raw fiber; (b) NCC sample

The powder was then subjected to several pre-treatments including dewaxing, delignification, mercerization and acid hydrolysis. The pretreated sample so obtained was dried in an oven and kept inside desiccator. Isolation of NCC was performed following the reported protocol with some modifications (Wijaya et al., 2019) . 20 ml of 60 % sulphuric acid was taken in a beaker and stirred in a magnetic stirrer. 1 g of pre-treated (MCC) sample was added slowly making the reaction mixture 1: 20 ratio g/ml. The reaction was carried on a magnetic stirrer at temperature of 40 °C for 45 minutes. The reaction was stopped by adding 20-fold of chilled distilled water. The solution was then kept for dialysis in a dialysis tube. Dialysis was done by continuous monitoring of pH and changing of distilled water for few days until the pH of dialysate becomes neutral. The neutral solution was then sonicated in an ultrasonic bath sonicator. The obtained turbid suspension was freeze dried to obtain NCC powder.

2.2.1. Morphological characterization of isolated NCC

2.2.1.1. Scanning electron microscopy (SEM)

The morphological characterisation of NCC was carried out using field emission scanning electron microscopy (FE-SEM). A small amount of NCC suspension was drop-cast onto a clean glass slide,

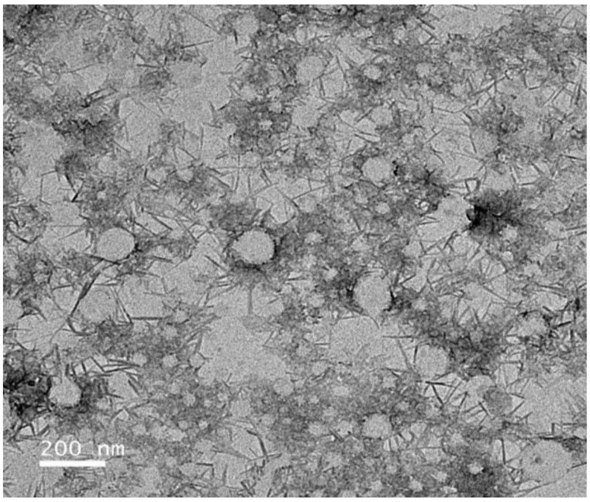

Figure 3. TEM micrograph of isolated NCC sample

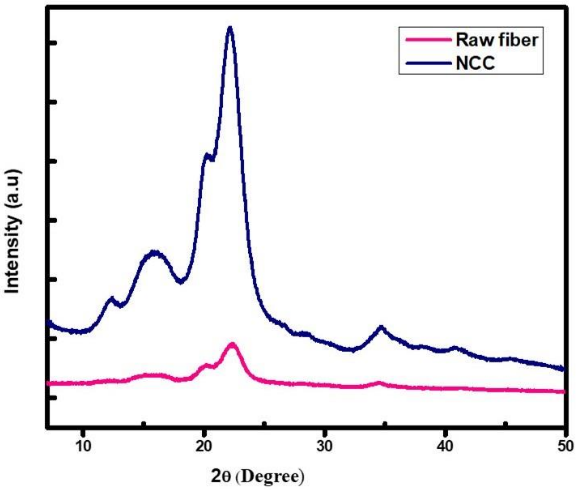

Figure 4. XRD diffractogram of the isolated NCC sample and raw fiber of bamboo

allowed to dry overnight at room temperature, and then sputter-coated with a thin layer of gold to enhance conductivity. Elemental composition analysis was performed using energy-dispersive X-ray spectroscopy (EDX) integrated with the FE-SEM instrument. For comparative evaluation, alpha cellulose and raw bamboo fibre samples were also prepared and examined under the same conditions to understand the extent of defibrillation and structural transformation during NCC production.

2.2.1.2. Transmission electron microscopy (TEM)

One of the most powerful tools to analyse nano-sized particle is TEM. For the analysis, the sample was diluted in proper concentration and then mounted on the copper grid. For better observation, the sample was negatively stained using a drop of 5 wt. % of uranyl acetate solution. The grid was allowed to dry at room temperature and finally observed in 200 Kv TEM machine to obtain the micrograph.

2.2.2. Physicochemical characterization of isolated NCC

2.2.2.1. X-ray Diffraction (XRD) patterns

X-ray diffraction (XRD) patterns were obtained using an X-ray diffractometer. The degree of crystallinity was determined and expressed as a percentage crystallinity index using the equation provided below (Segal et al., 1959).

$$CI = \left[\frac{(I_{oo2} - I_{am})}{I_{oo2}}\right] \times 100$$

Where, I_{oo2} is the peak intensity of the 002-lattice plane at 2θ value near 22.5° and I_{am} is the minimum intensity that lies near 2θ value 18°.

2.2.2.2. Fourier Transform Infrared (FTIR) spectroscopy

Fourier Transform Infrared (FTIR) spectroscopy was employed to investigate the chemical structure of the isolated NCC sample. The FTIR spectra were recorded using an FTIR spectrophotometer in the wavenumber range of 4000–400 cm^{-1}. Before analysis, the NCC sample was thoroughly dried to remove any residual moisture that could interfere with spectral interpretation. The acquired spectra were examined to identify characteristic absorption bands corresponding to specific functional groups in the cellulose structure. Particular attention was given to the bands associated with hydroxyl (–OH), alkane (C–H), and ether (C–O–C) stretching vibrations, as well as signals that reflect the purity and crystallinity of the cellulose. This analysis confirmed the presence of key chemical functionalities and validated the successful isolation and characterisation of the NCC sample.

2.2.3. Thermal characterization of isolated NCC

Thermal stability of the sample was evaluated using thermogravimetric analysis (TGA). The analysis was performed on a thermal analyser in the temperature range of 30 °C to 600 °C, with a constant heating rate of 10 °C/min under a nitrogen atmosphere to prevent oxidative degradation. The nitrogen gas flow rate was maintained at 100 mL/min throughout the run. Approximately 5 mg of sample was accurately weighed and placed in a standard aluminium pan, while an empty aluminium pan was used as the reference. The resulting thermograms were used to assess the thermal degradation behaviour and overall stability of the NCC sample.

3. Result and discussion

3.1. Scanning electron microscopy (SEM)

The surface morphology of NCC was examined using FESEM, as presented in Figure 1. The raw bamboo fibres (Figure 1a) exhibited a dense and irregular surface, whereas alpha cellulose (Figure 1b), obtained after alkaline and bleaching treatments, showed partial defibrillation. Following acid hydrolysis, the NCC sample (Figure 1c) displayed a network-like structure composed of rod-shaped nanofibres. Although some aggregation was observed, the fragmentation of larger cellulose structures into nanoscale elements was clearly evident. The nanofibres ranged in diameter from approximately 11.06 nm to 52.30 nm, aligning with the expected dimensions of nanocellulose. The marked reduction in fibre size and the increased surface area reflects the effectiveness of the hydrolysis process in removing non-cellulosic and amorphous components. The elemental composition of the samples was determined using EDX (Figure 2). The raw bamboo fibre (Figure 2a) predominantly comprised carbon (50.06 wt%) and oxygen (49.94 wt%), characteristic of lignocellulosic biomass. In contrast, the NCC sample (Figure 2b) contained carbon (32.80 wt%) and oxygen (56.11 wt%) as the primary elements, along with traces of sulfur (7.05 wt%) and gold (4.04 wt%). The presence of sulfur is attributed to sulphuric acid hydrolysis, while gold results from the sputter-coating process for FESEM analysis. The shift in elemental composition, particularly the increased oxygen-to-carbon ratio, coupled with the morphological transformation from dense, compact fibres to well-dispersed nanorods, provides strong evidence for the effective removal of non-cellulosic components such as lignin and hemicellulose during chemical treatment and the successful formation of nanoscale cellulose.

3.2. Transmission electron microscopy (TEM)

High-resolution TEM imaging enabled detailed observation of the morphological characteristics of the isolated nanocellulose. The hierarchical downsizing and defibrillation process, from larger microfibrils to nanoscale fibrils, is distinctly observed in the TEM micrograph (Figure 3). The image revealed well-defined, rod-like nanofibers with nanoscale dimensions. In addition, network-like

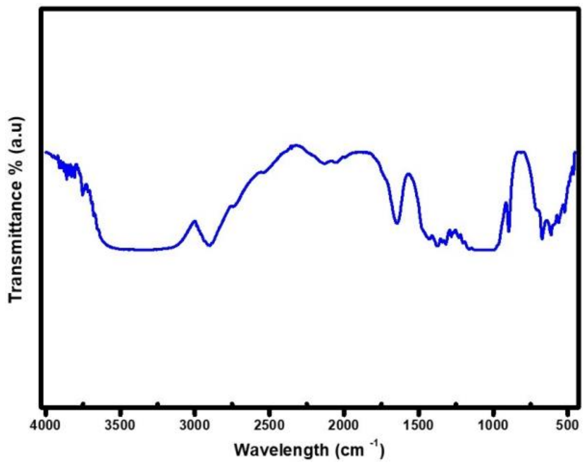

Figure 5. FTIR spectra of the isolated NCC sample

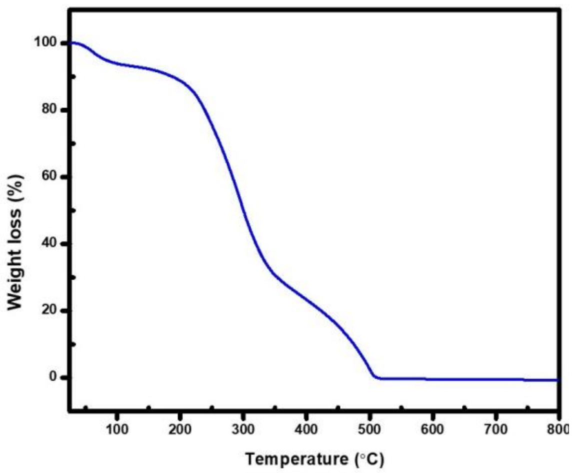

Figure 6. TGA thermogram of the isolated NCC sample

structures formed by interconnected nanofibers were clearly visible intricate arrangement. However, some degree of agglomeration is also noticeable, where individual nanofibers cluster together to form bundled formations, a common occurrence during the isolation process. One of the most significant observations from the TEM analysis is the high aspect ratio of the nanofibers, which refers to the longer length relative to their diameter. This characteristic is particularly important as a higher aspect ratio enhances the mechanical properties of the fibers, such as tensile strength, stiffness, and reinforcement potential. These findings are consistent with the work of Rasheed et al (2020), who also reported rod-shaped nanocellulose structures with slight agglomeration and high aspect ratio following acid hydrolysis of bamboo fibres.

3.3. X-ray Diffraction (XRD)

Among structural characterisation techniques, X-ray Diffraction (XRD) is widely used to investigate phase composition, crystallite dimensions, and lattice structure. The XRD patterns of raw fibre and NCC, as presented in Figure 4, confirms the crystalline nature of the samples. NCC exhibited sharper and more intense diffraction peaks compared to raw fibre, particularly around 2θ values of 15°, 20°, and 22°, which are characteristic of the cellulose I polymorph. The increased peak intensity and reduced background noise in NCC indicate a higher degree of crystallinity. Using Segal's equation, the crystallinity index was calculated to be 57.73% for raw fibre and 68.32% for NCC, confirming a significant reduction in amorphous regions following acid hydrolysis. The improved crystallinity plays a crucial role in determining the material's mechanical and thermal properties. These findings align with previously reported studies (Chan et al., 2012; Hachaichi et al., 2021), supporting the efficiency and reproducibility of the extraction process.

3.4. FTIR spectrum

The FTIR spectrum of bamboo-derived NCC, shown in Figure 5, revealed the structural and chemical transformations following the extraction process. A broad absorption band observed around 3345–3346 cm^{-1} corresponds to O–H stretching vibrations, indicative of hydrogen bonding within the cellulose matrix. The peak near 2900–2924 cm^{-1} is attributed to C–H stretching, reflecting the aliphatic nature of cellulose. The band around 1640–1646 cm^{-1} represents the bending vibration of absorbed water molecules. Distinct peaks at 1164, 1114, and 1058 cm^{-1} correspond to β-(1→4)-glycosidic linkages, C–O stretching, and C–C skeletal vibrations, respectively which are characteristic features of cellulose. Notably, the absence of peaks at 1739, 1371, and 1250 cm^{-1} confirms the effective removal of hemicellulose and lignin, supporting the chemical purity of the NCC. A slight shift and increased intensity in the O–H stretching region with ultrasonication suggest enhanced hydrogen bonding and possible nanocrystal re-aggregation. These spectral features are consistent with findings reported by Rasheed (2020), supporting the successful extraction of NCC while preserving the fundamental cellulose structure.

3.5. Thermogravimetric analysis (TGA)

The thermal decomposition profile of bamboo-derived NCC was assessed using *thermogravimetric analysis (TGA)*. The first decomposition event occurred with an onset temperature of 27.74 °C and a maximum degradation temperature of 128.49 °C, resulting in a weight loss of 6.99%, attributed to the evaporation of absorbed moisture. This relatively low moisture loss suggests moderate water retention. The second and primary decomposition phase began at 149.26 °C and peaked at 415.01 °C, with a significant weight loss of 71.19%, corresponding to the degradation of the cellulose backbone due to cleavage of glycosidic bonds. In the third stage, a further 19.33% weight loss was observed between 427.34 °C and 512.54 °C, representing the breakdown of more stable cellulose components. The temperature at 50% weight loss was recorded at 301.21 °C. These thermal characteristics align with previous findings, which report that nanocellulose with lower sulfate content typically decomposes at higher temperatures and leaves less char residue, indicating improved thermal stability and minimal structural alteration during processing. The current study showed better thermal stability than previously reported NCC samples in Rashid (2021), which degraded at much lower temperatures (180 °C) due to higher sulfate content (Figure 6).

4. Conclusion

The present study established a reliable method for extracting nanocrystalline cellulose (NCC) from bamboo using controlled acid hydrolysis, followed by extensive physicochemical characterisation. Morphological observations through SEM and TEM confirmed the formation of well-defined, rod-shaped nanocrystals with uniform distribution and nanoscale dimensions. EDX analysis verified the elemental purity of the material, predominantly composed of carbon and oxygen, with minimal inorganic residues. FTIR spectroscopy revealed the successful removal of non-cellulosic components, evidenced by the disappearance of lignin- and hemicellulose-related bands, while preserving the characteristic functional groups of cellulose. XRD analysis showed enhanced structural order, with a crystallinity index of 68.32%, indicating a substantial reduction in amorphous content when compared to the raw fibre. Thermal analysis through TGA demonstrated that the bamboo-derived NCC exhibited high thermal resistance, with a 50% mass loss occurring at 301.21 °C, suggesting a stable cellulose framework with low sulfate incorporation. These results confirm that bamboo serves as an efficient and sustainable source of NCC, yielding a material with promising morphological, structural, and thermal properties suitable for diverse industrial and biomedical applications.

Acknowledgements

The authors express their sincere gratitude to the Department of Botany and Department of Physics, Gauhati University, for providing the essential research facilities and infrastructure required to carry out this work.

Author Contributions

JP was responsible for data generation and preparation of the initial manuscript draft. NN and BT supervised the research activities and critically evaluated the manuscript. BT reviewed and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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