

## Research Article

# Bacterial Cellulose-Based Biofilm Forming Agent Extracted from Vietnamese Nata-de-Coco Tree by Ultrasonic Vibration Method: Structure and Properties

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Bacterial cellulose has recently received more attention in several fields including biology and biomedical applications due to its outstanding physicochemical properties such as thermal stability, biodegradability, good water holding capacity, and high tensile. Cellulose, the most abundant biomolecule on Earth, is available in large amounts in plants. However, cellulose in plants is accompanied by other polymers such as hemicellulose, lignin, and pectin. On the other hand, highly purified bacterial cellulose without impurities is produced by several microorganisms. In which, the most active producer is *Acetobacter xylinum*. A. This study developed a new process using sonication to isolate bacterial cellulose from nata-de-coco Vietnam. Sonicating time and temperature, two important engineering factors, were considered and discussed (Temperature: 55, 60, 65, 70°C; Time: 15, 30, 60, 90 min). Research results have established that the ultrasonic vibration time of 60 minutes at 65 degrees Celsius gives the best structural properties of BC. The morphology, structural, and thermal properties of the obtained films were investigated by SEM, FTIR, and TGA. Besides, tensile strength was also evaluated. The results show that sonication is not only a favorable technique to isolate cellulose nanofibers but it also enhances their crystallinity.

## 1. Introduction

Bacterial cellulose (BC) is abundant in nature and has excellent properties such as mechanical strength, biocompatibility, hydrophilicity, and relative thermal stability [1]. Cellulose has been used in various studies for applications such as pharmaceuticals [2, 3], filters [4, 5], drug delivery [6, 7]. In recent new studies, cellulose is also used as a food preservation composite film [8], an antibacterial cellulose biofilm [9], and an antibacterial film combined with Ag [10]. On the other hand, cellulose is also studied to make cellulose/gelatin films for wound dressings [11, 12]. Some other studies also study the use of cellulose in combination with other nanomaterials such as single-walled carbon nanotubes applied in biosensors [13, 14]. In addition, stabilizers or emulsifiers made from cellulose have been studied [15, 16].

On the other hand, with a structure including many hydroxyl groups in the molecular string, BC tends to prepare composites based on carbonized bacteria cellulose (CBC). CBC, with additional attractive features such as outstanding conductivity, high surface area, super flat density, and super-hydrophilization, is very desirable in areas related to electricity and magnetism [17].

A survey on Nano BC fibers and materials made from them showed that these materials have nanostructures with enhanced nanofibers that can cause changes that are comparable to carbon nanotubes in bone frame applications. Khan and Dahman study showed that polyurethane reinforced with bio-cellulose nanofibers with improvements in biological compatibility and mechanical properties has a strong potential for bone transplants and other tissue engineering applications [18]. Besides, some biodegradable

nanocomposite materials have been studied based on polymers such as polyvinyl alcohol [19], polylactic acid [20], polymethyl methacrylate [21], starch [22], and epoxy [23–25]. In addition to the polymer, various nanoparticles (titanium dioxide) [26, 27] and plant extracts have also been combined with BC to enhance its properties and impart new functions [28, 29]. As a result, polymer composite materials reinforced with BC achieved better mechanical and thermal properties. Therefore, the isolation of cellulose nanofibers has been given more attention to. It is especially true with isolations with low cost, eco-friendly methods and without serious degradation of cellulose nanofibers [30]. In particular, cellulose was isolated naturally by a bacterium called *Acetobacter xylinum* from cheap substrates [31, 32]. There were several methods for isolating BC from nature using chemical and mechanical means. However, these methods possessed some disadvantages, such as low yield, severe cellulose degradation, not being eco-friendly, and high energy consumption. The isolation of cellulose from plant resources can be by mechanical, chemical, or biological methods. Among the above methods, the method of extracting cellulose from plant sources through some bacterial strains produces a cellulose that has many good properties and has potential applications in many different fields [33]. Wang and Cheng, high-intensity ultrasound was used to isolate fibers from several cellulose sources [34]. Some other works have also investigated the effects of ultrasound on the formation of BC. Those results showed that the sonication method is an appropriate method for isolating BC nanoparticles from nature. This method can meet the requirements of natural structure preservation, low cost, eco-friendliness [35–37]. BC existing in nata-de-coco, a famous fermented product of coconut water using *Acetobacter* in South Vietnam, is a type of cellulose and has been used to prepare bio-composites [38–40]. Hasanin et al. conducted in situ synthesis studies of nanobioactive BC/NBG (nanobioactive glass) compounds by a new and simple method [41]; they prepared environmentally friendly cryogels using natural polymers such as hydroxyethyl cellulose (HEC) and bacterial cells (BC) [42]. In addition, Hasanin et al. also produced bacterial cellulose (BC) by *Gluconacetobacter xylinus* from potato peel waste (PPW) [43]. Continuous production of cellulose using *Gluconacetobacter xylinum* cells immobilized on bagasse (SCB) and Ca-alginate granules will be evaluated [44]. The brown algae, *Posidonia oceanica* (POBA), represent an abundant and renewable biomass in the waters of Algeria. Tarchoun et al. studied the chemical treatment of POBA through alkali reduction followed by acid hydrolysis to produce pure microcrystalline cellulose (MCC) [45]. Microcrystalline cellulose and cellulose are successfully extracted from cheap, fast-growing, and abundant giant reed using a multi-step alkaline treatment process, complete chlorine-free sterilization, and various types of hydrolytic media acid [46]. Research by Tarchoun et al. revealed that high-quality cellulose and microcrystalline cellulose can be prepared from giant reed by an environmentally friendly process followed by acid hydrolysis by using acid mixtures. This is considered a novel and adaptive method to control the properties of MCC.

Applications of giant reed microcrystalline cellulose will be explored in the future. Applications of giant reed microcrystalline cellulose will be explored in the future. A survey on Nano BC fibers and materials made from them showed that these materials have nanostructures with enhanced nanofibers which can make changes that are comparable to carbon nanotubes in bone frame applications. Some other works have also investigated the effects of ultrasound on the formation of BC [47, 48]. This work aimed to isolate BC fibers from nata-de-coco Vietnam. Bacterial cellulose was pretreated with NaOH 0.01 M solution for 90 min at 80°C. Water-soluble BC (BC/H<sub>2</sub>O ratio = 20/80) was treated with ultrasound in a sonicator at various times (15, 30, 60, 90 min) and temperatures (55, 60, 65, 70°C).

## 2. Experiments

**2.1. Materials.** Nata-de-coco is supplied by Dang Khoa coconut company, Ben Tre, Vietnam. Nata-de-coco Vietnam has a dry content of 10 wt%, 90 wt% of nata-de-coco is water. Ethanol, NaOH, and acetone were purchased from Sigma Aldrich (Vietnam).

### 2.2. Preparation Method

**2.2.1. Nata de Coco Purification.** The Nata de coco block (Dang Khoa coconut company, Ben Tre, Vietnam) was washed and soaked in distilled water. Then, the nata de coco was further purified by alkaline treatment to remove any residual bacterial cell debris, microorganisms, and other dissolved substances. Nata de coco was stirred in 0.01 M NaOH at 80 C for 90 min. Then, the nata de coco blocks were washed again with distilled water at room temperature until pH = 7. After vacuum filtration, the obtained BC was ground and homogenized in a 500 W blender for 20 min. Finally, the BC film was dried under a vacuum at 40 C.

**2.2.2. Sonication of Bacterial Cellulose Films.** The preparation procedure is shown in Figure 1.

After drying, the BCs were cut and aliquoted (20 wt% BC, 80 wt% water or medical grade ethanol) and treated with a hand blender for 10 min. Then, the mixture was sonicated in an Elmasonic S300H sonicator (Elma company, Germany) (frequency 37 kHz, power 300 W) at various temperatures (55, 60, 65, 70°C) and times (15, 30, 60, 90 min). The BC film was dried under a vacuum at 40°C.

**2.3. Characterizations.** The morphology of the samples was carried out by a scanning electron microscope (S-4800 FESEM, Hitachi, Japan).

Fourier transform infrared spectrum (FTIR) is recorded using FTS 2000 FTIR (Varian) using KBr Tablets, which are created by compressing KBr powder with a small amount of sample BC.

Thermal mass analysis (TGA) was performed on a DTG-60H, Shimadzu (Japan) using a heating rate of 10°C/min, under air with a flow rate of 20 cm<sup>3</sup>/min performed at the

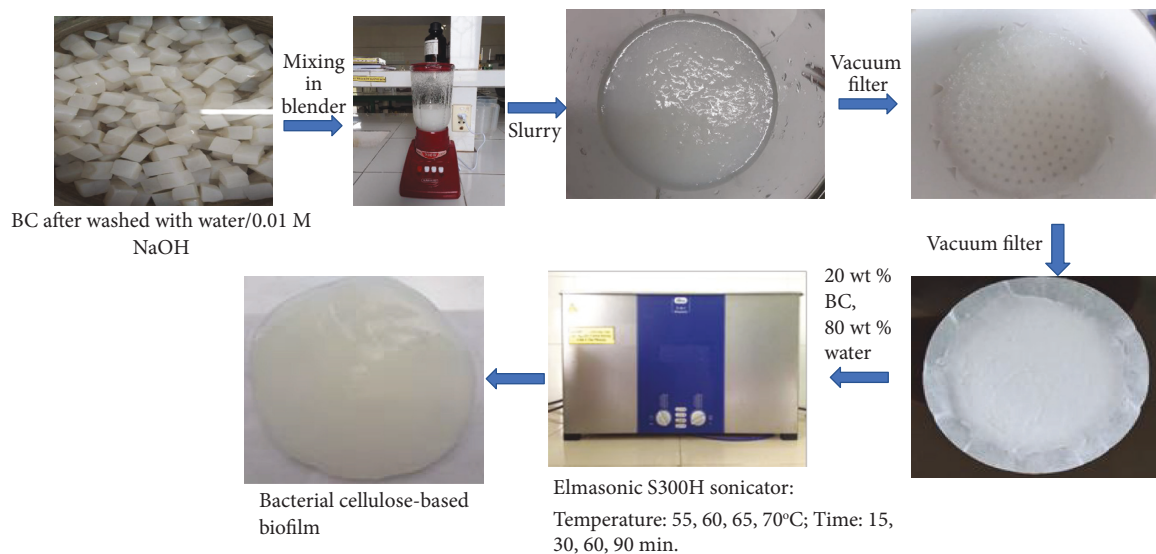


FIGURE 1: Procedure for isolating BC from nata de coco by the sonication method.

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The tensile strength of the film was determined according to ASTM D882, on a British LLOYD 0.5 KN meter with a tensile speed of 2 mm/min, room temperature, and humidity 50%.

### 3. Results and Discussion

**3.1. Morphology Analysis.** After sonication treatments for various times, cellulose was dried under vacuum at 40°C, and the morphology of the new film was initially observed by scanning electron microscopy (SEM), see Figure 2.

Figure 2 shows 3-D mesh structures of cellulose fibers containing interconnected pores of different sizes. These structures are similar to the typical cellulose structure produced by xylum. A in glucose medium by the static fermentation method. After sonication, we can observe that the surface of the fibers becomes smoother and cleaner. With a sonication time of 60 min and 90 min, we noticed that the bacterial cellulose fibers surface became more compact with fewer pores. These SEM images in Figure 2 clearly show that morphological changes of bacterial cellulose depend a lot on sonication time. This is the reason why bacterial cellulose nanofibers in Figures 2(a) and 2(b) are clearer than in Figures 2(c) and 2(d).

In Figure 3, the SEM image of native bacterial cellulose shows a wider band of microfibrils (ribbons) than that of sonicated bacterial cellulose. It is also observed that for native bacterial cellulose, the cellulose microfilaments aggregate to form thin and flat bands or bands of larger size. After sonication, we can observe that there is a decrease in the width of bands and the number of holes/density. Obviously, sonication effects make the surface more compact and the size distribution of the fibers more uniform (Figure 2). Further increasing the sonication time to 90 min makes the surface of the fibers smoother and cleaner.

However, fiber bundles appear (caused by the aggregated cellulose fibers-ribbons). Therefore, the sonication time of 60 min gives the best morphological structure results.

SEM images at 10.0 k magnification (Figure 4) clearly show the surface structure of BC with characteristic fibrous 3-D ultrafine networks of well-arranged nanofibers stabilized by hydrogen bonds existing in cellulose units [49–51]. The sample with 90 min of sonication has more voids (Figure 4(a)), while the sample with 60 min possesses a uniform structure without holes. These results indicate that the sonication changed the microfibrillar arrangement of BC, resulting in new films with a different nanostructure organization.

Figures 5 and 6 show the effects of sonication temperature on the structure of isolated bacterial cellulose. Sonication temperature plays a very important role in fiber homogenization. An appropriate sonication temperature results in a more thoroughly homogenized cellulose suspension with the cellulose fiber. From Figure 5, it can be seen that at various temperatures, the fiber structure morphologies are different. Sonication at 65°C gives a uniform fiber structure without the agglomeration of fibers (Figure 6). The higher the sonication temperature, the better the separation of the filaments from the cellulose-water suspension. Figure 6(b) shows that the fiber structure is uniform in size, and the fiber surface is smooth and clean. There is no fiber bundle formation, and the separated fibers intertwine to form a 3D structure. This structure can bring excellent properties to BC films, such as mechanical properties, thermal stability, etc.

### 3.2. Fourier Transform Infrared Spectroscopy (FTIR).

Figure 7 shows that there are absorption peaks located at 3342.45  $\text{cm}^{-1}$ , 2917.76  $\text{cm}^{-1}$ , and 1426.81  $\text{cm}^{-1}$  which correspond to valence vibrations of -OH, -CH and -CO groups, respectively. The spectra of samples with various sonication

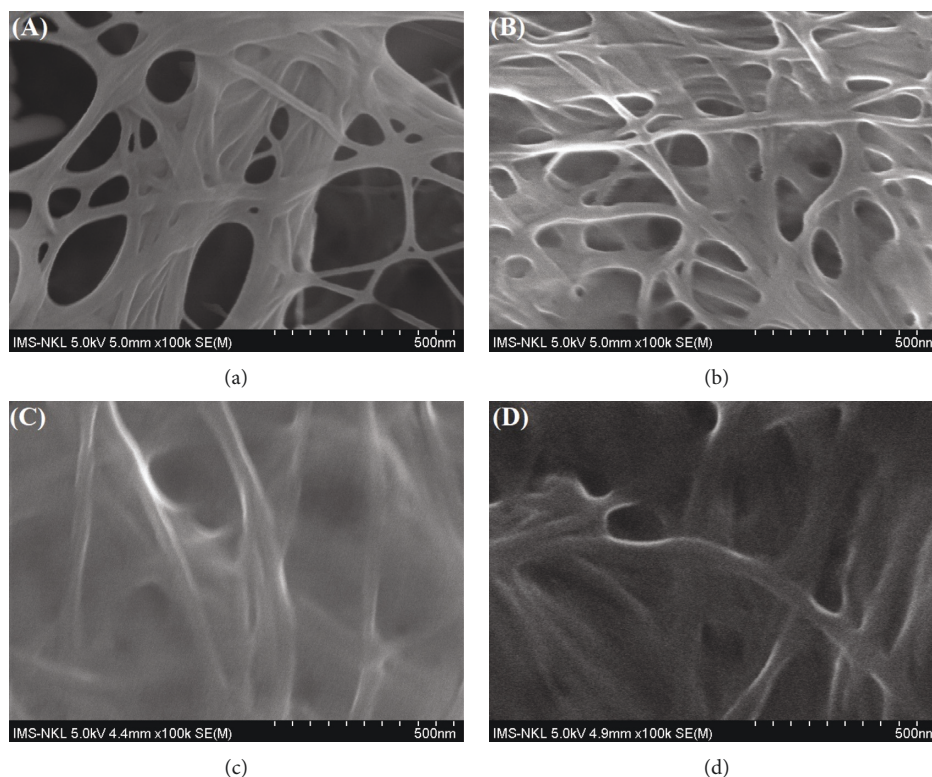


FIGURE 2: Scanning electron microscopy images of bacterial cellulose treated by sonication for 15 min (d), 30 min (c), 60 min (b), and 90 min (a).

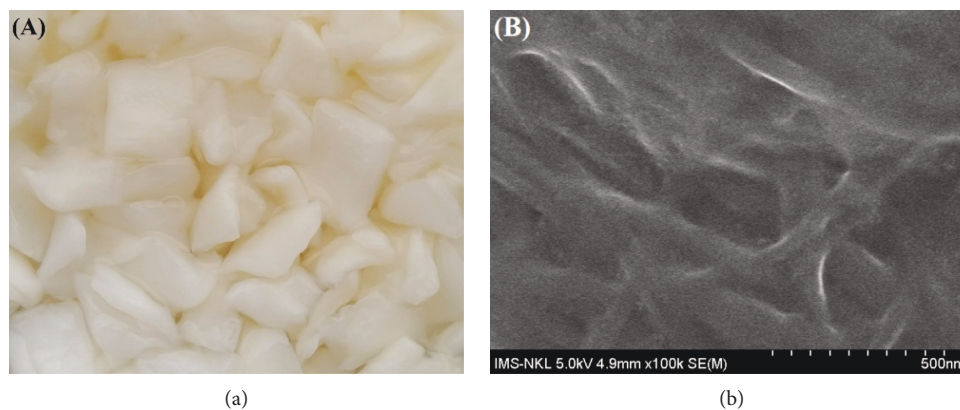


FIGURE 3: Scanning electron micrographs of bacterial cellulose, native (b); nata de coco (a).

times are similar, indicating that the molecular structure of BC does not depend on sonication time.

The FTIR spectra of samples with various sonication temperatures shown in Figure 8 also express a similar result. The molecular structure of BC does not depend on sonication temperature. In Figure 8, all samples have the absorption peaks located at  $3341.71\text{ cm}^{-1}$ ,  $2918.70\text{ cm}^{-1}$ ,  $1541.12\text{ cm}^{-1}$ , and  $1408.55\text{ cm}^{-1}$  which correspond to valence vibrations of the  $-\text{OH}$ ,  $-\text{CH}$ , and  $-\text{CO}$  groups, respectively. This result is completely consistent with the statement of Clasen et al. [52].

**3.3. Thermogravimetric Analysis (TGA).** TG and DTG curves of BC films isolated at various sonication times are shown in Figure 9.

The thermal decomposition of BC shows steps including degradation, dehydration, and decomposition of glycosic units (Figure 9(a)). The subsequent oxidation leads to the formation of the burnt residue. TG curves show that the temperature at which the degradation begins (Td) corresponds to about 10% weight loss. The temperature at which the decomposition of cellulose happens, called the maximum decomposition temperature (T<sub>dmax</sub>), is determined

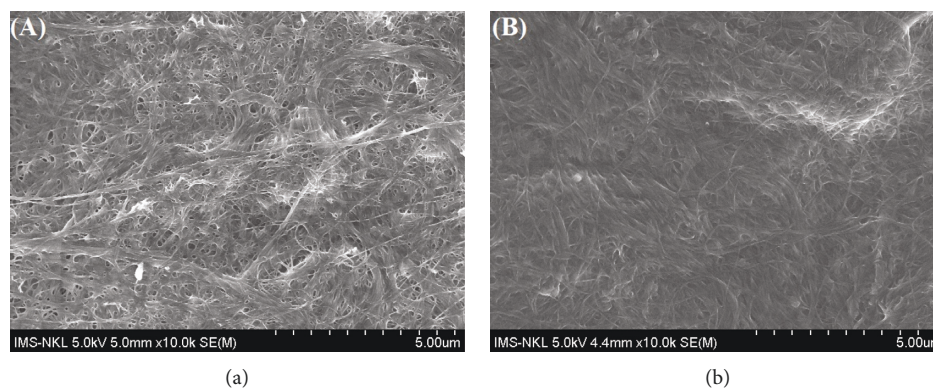


FIGURE 4: Scanning electron microscopy images of bacterial cellulose treated by sonication for 90 min (a) and 60 min (b), at magnification x10.0k.

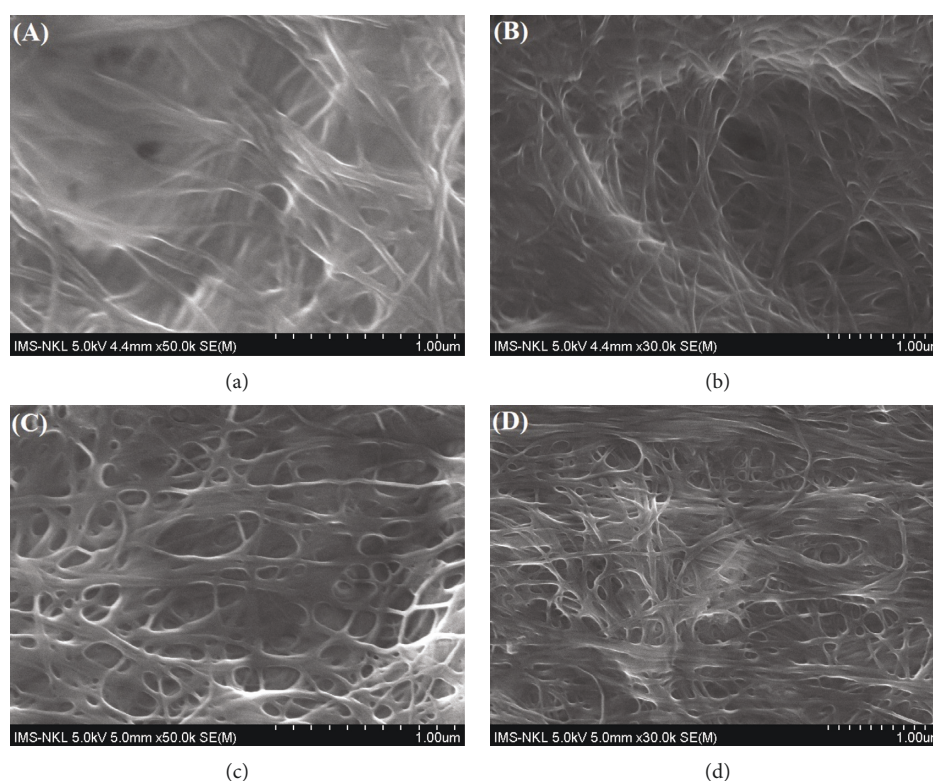


FIGURE 5: Scanning electron microscopy images of bacterial cellulose treated by sonication at various temperatures: 55°C (a), 6° (b), 65°C (d), and 70°C (c).

from the DTG curves which are similar for all samples. Tdmax values are observed at about 355.98°C (A), 353.23°C (Figure 9(b)), 363.59°C (C) and 359.07°C (D) (Figure 9(b)). These results are consistent with the results reported by Ullah et al [53].

TG and DTG curves of BC films isolated at various sonication temperatures are shown in Figure 10.

The TG and DTG curves in Figure 10 have quite similar shapes for all samples treated with various sonication temperatures. Tdmax values of the samples are observed at approximately 344.51°C (a0), 341.57°C (b0), 343.58°C (c0), and 335.67°C (d0).

From the TGA results, the thermal stability of the sonicated BC membrane is improved when isolated at the conditions of 65°C and 60 min. This improved thermal stability makes the sonicated BC membrane a high-potential material for medical and energy harvesting applications [30, 53].

**3.4. Mechanical Properties.** For tensile testing, after cutting into a paddled pattern, the BC films were dried at 100°C for 3 h. Then put it in a desiccator for 24 hours. BC is produced with a thickness of 2 to 3 mm. Bacterial cellulose fibers are

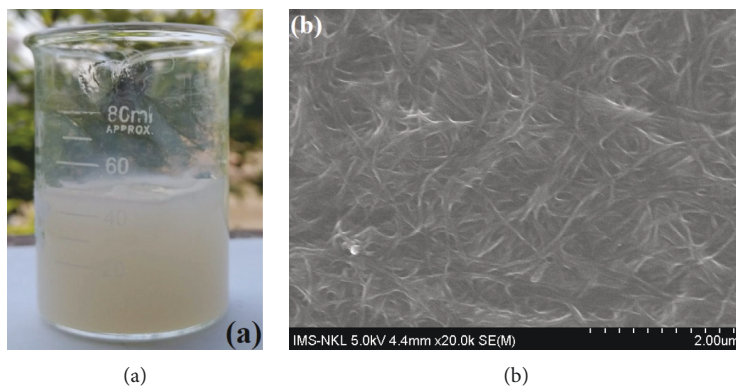


FIGURE 6: Scanning electron microscope images of bacterial cellulose treated by sonication at 65°C (b), BC/H<sub>2</sub>O (a).

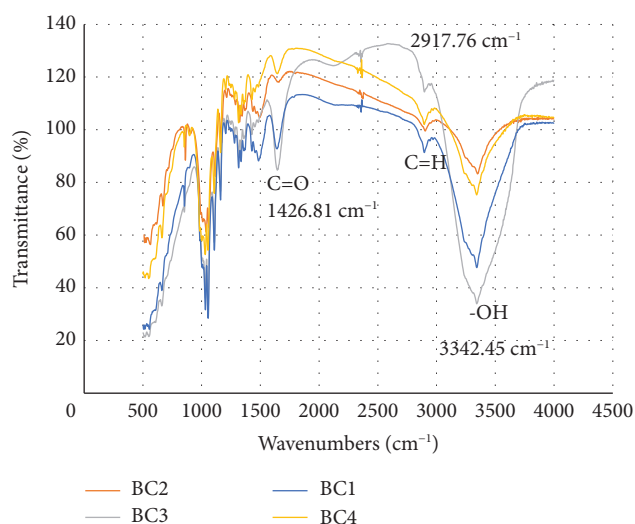


FIGURE 7: FTIR of bacterial cellulosic membranes under various sonication times: BC1—15 min, BC2—30 min, BC3—60 min, BC4—90 min.

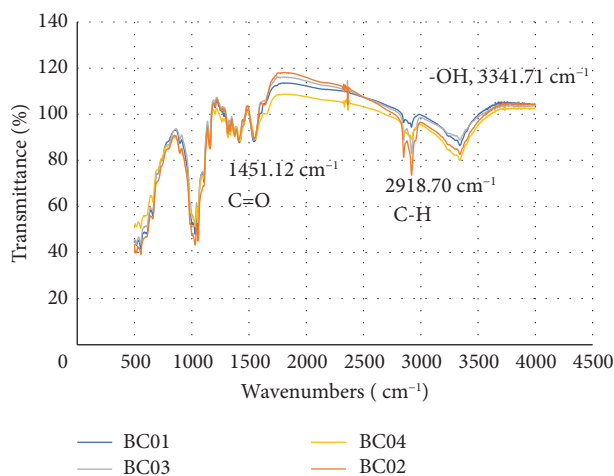


FIGURE 8: FTIR of bacterial cellulosic membranes under various sonication temperatures: BC01—55°C, BC02—60°C, BC03—65°C, and BC04—70°C.

dense and intertwined. The tensile strength of the films was determined according to ASTM D882, on a British LLOYD 0.5 KN meter with a tensile speed of 2 mm/min, at room temperature, humidity of 50%. The physico-mechanical properties changes depend on the nanofiber structure of the BC films.

The tensile strength of the BC films is shown in Figure 11.

From Figure 11, it is noticed that when ultrasonic vibration is done by the Elmasonic S300H sonicator (Elma company, Germany) (ultrasonic frequency 37 kHz, ultrasonic power 300 W) at various times (15, 30, 60, and 90 min), the tensile strength of the BC films is at high threshold [54–56]. When super-sonicating for 60 minutes, the tensile strength reached its maximum value which is consistent with the explanation in the morphological structure (SEM images). It can be further explained as follows; the compactness of the bacterial cellulose fibers, the uniform structure, the strong hydrogen bonding between the cellulose molecules and the high crystallinity lead to the highest strength. For the samples with sonication times of 15 min, 30 min, and

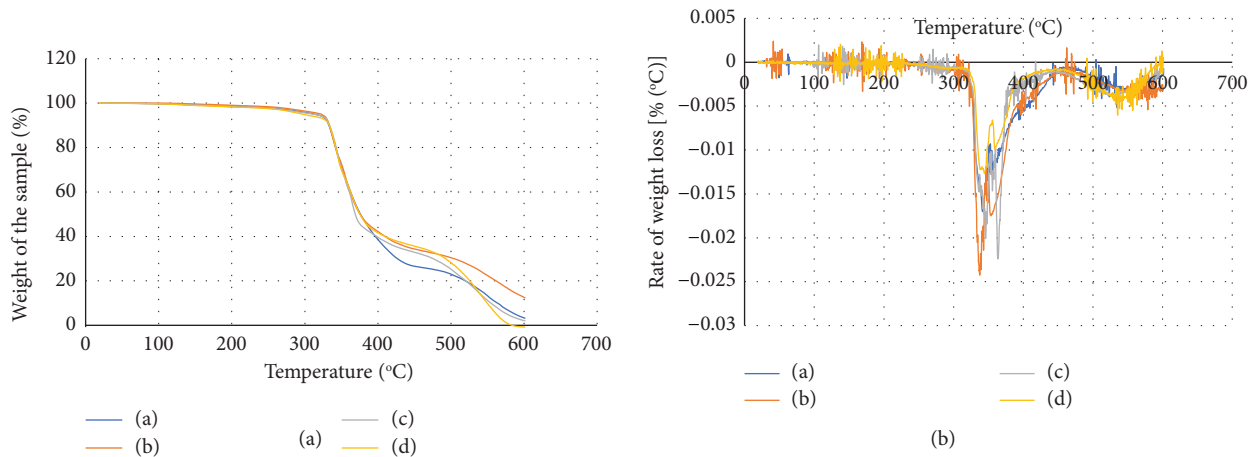


FIGURE 9: (a) TG and (b) DTG curves of BC fabricated under various sonicating time: 90 min (A), 60 min (B), 30 min (C), and 15 min (D).

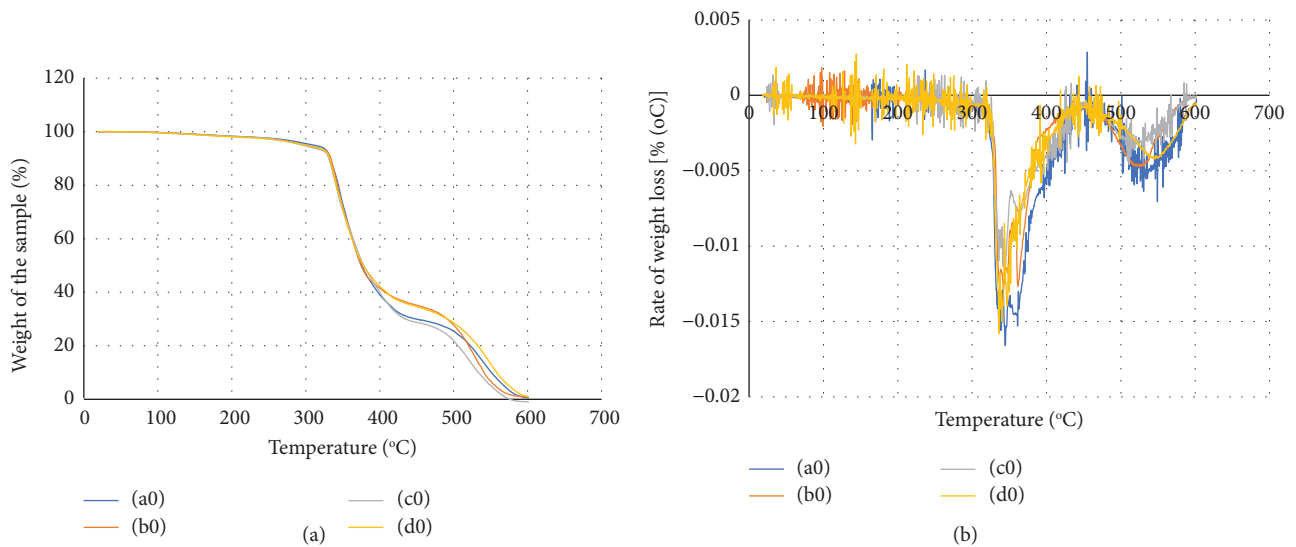


FIGURE 10: (a) TG and (b) DTG curves of BC fabricated under various sonicating temperatures: 70°C (a0), 65°C (b0), 60°C (c0), and 55°C (d0).

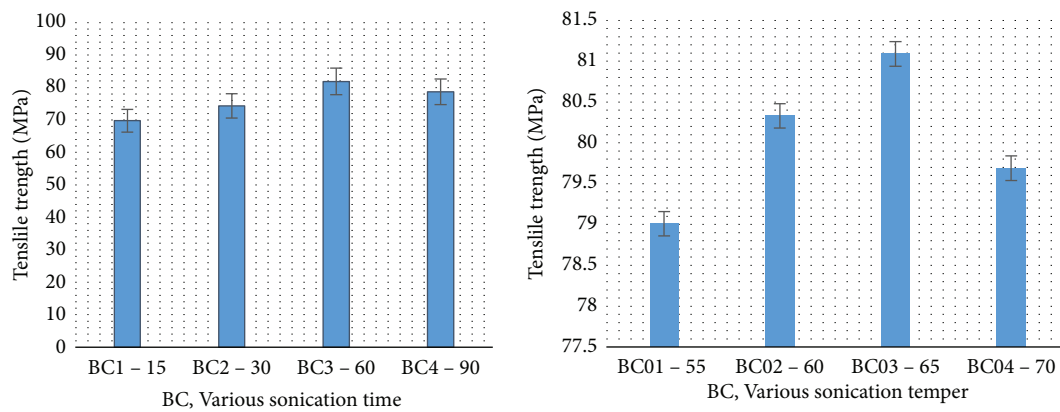


FIGURE 11: Tensile strength of BC films when isolated under different conditions.

90 min, as explained in section 3.1, the appearance of many holes and their structure is attributed to weakened hydrogen bonds and reduced crystallinity which results in reduced

tensile properties. This rule is also repeated in the sample with various sonication temperatures. As a result, the sample sonicated at 65°C achieves the best quality.

## 4. Conclusion

This study examined the feasibility of sonication in extracting BC microfibrils from Vietnamese nata de coco to fabricate BC biofilms. Two factors that have been studied are sonication time and temperature. The results showed that the appropriate sonication time and temperature are 60 min and 65°C, respectively. Evaluation of the structure of bacterial cellulose by SEM combined with infrared spectroscopy showed that the native structure of bacterial cellulose was preserved after treated with 0.01 M NaOH solution and sonication. It has been demonstrated that the sonication technique can significantly improve the mechanical properties and thermal stability of the material.

## Data Availability

All the data are included within the manuscript and are available for the readers.

## Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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