



RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF NANOCELLULOSE FROM BAMBOO FIBERS

Muhammad Akmal Kosnan¹, Rose Farahiyah Munawar^{1,*}, Muhamad Amer Kamli¹, Mohd Asyadi Azam¹, Intan Sharhida Othman¹, Jeefferie Abd Razak¹, Nur Ezyanie Safie²

¹*Fakulti Teknologi dan Kejuruteraan Industri dan Pembuatan, Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, Durian Tunggal, Melaka 76100, Malaysia.*

²*Fakulti Teknologi dan Kejuruteraan Elektrik, Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, Durian Tunggal, Melaka 76100, Malaysia.*

Abstract. Bamboo is recognized as a sustainable and renewable resource in Malaysia, with emerging potential for advanced material applications. To explore its viability for nanocellulose production, this study investigates the extraction of nanocrystals cellulose (NCC) from bamboo fibers through optimized chemical and mechanical processes, aiming to develop an efficient and environmentally conscious synthesis method. While there have been limited studies on bamboo, particularly *Phyllostachys Aurea*, this research uses acid hydrolysis with sulfuric (H₂SO₄) and hydrochloric (HCl) acids to prepare NCC. The study tested hydrochloric acid (HCl) concentrations between 50–70 wt% under controlled conditions (120 minutes, 45 °C). At 60% HCl, the hydrolysis yielded optimally dispersed crystalline cellulose with reduced aggregation, outperforming other concentrations. Specifying these conditions ensures methodological clarity and underscores the importance of acid concentration in nanocellulose synthesis. The resulting NCC exhibited a crystallinity index of 49.20% and a crystallite size of 4.04 nm, as confirmed by XRD and FTIR analyses. Additionally, Field Emission Scanning Electron Microscopy (FESEM) revealed a well-defined fibrous morphology and improved structural integrity, supporting the successful isolation of nanocellulose. The optimized process yields NCC with enhanced structural properties, demonstrating its potential as a reinforcing agent in nanocomposites. These findings position bamboo as a viable, eco-friendly resource for green material development.

Keywords: Bamboo nanocrystal cellulose (ncc), acid hydrolysis, sustainable extraction, nanocomposites.

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*Corresponding author: rosefarahiyah@utem.edu.my

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1. INTRODUCTION

Bamboo-derived nanocellulose has emerged as a promising biomaterial in recent years, owing to its exceptional physicochemical properties and versatile applications. As a renewable plant resource, bamboo offers distinct advantages for sustainable nanocellulose production, with potential uses across multiple industrial sectors [1]. Hence, this research focuses on using a two-stage chemical process to create nanocrystals cellulose (NCC) from bamboo fibers which involves an alkaline pre-treatment to remove pure cellulose, followed by acid hydrolysis to create NCC. The objective is to overcome difficulties in the synthesis and characterization of nanocellulose generated from bamboo while achieving high crystallinity and stable colloidal suspensions of NCC. Bamboo was selected because of its vast availability, sustainability, and quick growth, which make it a desirable resource for projects with renewable materials [2]. The absence of defined characterization techniques has hindered the commercialization of nanocellulose derived from bamboo fibers, despite its potential. Comparisons between studies are challenging due to variations in methods for evaluating characteristics such as particle size, surface area, crystallinity, and chemical composition, which prevent industrial adoption. Nanocellulose derived from bamboo offers advantages such as high strength, biodegradability, and excellent mechanical properties, making it suitable for applications in textiles, packaging, construction, electronics, and biomedicine. However, the high lignin content in bamboo fibers poses challenges in achieving optimal yield and purity, requiring precise control over acid hydrolysis parameters like acid concentration, hydrolysis time, and temperature. This study examines how acid concentration (50-70 wt% HCl) affects bamboo nanocellulose production. The optimal 60% HCl yielded well-dispersed nanocrystals with minimal aggregation. These findings help establish reproducible methods for quality nanocellulose synthesis. Additionally, addressing the specific characteristics of 'Phyllostachys Aurea' bamboo fibers may uncover unique process optimizations and further enhance the quality and potential applications of bamboo-derived NCC. Table 1 shows the properties of the nanocrystal cellulose by researchers [3].

Table 1: Characteristics of nanocrystals cellulose (NCC)

Properties	Desired level
Tensile modulus	131 GPa
Ratio	100-500 nm
Crystallinity	70-95% (XRD)
Level of whiteness	80-95%
Viscosity	500-10,000 mPa·s

The objective of this research is to optimize processing conditions for converting bamboo fibers into NCC through chemical modification. The methodology involves sequential alkaline pre-treatment to isolate cellulose, followed by controlled acid hydrolysis to transform the cellulose into NCC with enhanced crystallinity and tailored physicochemical properties. To evaluate the structural and chemical properties of the derived NCC, X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) analyses were employed to determine its crystallinity and functional group characteristics. Additionally, Field Emission Scanning Electron Microscopy (FESEM) was utilized to examine the surface morphology and structural features of the cellulose nanocrystals, providing insights into their nanoscale architecture [4]. These analyses collectively aim to validate the efficacy of the extraction process and characterize the resulting NCC for potential applications in various industries. According to one study, bamboo has outstanding physical and mechanical properties and can be cultivated practically everywhere in the world commonly used in civil construction. Figure 1 shows various places of bamboo planted around the world [5].

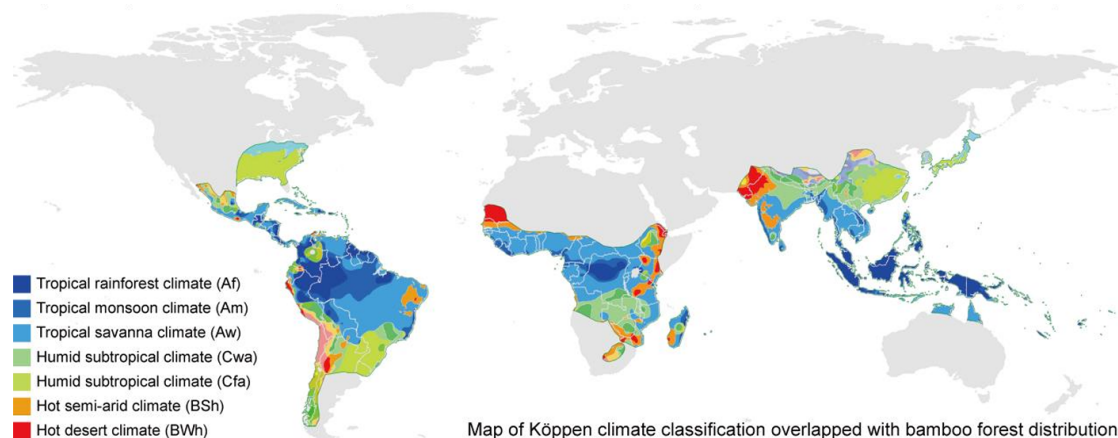


Figure 1: Mapping bamboo growth

The research seeks to develop standardized protocols for producing and characterizing nanocellulose from processed bamboo fibers (as opposed to raw bamboo culms). Table 2 presents the chemical composition of the specific bamboo fiber material used in this study, which underwent specific procedure prior to nanocellulose production [6].

Table 2: Chemical composition of bamboo fibers

Analysis	Composition (%wt)
Moisture	10.99%
Volatile	83.70%
Ash	1.4%
Fixed Carbon	3%
Lignin	1.5%
Cellulose	47.2%
Hemicellulose	23.9%

2. MATERIALS AND METHODS

This study investigates the use of bamboo as a sustainable and effective raw material for nanocellulose synthesis. Bamboo's rapid growth rate, minimal cultivation requirements, and high cellulose content make it a viable alternative feedstock [7]. The bamboo used in this study was obtained locally with appropriate permissions. Commercially available analytical-grade reagents were used in this study. During the pre-treatment phase, hemicellulose and lignin were expected to be partially removed through an alkali treatment using 5 wt% sodium hydroxide (NaOH). In the subsequent stages, sulfuric acid (H₂SO₄) and hydrochloric acid (HCl) were also employed; however, the precise concentration for the acid hydrolysis process differed. The trials were conducted using distilled water. The production of cellulose nanocrystals from bamboo fibers requires a number of experimental procedures, such as sample preparation, alkali pretreatment, and acid hydrolysis for cellulose nanocrystal separation. The fiber from *Phyllostachys Aurea* bamboo is the raw material used in this experiment. A total of about two kilograms of bamboo were prepared. Before being cleaned four or five times with hot water, the bamboo culms were initially sectioned into 2-4 cm segments using a stainless steel blade. Following a 24-hour equilibration period at ambient temperature (25±2 °C), the prepared segments were mechanically disintegrated using a high-speed rotary blender operating at 10,000 rpm for particle size reduction. This mechanical pretreatment effectively homogenized the lignocellulosic material while preserving structural integrity of the fibrous components.

2.1 Preparation of Sample from Bamboo Fiber

The bamboo was obtained with permission from a resident of the area. This experiment uses bamboo fiber, namely from "Phyllostachys Aurea," as the raw material. A total of about two kilograms of bamboo were prepared. First, the bamboo was divided into little pieces, about 2-4 cm in length, and then it was washed four or five times using hot water. The prepared bamboo segments were subjected to a 24-hour conditioning period at room temperature. Subsequently, the samples were comminuted using a high-speed blender operating at 10,000 rpm for 3 minutes to achieve homogeneous particle size reduction.

2.2 Pre-treatment by Alkali Treatment

A 5% concentration of NaOH was applied to the bamboo fiber sample for 24 hours at room temperature in order to break the hydrogen bonds between different cellulose chains and eliminate lignin and hemicellulose [8]. After that, the liquid was filtered and repeatedly cleaned with distilled water until the pH was neutral. After that, the mixture was dried for three hours at 100 °C in a drying oven.

2.3 Preparation of Nanocrystals Cellulose

Different quantities of H₂SO₄ and HCl, 50 wt%, 55 wt%, 60 wt%, 65 wt%, and 70 wt%, were used to carry out the acid hydrolysis, which lasted for 120 minutes. A temperature correlation of 45 °C was established. The optimal concentration was found to be 60 wt%, and prior research indicates that a concentration of 60 to 65 wt% is excellent for carrying out the acid hydrolysis method. A cellulose-to-acid solution ratio of 1:14 (w/v) was established. To find out if the concentration has an impact on the hydrolysis time, a new parameter was developed. H₂SO₄ and HCl have been combined with the bamboo fibers that have been alkali-treated. Different hydrolysis periods and concentrations were used in the experiment. Until the end of the hydrolysis time, the mixture was continuously mixed. Using pure water, the hydrolyzed cellulose was dialyzed several times until the pH reached 7. After that, the sample was dried at 50 °C in an oven. For analysis, the produced cellulose nanocrystals were kept at room temperature. After the bamboo fiber went through the processes, the nanocellulose could be obtained as shown in Figure 2, that then characterized by utilizing XRD, FTIR and FESEM

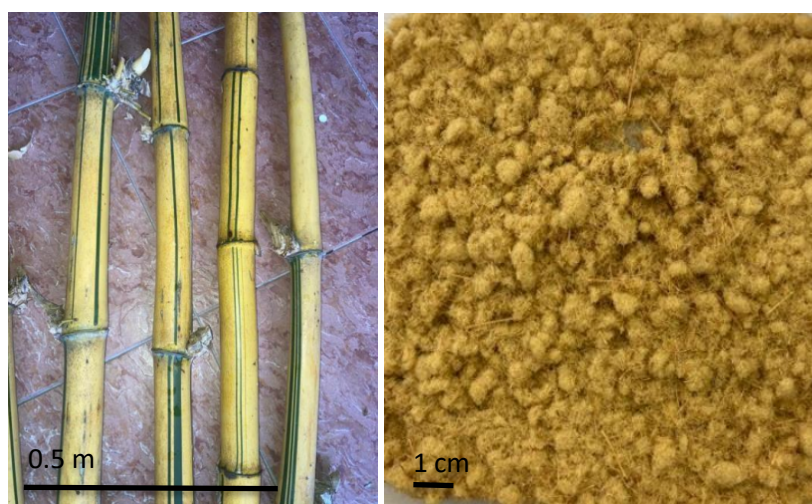


Figure 2: Images of the bamboo and derived nanocellulose obtained under selected parameters

An investigation into the extraction of NCCs from bamboo fiber was carried out using statistical analysis. The three crucial variables in this experiment were the temperature correlation, the hydrolysis time period, and the acid content. This led to the creation of nine samples under different conditions to carry out the acid hydrolysis process. Previous research indicates that the most widely used technique

for removing cellulose nanocrystals from different kinds of natural fibers is acid hydrolysis [9]. The chosen method was chosen due to its validity and dependability as well as prior research. Table 3 presents the experimental design for acid hydrolysis, detailing the specific reaction conditions tested for each sample.

Table 3: Arrangement of sample condition for acid hydrolysis

Sample	Acid concentration (w %)	Hydrolysis time (min)	Hydrolysis temperature (°C)
H ₂ SO ₄	50	120	45
	55		
	60		
	65		
	70		
HCl	50	120	45
	55		
	60		
	65		
	70		

Additionally, using different combinations of acid concentrations, temperature correlation, and hydrolysis duration, the acid hydrolysis strategy is one of the simplest ways to obtain bamboo fiber. Therefore, different concentrations of sulfuric and hydrochloric acid, as well as different hydrolysis times, were used to create the conditions needed to carry out this chemical treatment. In the meanwhile, 45 °C has been set as the temperature. An illustration of the general process is provided in Figure 3.

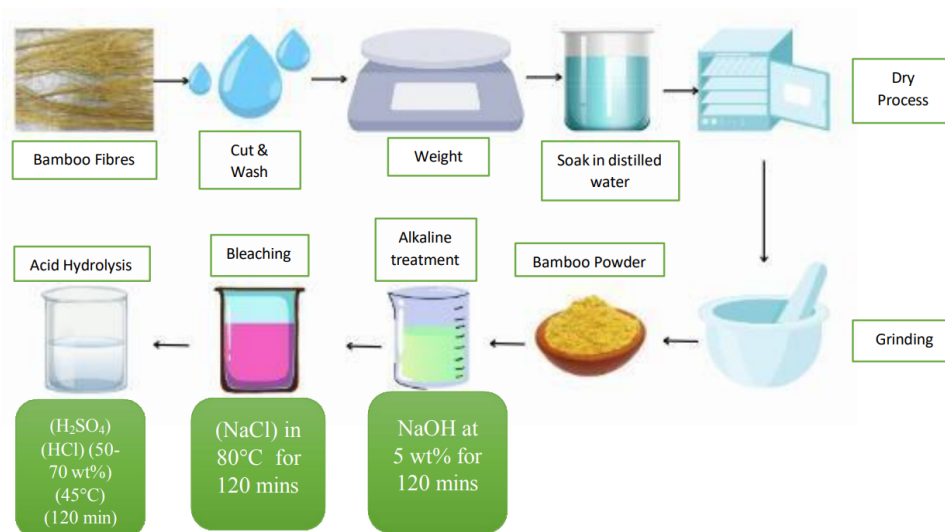


Figure 3: Illustration of Preparation of Nanocellulose from Bamboo Fibers

3. RESULTS AND DISCUSSION

3.1 X-Ray Diffraction (XRD) Analysis

The crystallinity index (CrI) of cellulose isolated from bamboo fiber were evaluated using X-ray diffraction (XRD) analysis. Figures 4(a) and (b) show the XRD of the samples that have been alkaline-treated differ with the untreated one. The sample's CrI was obtained using the Segal calculation in Equation 1. The highest peak intensity value for crystalline cellulose is provided by I200 at around

$2\theta = 22.0^\circ$ to 24° . In contrast, the peak intensity of diffraction of the amorphous area of cellulose is given by I_{am} .

$$CrI (\%) = (I_{200} - I_{am}) / I_{200} \times 100 \tag{1}$$

This formula is used to calculate the percentage of crystallinity [10]. The Scherrer Equation is then used to determine the crystallite size of the samples that were obtained from the XRD data. Equation 2 serves as the foundation for the Scherrer equation. The wavelength in this instance is λ (1.5418 Å), the full width at half maximum intensity (FWHM) is β , and the Bragg angle at peak maximum, stated in radians, is half equal to θ . The Scherrer constant that best fits the structure of the nanocrystal is K .

$$D_{hkl} = K\lambda / \beta \cos \theta \tag{2}$$

Bamboo nanocellulose was found to have a higher degree of crystallinity when the crystalline behavior of fibers was assessed using X-ray diffraction. The XRD results show that treated alkaline bamboo fiber has a larger peak than untreated bamboo fiber. The high peak intensity of $2\theta = 22$ in both the untreated and alkaline-treated bamboo fiber samples was caused by the crystalline structure of cellulose, but the existence of a broad peak at about 16.69° indicates the amorphous organization [11].

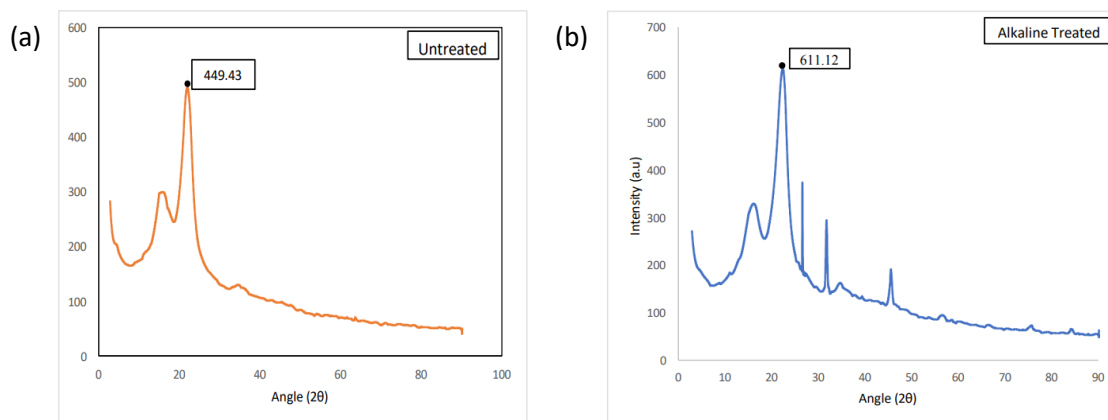


Figure 4: (a) X-ray diffraction before alkaline treated and (b) after alkaline treated bamboo fiber

As shown by XRD, both exhibit the same diffraction peaks, which are on $2\theta = 16.69^\circ$ for the amorphous component and on peak $2\theta = 22.52^\circ$ for the crystalline structure of cellulose for both treated and untreated bamboo fiber. According to one research, bamboo fibers that reflect type I cellulose have an estimated 61-67% crystallinity index [12]. This indicates that bamboo fiber, which is classified as type I nanocellulose, has undergone particular processes that can produce cellulose nanofibrils. It is possible to deduce that the crystallinity peak increases after an alkaline treatment. Table 4 depicts the results of this investigation, which included the calculation of the crystallite size and the crystallinity index (CrI) using the Segal and Scherer equations, respectively.

As shown in tabulated Table 4, untreated bamboo fiber has the lowest crystallinity at 35.75% and the lowest percentage of amorphous regions. However, as indicated by the crystallinity index, the percentage of CrI increases after purification with an alkaline treatment and treatment with an acid hydrolysis. When amorphous cellulose is removed by alkaline treatment, the crystallinity index (CrI) increases; if not, it may have increased as a result of the process' successful removal of amorphous cellulose and partial removal of non-cellulosic components, producing extremely pure nanocellulose [13]. The results showed that the diffraction peak of treated bamboo fiber was sharper than that of untreated bamboo fiber, with an intensity of $2\theta = 22.69^\circ$. The amorphous phase of cellulose is indicated by the presence of a large peak at a 2θ value of around 16° . Conversely, it is important to remove non-

cellulosic materials such as hemicellulose and lignin in order to get very pure nanocellulose. Research studies suggest that alkaline treatment is one way to get rid of lignin and hemicellulose residue in fiber [14]. A study reveals that an advanced process can be applied to the fiber after the alkaline treatment did removes the residual extractive less than 1%. In addition, it was discovered that the alkaline treatment's spectra included peaks about 1024.02 cm^{-1} , which corresponds to the C-O stretching peak. These peaks were marginally smaller than those of the untreated bamboo fibers, and 1020.16 cm^{-1} indicates much of the hemicellulose and lignin had been eliminated.

Table 4: Crystallinity Index (CrI) of untreated and alkaline treated bamboo fiber

Sample	2 theta (amorphous)		2 theta (crystalline)		Crystallinity index %
	Degree	Intensity	Degree	Intensity	
Untreated Bamboo Fiber	16.69	288.77	22.52	449.43	35.75
Alkaline Treated Bamboo Fiber	16.75	322.19	22.69	611.12	47.28

Figures 5(a) and (b) shows two samples, each with a distinct acid type for acid hydrolysis—HCl and H₂SO₄ with a concentration of 50–70 wt%, a set duration of 120 minutes, and a constant temperature of 45°—were compared. The XRD graph indicates that after acid hydrolysis and alkaline purification, the crystallinity peak rises. It is possible to conclude that both acids have cellulose type I structure based on the diffraction pattern, which shows the amorphous material to have a wide peak at about 17° [15]. The results indicate that there are three diffraction peaks for nanocellulose: $2\theta = 5^\circ, 17^\circ,$ and 22° . The sample for H₂SO₄ with acid concentration of 50-60 wt% does not show the presence of peak for the amorphous region, making the result for the crystallinity index could not be obtained. For this case, the sample was discarded from the project.

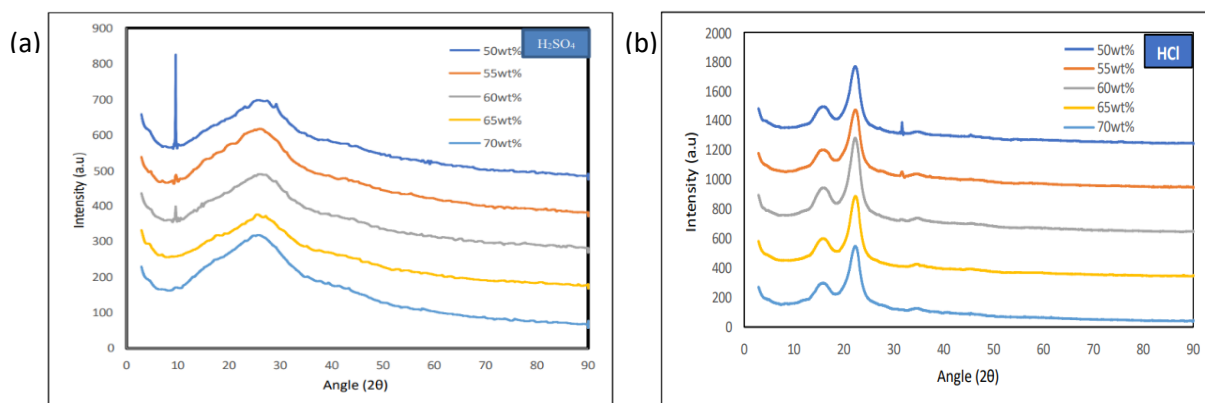


Figure 5: (a) XRD analysis of H₂SO₄ and (b) XRD analysis of HCl

During acid hydrolysis, the use of concentrated HCl, 70 wt%, due to its strong acidity, may lead to the degradation of both amorphous and crystalline regions of cellulose, thereby reducing the crystallinity index. In contrast, H₂SO₄ preferentially hydrolyzes the amorphous regions, preserving the crystalline structure and resulting in nanocellulose with a higher crystallinity index. The high reactivity and concentration of HCl can also cause thermal degradation of the samples during the process [16]. The bamboo fibers become black as a result and disintegrate in the acid. These circumstances make it impossible to do the characterization for the analysis.

3.2 Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectroscopy was employed to analyze the intrinsic chemical composition of the samples as shown in Figure 6. This analysis also supported the XRD findings by confirming the removal of

hemicellulose, lignin, and other impurities during the treatment process. The FTIR spectrum demonstrates that the pattern of treated bamboo fibers' spectra differs considerably from that of untreated bamboo fibers. The alkaline method used to eliminate lignin and hemicellulose before the chemical process of acid hydrolysis is the reason for this. Alkaline-treated bamboo fibers have a vibration peak at 3328 cm^{-1} , but untreated bamboo fibers have a vibration peak at 3315 cm^{-1} , which suggests that the hydrogen bonds O–H were bent as a result of water absorbed in the cellulose [17]. The peaks at 894.90 cm^{-1} indicate the C–H stretching of untreated bamboo fibers, while the peaks at 894.01 cm^{-1} indicate alkaline-treated bamboo fibers.

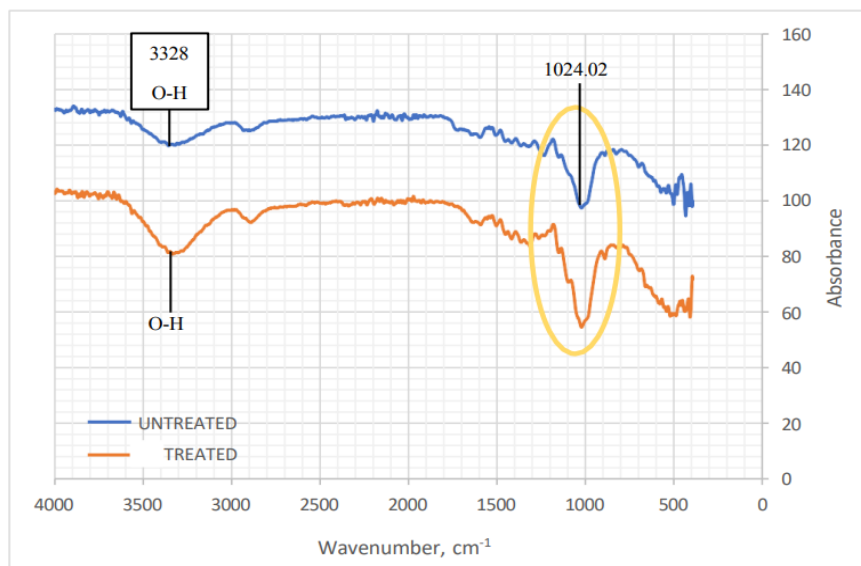


Figure 6: Untreated and Alkaline treated bamboo fibers under FTIR Spectra

Thus, it can be concluded that the FTIR spectra shows that the hemicellulose and lignin that were present in the sample were successfully removed by the alkaline treatment. According to studies, alkaline treatment helped to reduce the fiber's water absorption, and a clearer surface indicates that NaOH can show the elimination of hemicellulose and lignin.

The FTIR spectra of HCl and H_2SO_4 throughout acid hydrolysis are displayed in Figure 7. The spectra of nanocellulose acquired under various types of acid utilized for acid hydrolysis. The transmittance peak observed at 1144 cm^{-1} is characteristic of cellulose type I, indicating that the nanocellulose retained its native crystalline structure.

The peak at 1106 cm^{-1} indicates the C–O stretching mode. Two bands, 2107 and 2327 cm^{-1} , were discovered to generate precipitated calcium carbonate. These bands represent the O–C–O bending mode of calcite and the symmetric CO stretching mode, respectively [18]. However, following acid hydrolysis treatment, the intensity of the band at 1106 cm^{-1} decreased. The treatment effectively removed calcite, yielding acceptable purity nanocellulose. Additionally, the peaks in the absorption range of $3100\text{--}3300\text{ cm}^{-1}$ can be attributed to the stretching and bending vibrations of the cellulose's OH groups that absorb water. The identification of a wider peak at 2154 cm^{-1} , which corresponds to the O–H stretching vibration, indicated that there was an increase in water absorption following the treatment, which led to the amorphous cellulose being removed. Therefore, it may be inferred that the FTIR spectra difference between HCl and H_2SO_4 indicated that the acid hydrolysis procedure was successful in isolating the nanocellulose from the bamboo fiber. Thus, it is prudent to draw the conclusion that the practically pure nanocellulose produced in this work only contained trace amounts of lignin and other non-cellulosic ingredients.



Figure 7: Acid hydrolysis of H₂SO₄ and HCl under FTIR Spectra

3.3 Morphological Analysis by FESEM

Figure 8(a) displays a FESEM images of both untreated and alkaline-treated bamboo fiber. The untreated bamboo fiber's surface form was irregular and coated with impurities such as hemicellulose, lignin, wax, and other extractives, as seen in Figure 8(b). On the other hand, Figure 9 illustrates how the alkaline treatment changed the treated bamboo fiber's surface form. The surface of alkaline-treated fibers seems significantly cleaner than that of untreated fibers, but it also has a little roughness texture. According to a study, to improve fiber, surface impurities must be eliminated from the fibers through appropriate chemical treatment [19]. The process of acid hydrolysis, on the other hand, removes the remaining amount of amorphous lignin.

After alkaline treatment, acid hydrolysis was carried out by immersing in a 50–70 wt% HCl and H₂SO₄ solution. Following that, distilled water was used to filter and completely clean the fibers in order to achieve a pH of neutral. After that, the fiber is dried in an oven set to 60 °C for 12 hours. The small color changes of the fiber on H₂SO₄ are what give the bamboo fiber nanocellulose powder its white hue. HCl is what gives the powdered nanocellulose from bamboo fiber its dark grey color [20]. SEM analysis is being done on the acid hydrolysis sample to assess its surface shape.

The rod-like shape of the morphological surface in Figure 10 might be connected to the main breakdown of the amorphous cellulose. H₂SO₄ is the most widely used acid hydrolysis technique because, once amorphous components are removed, it yields extremely crystalline, stiff, and efficient products. Figures 10(a) and (b) depict the surface morphology of HCl under these conditions, where no cellulose fibers develop and only large-scale aggregation is shown. Higher corrosion resistance and a well-defined, typically large-scale aggregation of nanocellulose fibers are characteristics of HCl, in contrast to H₂SO₄, whose strong acidity dissolves bamboo fiber. H₂SO₄ was chosen as the best weight % acid hydrolysis technique to produce evenly distributed crystalline cellulose.

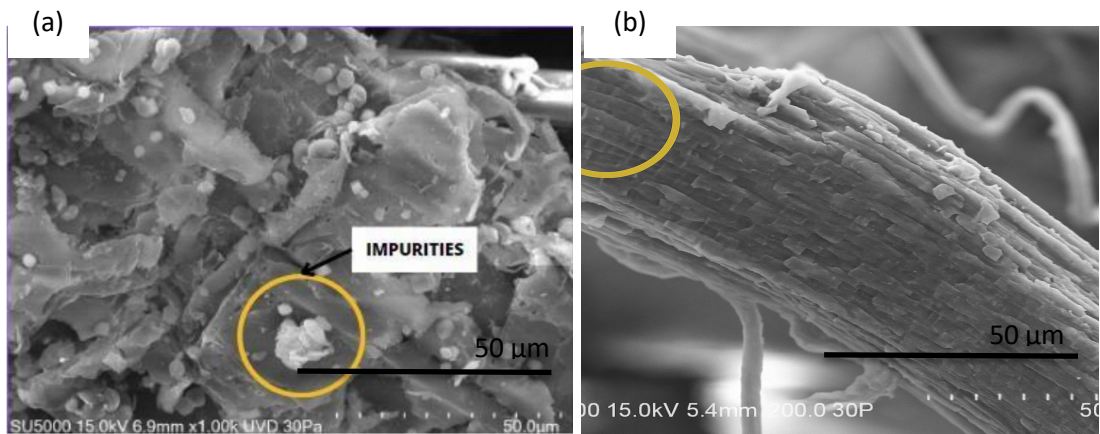


Figure 8: (a) FESEM micrographs of alkaline untreated sample and (b) alkaline treated sample

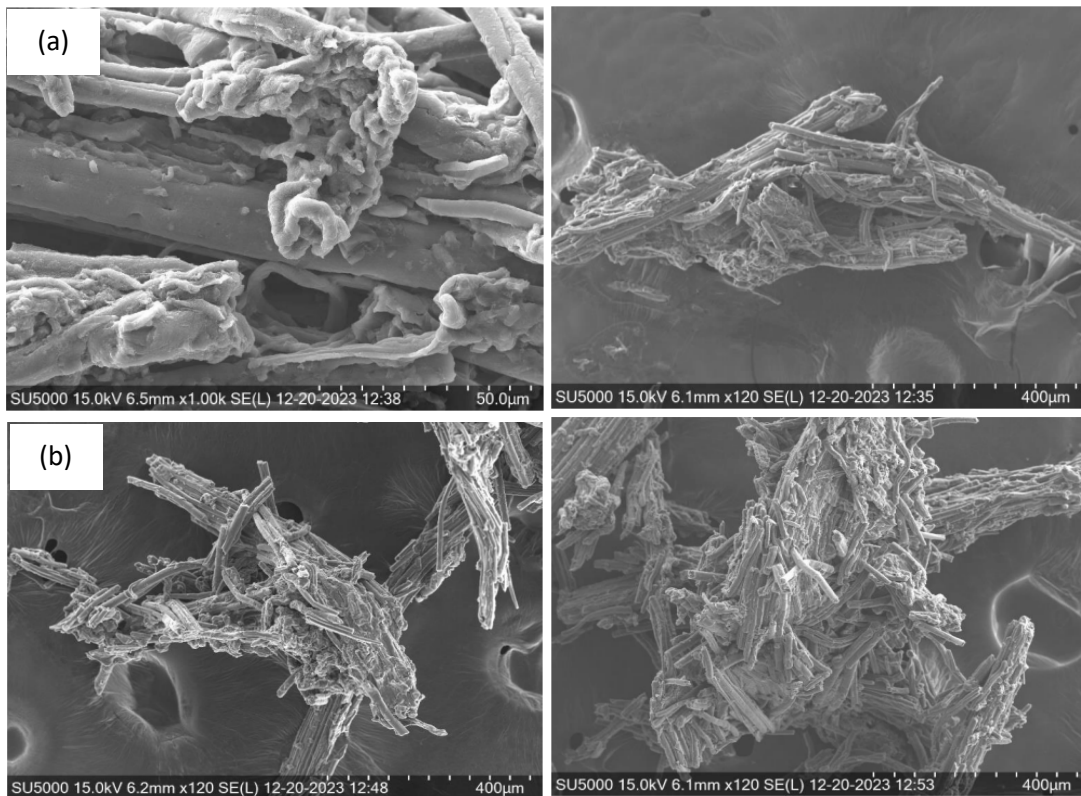


Figure 9: (a) 50 wt% surface morphology of H₂SO₄ on scale 50 µm and 400 µm and (b) 70 wt% surface morphology of H₂SO₄ on scale 400 µm

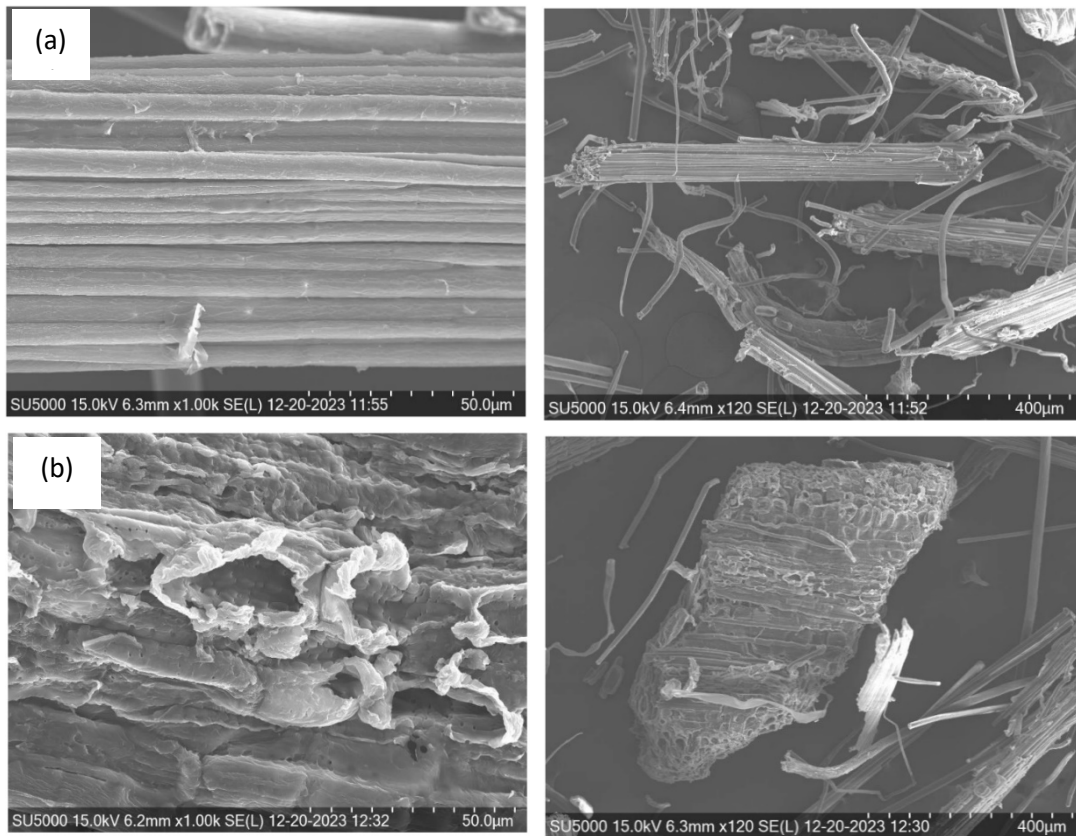


Figure 10: (a) 50 wt% surface morphology of HCl on scale 50 µm and 400µm, and (b) 70 wt% surface morphology of HCl on scale 50 µm and 400 µm

4. CONCLUSIONS

Nanocellulose was successfully extracted from bamboo fibers through a two-phase treatment process involving alkaline treatment with 5 wt% NaOH followed by acid hydrolysis using HCl and H₂SO₄. The optimal parameters for nanocellulose production were 60 wt% acid concentration, 120 minutes hydrolysis time, and 45 °C temperature, yielding high crystallinity and suitable crystallite size. XRD analysis using the Segal Equation confirmed the crystallinity index, while the Scherrer Equation validated the crystallite size. FTIR analysis demonstrated the removal of lignin and hemicellulose during the alkaline treatment. SEM revealed significant morphological changes, confirming the effective elimination of impurities and structural refinement of the bamboo fibers. These findings underscore the potential of bamboo as a sustainable and efficient raw material for nanocellulose production, contributing to the advancement of eco-friendly materials. Future work should explore the performance of the extracted nanocellulose in composite applications and investigate scalability for industrial production to support broader adoption in green material technologies.

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Author Contributions

All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure of Conflict of Interest

The authors have no disclosures to declare.

Compliance with Ethical Standards

The work is compliant with ethical standards.

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