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Preparation and characterization of bacterial cellulose film using coconut water and *Lentilactobacillus parafarraginis* by the casting method

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ABSTRACT

Bacterial cellulose (BC) is a natural biomaterial formed by bacteria. It has distinctive properties and enormous utilization in various fields. The present study focused on BC film preparation using *Lentilactobacillus parafarraginis* and coconut water as a fermentation medium. This study aimed to characterize BC with variations in coconut water storage and fermentation time. The coconut water was kept for 1 and 2 days, then fermented for 5, 7, and 9 days. The dry BC film was produced by the casting method. Besides characterization, BC films were also analyzed for their morphology based on Fourier transformed infra-Red, X-ray diffraction spectra, scanning electron microscopy, and atomic force microscopy images. The result showed that BC film with 2 days storage time of coconut water and 9 days fermentation time (BC29) possessed the best properties. It exhibited high wet weight (126.50 ± 0.24 g) and wet thickness (6.83 ± 0.18 mm) with pH, water vapor transmission, and moisture content value of 5.89 ± 0.01, 387.81 ± 0.35 g/m^2, and 6.12% ± 0.13%, respectively. The BC film showed a high swelling degree (125.64% ± 0.25%) and mechanical strength (tensile strength of 13.52 ± 0.01 mPa, elongation at break of 35.81% ± 0.03%, and Young's modulus of 99.08 ± 0.04 mPa). The result suggested that BC film can be developed as a new biomaterial with lower cost and better properties than existing films.

INTRODUCTION

Bacterial cellulose or biocellulose (BC) is an exopolymer composed of β-1,4-D-glucopyranose units. It is usually produced by aerobic bacteria, such as *Gluconacetobacter, Sarcina*, *Enterobacter, Pseudomonas*, and *Achromobacter* genera [1,2]. The biomaterial is biodegradable, environmentally friendly, nontoxic, nonallergenic, chemically stable, and biocompatible. BC film has high purity, crystallinity, degree of polymerization, tensile strength and Young's modulus, small size diameter of fiber, swelling capacity, and high hydrophilicity, compared to plant cellulose [3]. Bacteria synthesize BC as an extracellular polysaccharide, forming a protective sheath around the cell. Based on its morphological structure, BC is a ribbon-like cellulose nanofiber [4]. BC does not contain components that are difficult to separate in plants, i.e., lignin, pectin, and hemicellulose as found in plant cellulose [5]. After the microbial fermentation process, the BC pellicle is harvested. It contains bacterial cells, metabolic by-products, and nutrient residues, which can be simply separated from the BC network to produce a highly purified film. These distinct characteristics indicate that BC film is a highly advantageous biomaterial for application in various areas and industries [6].

The diameter of BC fibers is around 100 times smaller than plant cellulose fibers, exceptionally permeable, and highly

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porous. The characteristic allows for the diffusion of various active ingredients into the wound or skin. It absorbs body fluids, such as exudates, because of its swelling capacity. The BC film functions as a physical barrier that effectively eliminates the infiltration of contaminants. Therefore, BC is widely used for wound healing applications [6]. Many types of injuries, such as traumatic injuries, pressure sores, skin tears, diabetic wounds, and donor skin graft sites, use BC as a temporary dressing. BC enhanced the healing process, leading to decreased pain at the wounded sites [7]. Several investigations have demonstrated that composites of BC enhanced the proliferation of corneal stromal cells [8]. BC has been studied for its potential use as a contact lens as well as for its therapeutic application in ocular surgery as a dressing with ciprofloxacin. It has also been explored for its ability to promote bone regeneration in bone transplants and as an implant in cardiac and vascular surgery [9–11]. In the field of cosmetic and personal care, BC as a sheet mask's base material is loaded with compounds with cleansing, moisturizing, or anti-inflammatory properties, as well as plant extracts [12–15]. BC exhibited excellent swelling characteristics in various chemical compositions, indicating its potential as a carrier for several useful active compounds employed in cosmetics [16]. Furthermore, in drug delivery, the combination Ag-BC demonstrated significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria [7,17]. BC was employed in the production of film-coated paracetamol [18], as well as in protein delivery, macromolecular prodrug delivery, and transdermal drug administration. It is also used in the food industry for low-cholesterol diets, traditional desserts, vegetarian meat substitutes, and food packaging materials, among other uses [6].

Bacteria type or strain affects BC yield and characteristics [19]. One of the bacteria potentially developed in BC production is *Lentilactobacillus parafarraginis* (basionym: *Lactobacillus parafarraginis*). Several *Lactobacillus* species have been widely used in BC production, i.e., *Lactobacillus lactis, Lactobacillus plantarum, Lactobacillus brevis*, and *Lactobacillus acidophilus* [20,21]. *Lactobacillus* sp. is classified as nonpathogenic, safe, and defined as generally recognized as safe [22]. The co-culture of *Lactobacillus mali* and *Gluconoacetobacter xylinus* was reported as an appropriate combination to increase BC production [23]. The combination of cultures between *Gluconacetobacter* sp. gel_SEA623-2 and *L. plantarum* KCCM 80077 increased the weight and thickness of dry BC (37.83 ± 6.81 g/l and 10.33 ± 0.58 mm) compared to the single culture of *Gluconacetobacter* sp. gel_SEA623-2 [24]. Under the same physiological conditions, *L. hilgardii* IITRKH159 produced more BC with finer and thinner fiber, higher crystallinity index (CrI), and smaller crystal size than *Komagataeibacter xylinus* [25]. Prior research has demonstrated that *L. parafarraginis* has been utilized to generate BC using the oven-dried technique without casting, yielding desirable properties [26].

Other factors that affect BC quality are fermentation conditions (production method, fermentation time, and inoculum size), source of nutrition, and so on [19,27,28]. The selected method for BC production is a static condition because it produces BC film with better characteristics compared to other methods [29]. Culture medium is one of the essential factors because it provides essential nutrients for bacterial growth and BC production, and influences BC yield as well as structure significantly, including its mechanical and physical properties [30]. Coconut water was chosen as the primary ingredient in the fermentation medium due to its high mineral content and significant amounts of fructose (32.52 ± 0.227–39.04 ± 0.824 mg/ml), glucose (29.96 ± 0.243–35.43 ± 0.510 mg/ml), and sucrose (6.36 ± 0.06–0.85 ± 0.010 mg/ml) [31]. The medium contained the best type of carbon source for high BC yields [32]. Furthermore, applying coconut water as a medium for fermentation yielded BC with enhanced properties compared to the Hestrin Schramm medium and the combination of Hestrin Schramm-coconut water [33,34]. As one of the ingredients of bacterial culture medium, coconut water also lacks of need for pre-treatment [31].

The coconut water used in this study was sourced from the Bondowoso district in East Java, Indonesia. In 2023, East Java ranked as the third largest province in Indonesia in terms of coconut production, with a total of 233.70 thousand tons. Bondowoso district contributed 2,415 tons of coconut production in 2022 [35,36]. Coconut water has traditionally been regarded as waste in coconut processing industries, such as desiccated coconut factories and coconut milk factories [37]. Consequently, there is an abundance of coconut water that can be used as a bacterial growth medium for BC production. Due to its cost-effectiveness, utilizing coconut water as a fermentation medium enhances the economic value of wasted coconut water, particularly in traditional markets [38].

Nevertheless, there may be quality alteration during coconut water storage because of the fermentation performed by natural bacteria, such as *L. plantarum, L. paracasei*, and *Pediococcus* sp. [39]. It increased lactic acid bacteria (LAB) quantity and decreased pH value [40]. The microbes caused a content deterioration of coconut water, including sugar as a carbon source of microbial growth [41]. Previous studies showed that different medium conditions and fermentation times affected the yield of BC, and its characteristics [28,42,43]. Furthermore, the application of the casting method in BC manufacturing involves blending the obtained BC, casting it onto a glass plate, and drying it to form a film layer with uniform fiber mass and thickness. This process allows for easier modification of BC with enhanced properties. Hence, this work produced BC by employing different coconut water storage times and fermentation times through the casting method, resulting in cost-effective BC with good characteristics suitable for various uses.

MATERIALS AND METHODS

Materials

Coconut water was obtained from coconut (*Cocos nucifera* L.) fruit grown in Bondowoso, Indonesia, and harvested in 11–12 months. *Lentilactobacillus parafarraginis* was obtained from PT Biotechno, Serang, Indonesia, and identified using molecular analysis of the chromosomal 16S rRNA gene at Professor Nidom Foundation, Surabaya, Indonesia (certificate number of 071122/PNF-XI/2022). The polymerase chain

reaction method was applied to isolate and amplify the DNA sample. This result was determined using Sanger sequencing. The sequences were compared to data from NCBI BLAST and identified as *L. parafarraginis* strain F0439 with a homology of 99.67% (Accession number HM596288.1). KH_2PO_4, NaOH, sucrose, acetic acid, and ammonium sulfate were supplied by Merck (Darmstadt, Germany).

Preparation of inoculum

Lentilactobacillus parafarraginis, as a starter in producing BC film, was grown in the culture medium consisting of coconut water (94%) as the main ingredient rich in nutrients, suspended culture (2%) as a bacterial source, sucrose (2.5%) as a carbon source, acetic acid (1%) for pH adjustment and BC yield enhancement, and ammonium sulfate (0.5%) as a nitrogen source [26,44,45]. The coconut water was left at 25°C for 48 hours, subsequently filtered, and then boiled at 100°C. The heated coconut water was added with sucrose, ammonium sulfate, and acetic acid. The mixture was then stirred and sterilized at 121°C for 15 minutes. The starter bacteria were introduced into the growth medium and inoculated under static incubation at 26°C–30°C for 7 days.

Preparation of BC film

The BC film production used the same method as the preparation of inoculum with different coconut water storage times and fermentation times, i.e., BC with 1-day storage of coconut water and 5 days fermentation time (BC15), BC with 1-day storage of coconut water and 7 days fermentation time (BC17), BC with 1-day storage of coconut water and 9 days fermentation time (BC19), BC with 2-day storage of coconut water and 5 days fermentation time (BC25), BC with 2-day storage of coconut water and 7 days fermentation time (BC27), and BC with 2-day storage of coconut water and 9 days fermentation time (BC29). However, coconut water was placed at room temperature for 1 day and 2 days before preparation. pH of all mediums was determined using a pH meter. Two percent (v/v) inoculum was utilized for all experimental procedures. The inoculum adjusted its turbidity using a spectrophotometer (Thermo Scientific Genesys, Mississauga, Canada) was equivalent to 0.1 at OD_{600} (approximately 1×10^7 CFU/ml) [46]. The fermentation was carried out at 26°C–30°C for 5, 7, and 9 days statically. pH of the remnant medium was recorded after fermentation completion, and the BC pellicle was harvested, rinsed in running water, and boiled in distilled water for 60 minutes. The wet BC was treated with purification using 0.5 M NaOH at 100°C for 30 minutes. It was then rinsed with running water and immersed several times in distilled water until it reached a neutral pH of 7.0. BC film was blended until smooth, filtered, weighed 13 g, cast onto a glass plate [47] (width of 14 cm and length of 15 cm with 4 mm thickness), and dried at 60°C for 72 hours.

BC film characterization

Determination of organoleptic properties and pH

The organoleptic test of wet and dry BC film was conducted using a human visual appearance at 25°C to observe its color, smell, and surface flatness. The pH determination was performed using a pH meter by immersing 1% of the dry BC film in distilled water [48].

Determination of weight and thickness

Wet BC weight was determined using an analytical balance. Meanwhile, BC thickness measurement was performed using calipers in 5 distinct positions. Moreover, the weight and thickness of dry BC film were determined using the same procedures [48].

Determination of water vapor transmission (WVTR)

Five grams of anhydrous calcium chloride was placed into a weighing bottle. The dry BC was placed onto the top of the bottle. It was kept in desiccator at 75% RH and 25°C for 24 hours. The WVTR was determined using this specific equation:

$$WVTR = W / S \quad (1)$$

W represents the weight of the sample (gram), whereas S represents the area of the sample (m^2) [48].

Determination of moisture content

The dry BC film of 2 × 2 cm size was heated at 90°C for 24 hours. Afterward, the moisture content was determined utilizing the specified equation:

$$\text{Moisture content} = (m_1 - m_2) / m_2 \times 100\% \quad (2)$$

m_1 is the initial mass of the sample (gram), whereas m_2 is the final mass of the sample (gram) [48].

Determination of swelling degree

The BC film with 1.5 × 1.5 cm size was weighted and immersed in a 25 ml solution of phosphate buffer at a pH of 7.4 at 25°C for 1, 4, and 6 hours. After each immersion period, the BC film was promptly moved and dried using filter paper to remove any remaining solution on the surface. The film was weighted and calculated for a swelling degree using the following equation:

$$\text{Swelling degree} = (W_2 - W_1) / W_1 \times 100\% \quad (3)$$

W_1 is the initial weight of the sample (gram), while W_2 is the weight of the sample after swelling for certain times (gram) [48].

Determination of mechanical strength

The mechanical strength was measured using a universal testing machine (Huang Ta HT-2328, Taichung City, Taiwan) at room temperature to determine tensile strength, elongation at break, and Young's modulus value. The instrument was operated at a deformation rate of 10 mm/minute [44]. The BC film was cut in 6 cm × 2 cm size for the evaluation.

Analysis of Fourier Transformed Infra-Red (FTIR) spectroscopy

BC film was evaluated for its functional groups using an FTIR spectrometer (Bruker Alpha Eco-ATR, Esslingen, Germany) in ATR (Attenuated Total Reflection) mode. The FTIR spectra of dry BC film were recorded in

4,000–600 cm^{-1} wavenumbers and the baseline of all spectra was normalized [49].

Analysis of X-Ray Diffraction (XRD)

The X-ray diffractograms were obtained at room temperature using a diffractometer (Rigaku Miniflex 600, Tokyo, Japan) at the Cu Kα radiation wavelength of 1.54 Å. The voltage and current used for operation were 40 kV and 15 mA, respectively. The data were obtained using reflection mode within the 5–90° 2θ range, with a step of 0.02° 2θ intervals. The scan speed was 10°/minute. The CrI and percentage of crystallinity (%crystallinity) of BC films were determined using the following equations:

$$CrI = \frac{I002 - Iam}{I002} \quad (4)$$

$$\%crystallinity = \frac{I002}{I002 + Iam} \times 100 \quad (5)$$

I_{002} is the highest value of intensity observed in the diffraction spectrum of the lattice plane (002) at an angle of 2θ between 22° and 23°. I_{am} is the minimum intensity at 2θ of 18° to 19° [50].

Morphological analysis of scanning electron microscopy (SEM)

The surface morphology of the dried BC film was examined using SEM (Hitachi TM3000, Tokyo, Japan) imaging. The film was attached to a carbon type and covered with platina using an ion sputter coater (Hitachi E-1045, Tokyo, Japan). Photomicrographs were taken at 5,000 magnifications operated at 5 kV [49].

Texture analysis of atomic force microscopy (AFM)

BC film with the best characteristics (BC29) was observed for its morphology with an AFM (Bruker N8 Neos, Santa Barbara, USA). Ambient atmosphere measurement was performed in tapping mode with rectangular silicon cantilevers. The height mode of the image was 5 × 5 μm^2 and scanned with the speed of 1–3 Hz [51].

Data analysis

The tests were conducted in triplicates. The results were reported as the mean of all measurements ± the standard deviation. One-way analysis of variance and the least significance difference method were utilized to analyze significant differences between groups, with a significance level (α) of 5%.

RESULTS AND DISCUSSION

BC film production

As shown in Table 1, it was known that the pH of all mediums was around 3.00. The results followed the previous study, which reported that *L. parafarraginis* strain A1 (KU495926) exhibited higher growth under acidity at pH 3.00 than at pH 7.00 and pH 9.00 [52]. The pH is important for appropriate nutrient uptake, oxidative reaction, solubility, and enhancement in the production of BC film [53]. pH before fermentation of all mediums was higher than the pH after fermentation. Furthermore, the longer fermentation, the more acidic the pH. Therefore, the medium of BC29 showed the most acidic pH. It indicated that fermentation time affected medium acidity. Several studies reported that LAB, such as *L. parafarraginis* metabolized sugars, including sucrose through a fermentation process to produce several acidic-by products, i.e., lactic, propionic, formic, and acetic acid causing the pH of the medium to decrease [54]. This result concorded with the previous study that used *Acetobacter xylinum* and oil palm frond juice as a growth medium for the production of BC film. The study showed that the enhancement of fermentation time increased the acidic-by-product concentration and acidity of the medium [55]. Moreover, the pH of medium from coconut water kept for 2 days was lower than 1 day of coconut water. It indicated that coconut water storage caused initial fermentation that decreased the pH value of the medium.

The fermentation from the early day to the ninth day is shown in Figures 1 and 2. After several days of fermentation, the pellicles form on the surface of the coconut water medium. This is because *L. parafarraginis* requires oxygen for its growth (as an aerobic bacteria). The initial stages of pellicle formation varied among media for different times of coconut water storage. Production of BC film was started on the fourth day for coconut water kept for 1 day and the third day for coconut water kept for 2 days. An increase in fermentation time caused an enhancement of the cellulose pellicle's thickness. It was suggested that optimal BC production was started at the log phase of bacterial growth as the primary metabolite. At this time, sugars as substrates were metabolized to produce energy for its growth and conversion of glucose into cellulose [56].

Table 1. pH of the medium in BC production.

pH of medium	BC15	BC17	BC19	BC25	BC27	BC29
Before fermentation	3.48 ± 0.02^{a1}	3.46 ± 0.01^{ab1}	3.44 ± 0.01^{b1}	3.41 ± 0.01^{c1}	3.35 ± 0.01^{d1}	3.34 ± 0.01^{d1}
After fermentation	3.43 ± 0.01^{a2}	3.39 ± 0.01^{b2}	3.35 ± 0.01^{c2}	3.32 ± 0.01^{d2}	3.29 ± 0.01^{e2}	3.26 ± 0.01^{f2}

The data represents the mean of three samples, with the standard deviation (SD) indicated as the mean ± SD. Significant differences were observed in the different superscript letters within the same row and the different superscript numbers within the same column, as determined by the LSD test ($p < 0.05$). BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

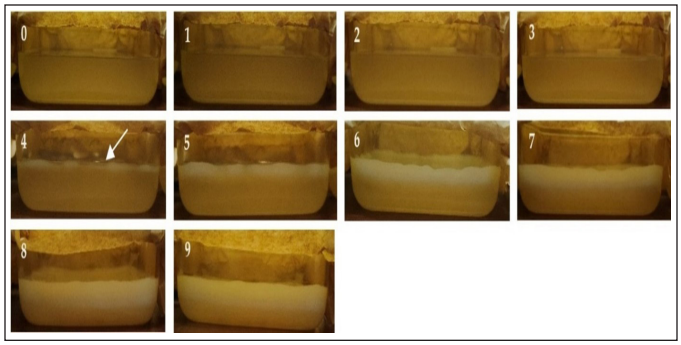

Figure 1. Fermentation of coconut water kept for 1 day at (0) early day, (1) first day, (2) second day, (3) third day, (4) fourth day, (5) fifth day, (6) sixth day, (7) seventh day, (8) eighth day, and (9) ninth day. The arrow sign indicated the formation of the first cellulose pellicle.

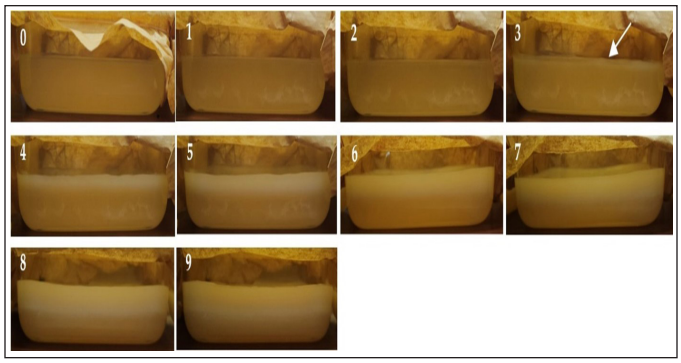

Figure 2. Fermentation of coconut water kept for 2 days at (0) early day, (1) first day, (2) second day, (3) third day, (4) fourth day, (5) fifth day, (6) sixth day, (7) seventh day, (8) eighth day, and (9) ninth day. The arrow sign indicated the formation of the first cellulose pellicle.

Determination of organoleptic properties and pH

The physical appearances of wet and dry BC film were demonstrated in Figures 3 and 4. The wet BC was yellowish–white, odorless, and had a smooth surface. Its organoleptic properties were similar to wet BC produced by *L. hilgardii* IITRKH159. The harvested BC turned from pale yellow to a yellowish-white film after purification treatment to remove the residual bacterial cells and the medium [25]. BC film fermented for 5 days was slightly more translucent than longer fermented samples. The dry BC films showed brownish color, especially at the edge of the films. The heat from the drying process using the oven probably caused discoloration. Nevertheless, the dry BC was odorless, flat, and smooth. As shown in Table 2, the pH of all dry BC films was in the range of 5.67 ± 0.02–5.89 ± 0.01. The pH value was in the skin pH range (4.5–6.5), indicating that the use of BC films does not cause any irritations, especially for skin usage, such as wound dressing, sheet masks, and so on [57].

Determination of weight and thickness

For equal fermentation time, wet BC film from coconut water kept for 2 days possessed a greater weight and thickness than BC film from 1 day storage time of coconut water (Table 2). The longer the fermentation, the weight and thickness increase. Bacteria produced an increasing amount of cellulose exopolymers as fermentation time increased, which then aggregated to form the fibers of BC. For wet BC with coconut water kept for 1 day, BC19 had the highest weight and thickness (105.69 ± 0.14 g and 6.36 ± 0.06 mm). Whereas, in wet BC film from coconut water kept for 2 days, the weight and thickness of BC27 (125.48 ± 0.07 g and 6.47 ± 0.31 mm) and BC29 (126.50 ± 0.24 g and 6.83 ± 0.18 mm) were not significantly different, but higher than BC25. A previous study also exhibited that BC film produced in 9 days resulted in the highest yield and there was BC yield reduction for 12 and 14 days [55].

Determination of WVTR and moisture content

BC films with coconut water kept for 2 days had a higher WVTR value than BC films from 1 day of coconut water storage (Table 2). The longer the fermentation, the WVTR value increases. Wet BC films produced for 9 days of fermentation (BC19 and BC29) had higher WVTR characteristics than other BCs with equal coconut water storage time. In addition, all BC films showed moisture content characteristics that were not significantly different. The expected value of WVTR was between 300–840 g/m^2 to have sufficient occlusive and moisture-retentive characteristics [58]. The expected moisture content value was 5%–10% for dry BC film [59]. If the moisture content was under 5%, the hydrophilic and absorption properties of BC films would decrease. From this study, it was known that all BC films met the requirements of WVTR and moisture content value.

Determination of swelling degree

As shown in Table 2, all dry BCs swelled rapidly in the first hour. The swelling degree value increased until 6 hours, even reaching more than 100% for all BC films with a coconut water storage time of 2 days. Generally, BC films from coconut water kept for 2 days had a higher swelling degree value than BC films from 1 day of coconut water storage. BC19 (90.45% ± 0.66%) and BC29 (125.64% ± 0.25%) resulted in a higher swelling degree for 6 hours than BC films with equal storage time of coconut water. Nevertheless, the values were not significantly different with BC17 (91.96% ± 0.31%) and BC27 (125.19% ± 1.02%). A similar effect of swelling ability was suggested due to similar arrangement and characteristics of fibers and pores that formed the three-dimensional structure of BC films, as shown in SEM images (Fig. 7). BC film with a high swelling degree value showed a high absorption characteristic [60]. Therefore, BC film can be applied as wound dressing preparation that absorbs a lot of exudates or as a cosmetical mask impregnated in serum or essence containing active ingredients [13,60].

Determination of mechanical strength

The mechanical strength of all BC films is shown in Table 2. In this study, BC film from coconut water kept for 2 days showed higher tensile strength and Young's modulus value compared to BC film from 1 day of coconut water storage. The longer the fermentation time, the higher the tensile strength and Young's modulus value. The fermentation duration directly

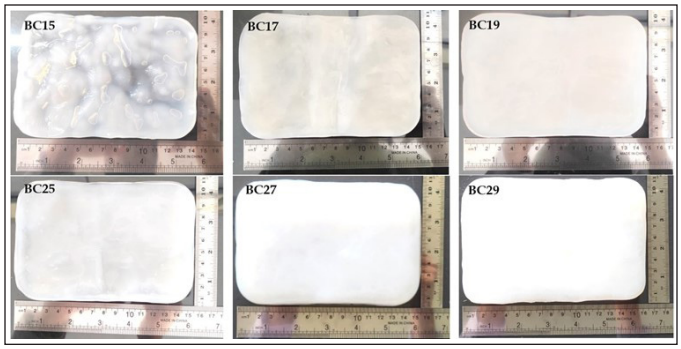

Figure 3. Wet BC films with various coconut water storage and fermentation times. BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

Figure 4. Dry BC film after casting onto glass plates.

impacts the properties of BC, such as weight and thickness. The weight and thickness of BC increase because of the higher number of fibers produced as fermentation time increases. This will enhance the mechanical strength of BC. BC29 had the highest value of tensile strength and Young's modulus, whereas BC15 exhibited the lowest value. Additionally, fermentation time enhancement from 5 to 7 days was suggested to increase elongation at break properties. Nevertheless, there was a slight reduction in value for BC film with 9 days of fermentation time. Generally, elongation at break reduction is common for polymers, mainly natural polymers, as it is inversely related to tensile strength [61]. The previous study recommended values of tensile strength and elongation at break for wound dressing were more than 1 mPa and 10% [48]. However, the optimal tensile strength value of the hydrogel mask was more than 0.1 kgf/cm^2 [62]. Elongation at break value for sheet mask was more than 30% [63].

Analysis of FTIR spectroscopy

The FTIR spectra of all samples showed several peaks corresponding to the typical BC peaks (Fig. 5). The spectra analysis is shown in Table 3 [25,49,64–69]. Based on its typical peaks, the BC spectra of *L. parafarraginis* showed similarity with dry BC spectra from *L. hilgardii* IITRKH159 strain. BC film of *L. hilgardii* IITRKH159 strain resulted in peaks at around 3,340, 2,900, 1,646, 1,424, 1,360, 1,060, 893, and 750 cm^{-1} [25]. Dry BC of *L. parafarraginis* without casting method using 2 days storage time and 10% inoculum size also showed a similar spectrum. Most of the peaks have similar wavenumbers with insignificant shifts [26]. Based on the spectra, it was known that the BC structure had plentiful hydrogen bonds because of many hydroxyl groups and oxygen atoms [70]. The identical spectra of BC film from both bacteria exhibited similar chemical structures. Moreover, the FTIR spectra of BC15, BC17, BC19, BC25, BC27, and BC29 were also identical, implying that the different BC films had similar chemical structures.

Analysis of XRD

The XRD pattern of BC films is presented in Figure 6. The pattern was similar to previous studies that used coconut water as a medium and casting method for its preparation [71,72]. All samples exhibited similar peaks at 2θ of 14.1°, 16.4°, and 22.3°. These diffractions indicated three main peaks representing crystal planes 101, 10$\bar{1}$, and 002, respectively. These peaks are typical of the distinctive structure of cellulose I [73]. There was an insignificant shifting of the main peaks for all BC films. The study demonstrated that alterations in the storage times of coconut water in inoculum and fermentation times may affect the crystallinity of BC films. The X-ray diffractograms revealed the strongest primary peaks in BC27 and BC29. This means that the crystallinity increased because there were more crystal components and fewer amorphous components. BC27 and BC29 exhibited the highest CrI values of 0.9205 ± 0.0014 and 0.9232 ± 0.0018 (Table 4). The films also showed the greatest %crystallinity value of 92.6363% ± 0.1171% and 92.8677% ± 0.1556%, respectively (Table 4). Meanwhile, BC15 exhibited the lowest values for CrI (0.9094 ± 0.0010) and %crystallinity (91.6901 ± 0.0804). The study found that increasing the storage times for coconut water and prolonging the fermentation time resulted in a higher level of BC film crystallinity. Overall, BC exhibited a high degree of crystallinity. The BC films produced by *A. xylinus* had CrI of 0.81 and %crystallinity of 84% in the Hestrin–Schramm medium. In the hot water extract medium, the BC films had CrI of 0.73 and %crystallinity of 79% [50]. Furthermore, another study produced BC film using *Acetobacter* sp. bacteria, coconut water as the growth medium, and the casting method, resulting in a CrI of 88.58% [74].

Morphological analysis of SEM

The morphological structures of the dry BC film surface were illustrated using SEM analysis at 5,000 magnifications (Fig. 7). BC structure showed irregular fine cellulose fibers arranged in a 3D porous network, which is similar to previous studies [75,76]. The average width of BC fiber using *L. parafarraginis* was about 0.25–1 µm. It was bigger than BC fiber produced by *L. hilgardii* IITRKH159 with about 0.039 µm width size [25]. It was suggested that different bacteria, medium, and fermentation conditions affected BC fiber structures.

BC film produced from coconut water with 1 day storage time exhibited a very fine fiber structure with an irregular arrangement and many pores. Whereas BC film with longer fermentation time produced fine fiber structure in smaller quantities and more fiber structure with larger sizes. The result was similar to BC film from coconut water with a storage time

Table 2. Characterization of BC films with various coconut water storage and fermentation times.

Characteristics	BC15	BC17	BC19	BC25	BC27	BC29
pH	5.67 ± 0.02^{a}	5.77 ± 0.02^{b}	5.83 ± 0.02^{cd}	5.84 ± 0.02^{cde}	5.87 ± 0.01^{cde}	5.89 ± 0.01^{de}
Wet weight (gram)	24.43 ± 1.59^{a}	84.11 ± 1.04^{b}	105.69 ± 0.14^{c}	61.94 ± 0.01^{d}	125.48 ± 0.07^{e}	126.50 ± 0.24^{e}
Wet thickness (mm)	1.82 ± 0.07^{a}	4.19 ± 0.22^{b}	6.36 ± 0.06^{c}	3.47 ± 0.11^{d}	6.47 ± 0.31^{c}	6.83 ± 0.18^{c}
WVTR (g/m^2)	365.35 ± 1.24^{a}	365.46 ± 2.13^{a}	374.99 ± 2.60^{b}	367.93 ± 1.94^{a}	376.75 ± 1.78^{b}	387.81 ± 0.35^{c}
Moisture Content (%)	5.64 ± 0.16^{a}	5.77 ± 0.33^{a}	6.04 ± 0.15^{a}	5.89 ± 0.16^{a}	5.96 ± 0.22^{a}	6.12 ± 0.13^{a}
Swelling Degree (%):						
1 hour	44.38 ± 0.19^{a1}	44.54 ± 0.17^{a1}	32.57 ± 0.63^{b1}	68.94 ± 0.54^{c1}	80.55 ± 2.09^{d1}	76.92 ± 1.36^{e1}
4 hours	40.94 ± 0.49^{a2}	55.99 ± 0.41^{b2}	58.37 ± 0.22^{b2}	84.41 ± 1.38^{c2}	93.40 ± 2.83^{d2}	93.36 ± 4.03^{d2}
6 hours	66.88 ± 0.43^{a3}	91.96 ± 0.31^{b3}	90.45 ± 0.66^{b3}	121.43 ± 1.00^{c3}	125.19 ± 1.02^{d3}	125.64 ± 0.25^{d3}
Mechanical Strength:						
Tensile Strength (mPa)	1.57 ± 0.00^{a}	11.37 ± 0.02^{b}	12.05 ± 0.08^{c}	8.62 ± 0.11^{d}	11.66 ± 0.02^{e}	13.52 ± 0.01^{f}
Elongation at Break (%)	30.83 ± 0.00^{a}	35.82 ± 0.01^{b}	27.59 ± 0.19^{c}	29.24 ± 0.00^{d}	35.89 ± 0.05^{b}	35.81 ± 0.03^{b}
Young's Modulus (mPa)	7.94 ± 0.08^{a}	78.79 ± 0.13^{b}	82.03 ± 0.10^{c}	70.36 ± 0.05^{d}	98.29 ± 0.08^{e}	99.08 ± 0.04^{f}

The data represents the mean of three samples, with the standard deviation (SD) indicated as the mean ± SD. Significant differences were observed in the various superscript letters within the same row and the different superscript numbers within the same column, as determined by the LSD test ($p < 0.05$). BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

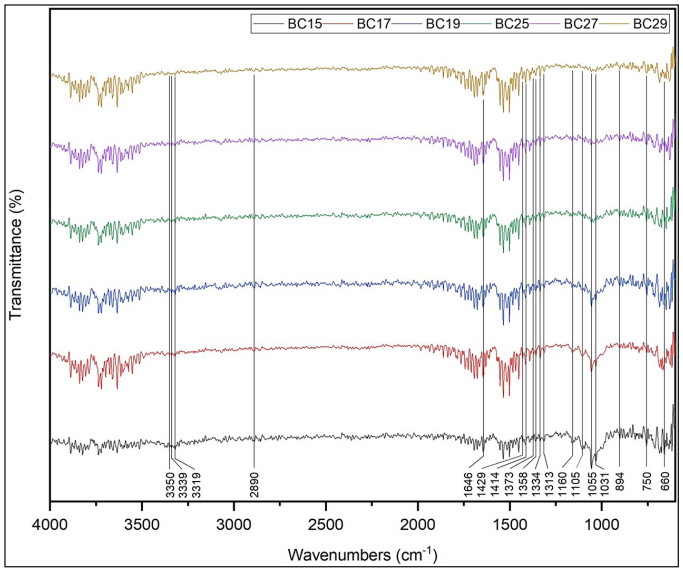

Figure 5. FTIR spectra of BC films with various coconut water storage and fermentation times. BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

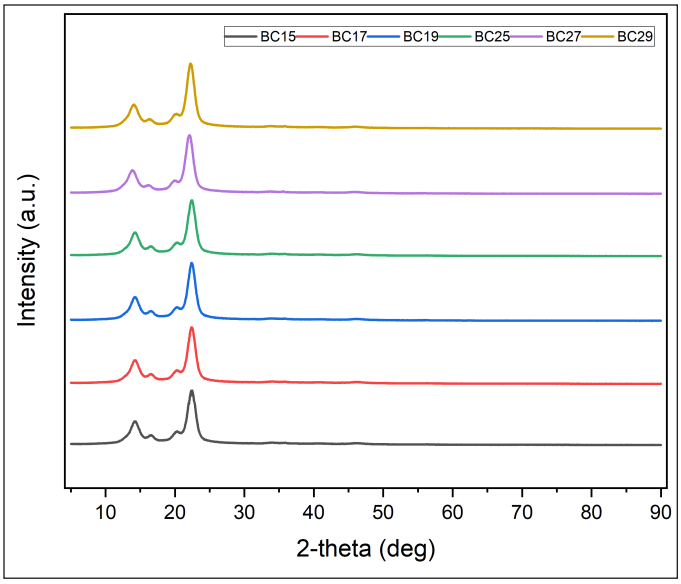

Figure 6. X-ray diffraction spectra of BC films with various coconut water storage and fermentation times. BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

of 2 days, but the structure of very fine fiber was less than that of BC film from 1-day storage of coconut water. The BC film with 2 days of storage showed that the fine fiber aggregated to form ribbons. BC25 still had very fine fiber, but it was less than BC27 and BC29. The fiber structure of BC27 and BC29 were larger and the number was more than BC25. Both BC films showed similar structures consisting of overlapping, folding, and random fiber with a slight difference in fiber arrangement and pores. It caused a slight difference in all measured parameters for BC27 and BC29. The larger and more numerous fiber structures in BC27 and BC29 gave better advantages, as mentioned in the previous studies [55,75]. It exhibited better WVTR, moisture content, swelling degree, and mechanical strength properties

Table 3. Analysis of FTIR spectra of BC films.

Wavenumbers (cm-1)	Analysis of functional groups	Ref.
3350	Inter and intra -OH stretching vibrations, corresponding to cellulose type I	[65–67]
3339 3319	Inter and intra -OH stretching vibrations	[66,67]
2890	C-H stretching vibration of CH_2 and CH_3 bonds or CH_2 symmetric and asymmetric stretching vibration	[25]
1646	C-O stretching	[49]
1429	-OH bending, corresponding to cellulose type I	[49,65,66]
1414	CH_2 bending	[49,66]
1373	C-H bending, corresponding to crystalline regions of BC structure	[49]
1358	C-H bending	[68]
1334	O-H in-plane bending, corresponding to crystalline regions of BC structure	[49]
1313	CH2 wagging, corresponding to crystalline regions of BC structure	[49]
1160	C-O-C asymmetric bridge stretching of 1,4-β-glucoside, corresponded to cellulose type I	[49,65,66,68]
1105	C-O stretching	[49]
1055	C-O-H bond from carbohydrates	[69]
1031	C-O-C ring vibration	[49]
894	C-O-C stretching of the pyranose ring and bending vibration of (1-4) β linkage, corresponding to cellulose type I	[25,65]
750	Indicating Iα allomorph of cellulose type I (predominantly resulted from cellulose of bacteria and algae)	[70]
660	O-H out-of-phase bending	[65]

Table 4. CrI and %crystallinity of BC films.

Samples	CrI	%crystallinity
BC15	0.9094 ± 0.0010^{a}	91.6901 ± 0.0804^{a}
BC17	0.9138 ± 0.0008^{b}	92.0605 ± 0.0714^{b}
BC19	0.9155 ± 0.0008^{c}	92.2063 ± 0.0686^{c}
BC25	0.9119 ± 0.0009^{d}	91.9036 ± 0.0744^{d}
BC27	0.9205 ± 0.0014^{e}	92.6363 ± 0.1171^{e}
BC29	0.9232 ± 0.0018^{e}	92.8677 ± 0.1556^{e}

The data represents the mean of three samples, with the standard deviation (SD) indicated as the mean ± SD. Significant differences were observed in the different superscript letters within the same column, as determined by the LSD test ($p < 0.05$). BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

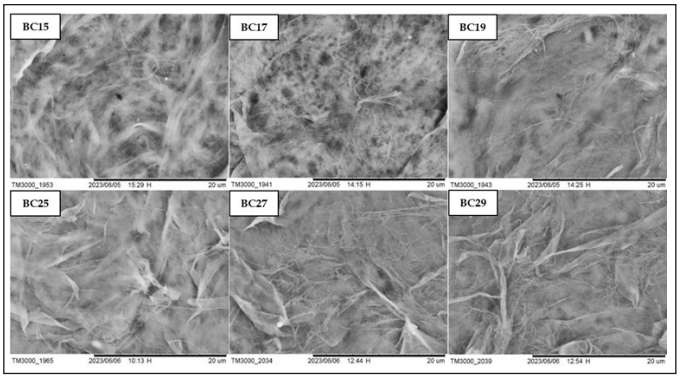

Figure 7. Scanning electron micrograph of BC films with various coconut water storage and fermentation times. BC15: Bacterial cellulose from coconut water kept for 1 day and fermented for 5 days. BC17: Bacterial cellulose from coconut water kept for 1 day and fermented for 7 days. BC19: Bacterial cellulose from coconut water kept for 1 day and fermented for 9 days. BC25: Bacterial cellulose from coconut water kept for 2 days and fermented for 5 days. BC27: Bacterial cellulose from coconut water kept for 2 days and fermented for 7 days. BC29: Bacterial cellulose from coconut water kept for 2 days and fermented for 9 days.

than other samples. Furthermore, the presence of irregular and folded fiber structures was suggested due to blending and casting processes in the production of dry BC film [26].

Texture analysis of AFM

Figure 8 depicts the micromorphology and microscopic characteristics of the surface of BC29, which is the BC film with the most promising properties. The AFM image exhibited a reticulated structure consisting of an extensive number of randomly orientated and overlapping fibers with irregular ordering. The presence of numerous fibers appeared to aggregate and form a dense bundle and ribbon structure consisting of very fine fibers. Unlike SEM, AFM was able to capture images of fibers that were significantly smaller in width, measuring approximately 0.05 µm. The surface topography of BC29 exhibited a low average roughness (Sa), with a value of 41.83 ± 6.96 nm. A previous study demonstrated that the roughness of BC film produced by using the Hestrin and Schramm medium and molasses medium was 50–90 nm

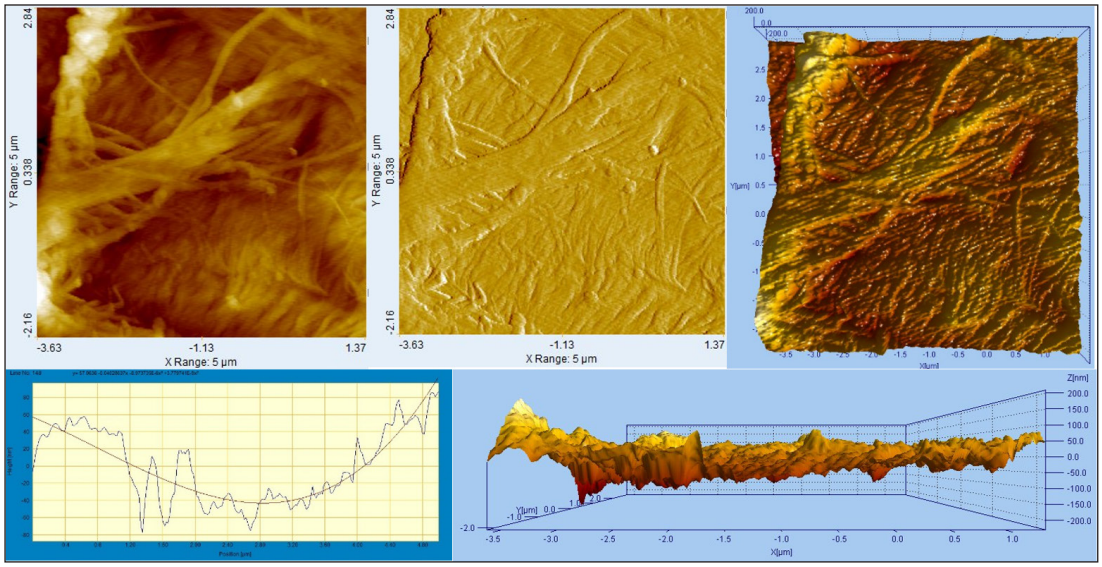

Figure 8. AFM topography image of BC29.

[77]. BC film using the oven-dried method without casting showed a roughness of 21.40 ± 1.80 nm [78]. It seemed that manually preparing BC film with the casting method increased its degree of roughness. In this study, the 3D-AFM image of BC29 confirmed the surface roughness' homogeneity. The relatively smooth texture of BC29, due to its low degree of roughness, made it suitable for various applications.

CONCLUSION

This study has successfully developed BC film using *L. parafarraginis* and coconut water as fermentation medium by casting method. BC film preparation with various storage times of coconut water and fermentation times was studied and concluded that the research design had significant effects on several properties, including its morphological characteristics. Generally, BC film with 2 days storage of coconut water had better characteristics than BC with 1 day storage of coconut water. Furthermore, the longer the fermentation, the better the characteristics. In summary, it was recommended that BC29 had the best conditions to produce the best BC film properties. The FTIR spectra indicated several functional groups of BC film in general. The XRD spectra showed that the crystallinity of all BC films was high. Additionally, the SEM and AFM images of BC29 showed more fiber structure with many pores and its smooth texture giving more advantages, as indicated by its good characteristics.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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