Pharmacy Education (2024) 24(3) 75 - 81
https://doi.org/10.46542/pe.2024.243.7581

IGSCPS SPECIAL EDITION

RESEARCH ARTICLE

Impact of different NaOH treatments on biocellulose properties from coconut water fermented by *Lentilactobacillus parafarraginis*

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Keywords
Biocellulose property
Coconut water
NaOH treatment

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Abstract

Background: Biocellulose is a natural polymer produced by bacteria with distinct physicochemical properties, although it has a chemical composition identical to plant cellulose. Before use, biocellulose was purified by chemical treatment to increase its properties. **Objective:** The study aimed to evaluate biocellulose properties using NaOH solution as a purification agent. **Method:** After harvesting, all samples were purified using NaOH of 0.25 M (BC0.25), 0.5 M (BC0.5), and 1 M (BC1) and determined for their properties, such as mechanical strength, swelling degree, water vapour transmission, and moisture content. Analysis of scanning electron microscope (SEM) images and Fourier Transform Infrared Spectroscopy (FTIR) spectra were also performed for characterisation. **Result:** BC0.5 exhibited the highest tensile strength of 15.38±0.45 MPa and elongation at a break of 38.40±0.58%. BC1 had the highest swelling degree of 131.24±0.70%, water vapour transmission of 400.00±0.36 g/m^2, and moisture content of 6.44±0.14%. Nevertheless, there were slight alterations in morphological structure and spectral peaks because of the high concentration of NaOH. **Conclusion:** The use of NaOH treatment of 0.5 M in biocellulose purification removed more contaminants and resulted in better properties than other concentrations.

Introduction

Biocellulose is a gelatinous membrane that results from the fermentation of certain bacteria at the liquid-air interface of a medium (Kamide *et al.*, 1990). Brown conducted the first biocellulose production study in 1886 using *Acetobacter xylinum* (Brown, 1886). Nowadays, various bacterial genera such as *Gluconacetobacter, Achromobacter, Agrobacterium, Aerobacter, Azotobacter, Alcaligenes, Salmonella*, *Rhizobium,* and *Lactobacillus* have been developed to produce biocellulose (Adetunji & Adegoke, 2007; Sumardee *et al.*, 2020). Biocellulose was characterised by ultrafine fibre structure, high porosity, water absorption, and mechanical strength (Rusdi *et al.*, 2022). Due to its unique characteristics, biocellulose was potentially utilised in many fields, e.g., food, pharmaceutical, and cosmetic industries (Bäckdahl *et al.*, 2006; Hsieh *et al.*, 2008; Fang & Catchmark, 2015).

Biocellulose is essential for purifying the pellicle for various purposes. At the end of the fermentation process, the pellicle contained impurities that affect its properties. Several methods have been conducted to purify biocellulose, such as the addition of an alkaline and organic acid solution or continuous washing with distilled water (Chawla *et al.*, 2009). Alkaline treatment increased the fibre density and modified the crystallinity of cellulose structure to enhance its properties (Ouarhim *et al.*, 2019). The treatment method affected biocellulose morphology structure, mechanical properties, porosity, and cell attachment

without causing alteration of the chemical structure and biocellulose shape (Moniri *et al.*, 2017). NaOH treatment, as a low-cost and environmentally friendly purifying agent, enhanced the diameter of biocellulose fibre and resulted in an effective pore size of 45-130 nm (Al-Shamary & Al-Darwash, 2013). Hence, the study was performed to determine biocellulose properties with different NaOH treatments, i.e. 0.25 M, 0.5 M, and 1 M. The biocellulose was produced using coconut water as the main medium ingredient and Lentilactobacillus parafarraginis as the fermentation bacteria. The basionym of the bacteria is *Lactobacillus parafarraginis*. Based on literature searches, no publications use the bacteria to produce biocellulose. *Lactobacillus* species is widely known as a non-pathogenic and safe bacteria (Belicová *et al*., 2013). Other *Lactobacillus* species have been developed in biocellulose production, such as *L. acidophilus, L. brevis, L. lactis*, and *L. plantarum* (Adetunji & Adegoke, 2007; Sumardee *et al*., 2020). Moreover, *L. hilgardii* IITRKH159 produced biocellulose with higher properties than *Komagataeibacter xylinus* (Khan *et al*., 2020). The mixed culture of *L. plantarum* KCCM 80077 and *Gluconacetobacter* sp. gel_SEA623-2 produced dry biocellulose with higher characteristics than the mono-culture of *Gluconacetobacter* sp. (Kim *et al*., 2014).

Methods

Materials

Coconut water was obtained from coconut (*Cocos nucifera* L.) grown in Bondowoso, East Java, Indonesia. Lentilactobacillus parafarraginis was supplied by PT Biotechno (Serang, Banten, Indonesia). The molecular analysis of 16S rRNA was performed to identify the bacterial rRNA gene at Professor Nidom Foundation (Surabaya, East Java, Indonesia) (certificate number 071122/PNF-XI/2022). The chemical materials, i.e. sucrose, acetic acid, ammonium sulfate, NaOH, and KH_2PO_4, were supplied by Merck (Darmstadt, Germany).

Biocellulose preparation and purification

The culture medium contained 0.25 mL ammonium sulfate, 0.5 mL acetic acid, 1.25 mL sucrose, and 46 mL coconut water stored at 25 °C for two days. After filtration, coconut water was heated and stirred with other ingredients. The medium was sterilized at 121 °C for 15 min. Inoculum of *L. parafarraginis* (2 mL) was added and fermented at 26-30 °C under static conditions. After seven days, the pellicle was removed from the medium, rinsed in running water, and boiled for 60 min. The biocellulose was purified in NaOH of 0.25 M, 0.5 M, and 1 M at 100 °C for 30 min. Each sample was soaked in distilled water several times until a pH of 7.0 was reached (Kamaruddin *et al.*, 2021). The biocellulose was blended, filtered, cast using glass plates, and dried at 60 °C for 72 h (Kuswandi *et al.*, 2014).

Determination of biocellulose properties

Mechanical strength

The mechanical strength measurements were conducted using a universal testing machine (Huang Ta HT-2328, Taiwan) at a 10 mm/min stretching rate and 25 °C for each sample. The biocellulose was cut into 6 cm x 2 cm size (rectangular shape) (Sulastri *et al.*, 2021).

Swelling degree

The dried biocellulose in 1.5 cm x 1.5 cm size was immersed in phosphate buffer at a pH of 7.4 for six hours (h). The weight of dried (Wd) and swollen (Ws) biocellulose was determined to calculate the swelling degree using equation (1) (Sulastri *et al.*, 2021):

$$Swelling\ degree = \frac{Ws-Wd}{Wd}\ x\ 100\% \quad (1)$$

Water vapour transmission

The water vapour transmission was determined using a weighted bottle filled with 5 g of $CaCl_2$ anhydrate. The biocellulose, with certain area (S), was placed on the top of the bottle and stored in a desiccator at 25 °C and 75% RH. The sample was weighed (W) after 24 h, and the water vapour transmission was calculated using equation (2) (Sulastri et al., 2021):

$$Water\ vapour\ transmission = \frac{W}{S} \quad (2)$$

Moisture content

The dried biocellulose was cut into 2 cm x 2 cm size, weighed (W1), heated at 90 °C for 24 h, and weighed (W2). The moisture content was determined using equation (3) (Sulastri *et al.*, 2021):

$$Moisture\ content = \frac{W1-W2}{W2}\ x\ 100\% \quad (3)$$

Scanning Electron Microscopy (SEM) images

The morphological structure of the biocellulose surface was observed using SEM (Hitachi Tabletop Microscope TM3000, Japan) at 5 kV. The biocellulose was cut in 0.5 cm x 0.5 cm size, attached to carbon type, and coated with platinum using an ion sputter (Hitachi E-1045, Japan).

Fourier Transformed Infra-Red (FTIR) spectra

The functional groups of all samples were examined using an FTIR spectrometer (Bruker Alpha, Germany). The FTIR spectra were recorded at 4,000-600 cm^{-1} wavenumbers with baseline corrections at 25 °C.

Statistical analysis

The data were presented as mean values ± SD (standard deviation) (n=3). One-way analysis of variance (ANOVA) with the Least Significance Difference (LSD) at $p < 0.05$ (α=0.05) was used to assign significant differences.

Results

Mechanical strength determination

The mechanical strength of all samples was tested for tensile strength and elongation at break (Table I). All samples indicated good mechanical strength, but BC0.5 showed higher mechanical properties than BC1. Okur *et al*. (2019) and Pansara *et al*. (2020) reported that tensile strength and elongation at break recommendation value for wound dressing is > 1 mPa and > 10%.

Table I: Biocellulose properties with different NaOH treatments

Parameters	BC0.25	BC0.5	BC1
Tensile strength (mPa)	12.82±0.80[a]	15.38±0.45[b]	10.25±0.38[c]
Elongation at break (%)	28.74±0.57[a]	38.40±0.58[b]	25.16±0.32[c]
Swelling degree (%)	121.12±0.88 [a]	126.84±0.83 [b]	131.24±0.70 [c]
Water vapour transmission (g/m^2)	376.85±0.74 [a]	350.98±0.62 [b]	400.00±0.36 [c]
Moisture content (%)	5.55±0.09[a]	5.95±0.12[b]	6.44±0.14[c]

*Data are represented as mean ± SD of triplicate measurement. The different superscript letters for the same row indicated significant differences ($p < 0.05$). BC0.25: Biocellulose with 0.25 M NaOH treatment. BC0.5: Biocellulose with 0.5 M NaOH treatment. BC1: Biocellulose with 1 M NaOH treatment.

Swelling degree determination

BC1 showed the highest swelling degree (121.12±0.88%). The study exhibited that enhancement of NaOH concentration increased swelling degree. The swelling degree is related to film ability in exudate absorption for wound dressing. The higher the swelling degree, the higher its ability to absorb exudate (Sulastri *et al*., 2021).

Water vapour transmission determination

Table I shows that the water vapour transmission of BC1 is higher than that of BC0.25 and BC0.5. It shows that a high NaOH treatment enhances its water vapour transmission. All samples fulfilled water vapour transmission requirements for wound care in 300-800 g/m^2 (Seaman, 2002).

Moisture content determination

The study exhibited that the moisture content of BC0.25 was lower than that of other samples, which revealed that a low NaOH concentration might cause a decrease in the water content of biocellulose. Clasen *et al.* (2006) reported that moisture content requirements for dry biocellulose is 5-10%.

Analysis of FTIR spectra

The spectra of all biocelluloses are shown in Figure 1 and identified in Table II. BC0.25, BC0.5, and BC1 exhibit similar peaks with few shifting (less than 6 cm^{-1}).

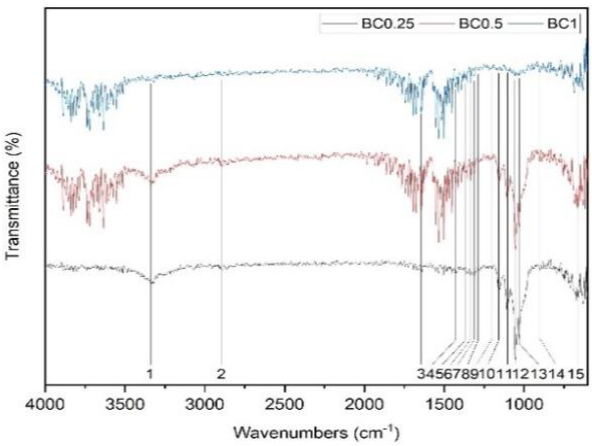

Figure 1: FTIR spectra of biocellulose purified with different NaOH treatments

Table II: FTIR analysis of biocellulose purified with different NaOH treatments

Peak number	Wavenumbers (cm^{-1})			Functional groups	References
	BC0.25	BC0.5	BC1		
1	3338.81	3338.81	3337.39	O-H stretching intramolecular hydrogen bonds of cellulose	(Kumar *et al.*, 2014; Meftahi *et al.*, 2015)
2	2889.76	2889.76	2889.76	C-H stretching of sugar rings	(Kumar *et al.*, 2014; Akintunde *et al.*, 2022)
3	1643.20	1646.03	1646.03	C-O stretching	(Akintunde *et al.*, 2022)
4	1427.88	1430.72	1430.72	CH_2 scissoring motion in cellulose	(Kumar *et al.*, 2014)
5	1374.05	1368.39	1369.81	C-H bending	(Nelson & O'Connor, 1964; Akintunde *et al.*, 2022)
6	1334.39	1334.39	1334.39	O-H in plane bending or C-H deformation	(Nelson & O'Connor, 1964; Wang *et al.*, 2017; Akintunde *et al.*, 2022)
7	1313.14	1313.14	1313.14	CH_2 wagging	(Akintunde *et al.*, 2022)
8	1286.23	1284.81	1284.81	C-H bending	(Wang *et al.*, 2018)
9	1199.82	1199.82	1199.82	C-H bending	(Wang *et al.*, 2018)
10	1158.74	1158.74	1162.99	C-O-C asymmetric bridge stretching	(Meftahi *et al.*, 2015)
11	1104.91	1104.91	1104.91	C-O bending or C-C bonds of polysaccharide monomer units	(Wang *et al.*, 2017)
12	1053.91	1053.91	1055.33	C-O-C pyranose ring stretching or C-O-H bond of carbohydrate bending	(Kumar *et al.*, 2014; Wang *et al.*, 2018; Akintunde *et al.*, 2022)
13	1031.25	1031.25	1031.25		
14	900.93	902.34	902.34	Cellulosic β-glycosidic linkage	(Kumar *et al.*, 2014)
15	661.53	664.36	658.70	O-H out-of-phase bending	(Wang *et al.*, 2018)

*BC0.25: Biocellulose with 0.25 M NaOH treatment. BC0.5: Biocellulose with 0.5 M NaOH treatment. BC1: Biocellulose with 1 M NaOH treatment

Analysis of SEM images

SEM images of BC0.25, BC0.5, and BC1 are shown in Figure 2. BC 1 shows larger pores than other biocellulose with irregular and overlapping fibre.

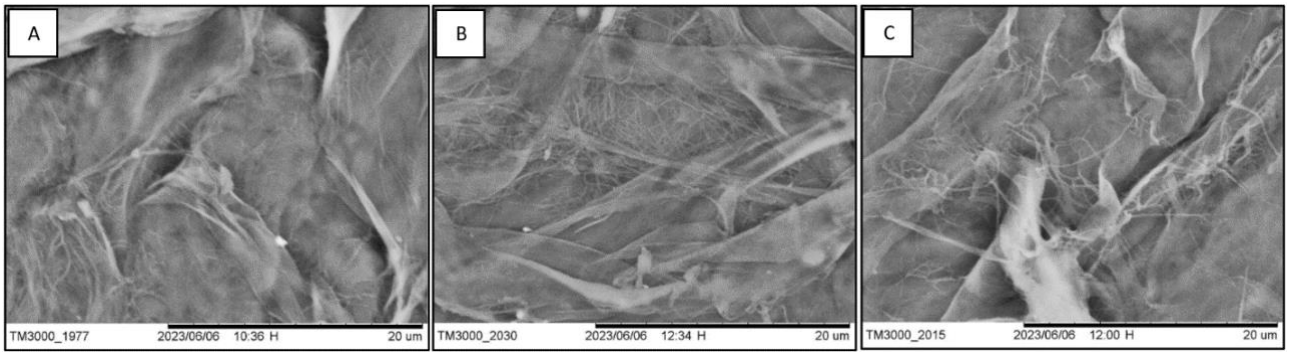

Figure 2: Micrographs of (A) Biocellulose with 0.25 M NaOH treatment, (B) Biocellulose with 0.5 M NaOH treatment, and (C) Biocellulose with 1 M NaOH treatment in 5,000x magnification

Discussion

NaOH solution purifies biocellulose by hydrolysing and removing cell impurities, even at low concentrations. Nevertheless, the study showed that NaOH treatment caused different properties of biocellulose. Mechanical strength shows the film's ability to resist loads or pull. The swelling degree indicates the film's ability to absorb liquid. The water vapour transmission is related to its ability to provide a moist environment and gas exchange on a certain surface. Moisture content shows the film's ability to retain moisture in its structure (Sulastri *et al.*, 2021). Furthermore, FTIR spectra were

used to identify characteristic functional groups of biocellulose and analyse differences among samples because of different NaOH concentrations.

It was known that biocellulose purified with different NaOH treatments resulted in similar FTIR spectra, as shown in Figure 1. All samples showed several typical peaks with little differences, indicating the same chemical structures. Based on previous studies, all peaks have been assigned to functional groups of biocellulose, as shown by the identification of each sample in Table II. Peaks at around 3338, 1430, 1160, and 900 cm^{-1} exhibited that BC0.25, BC0.5, and BC1 were mainly composed of cellulose I. Meanwhile, other weak peaks at about 1335, 1313, and 1284 cm^{-1} revealed that all samples contained less cellulose II (Wang *et al.*, 2017).

Peaks around 3338, 1053, and 1031 cm^{-1} (peaks 1, 12, and 13) significantly differed for BC1, compared to BC0.25 and BC0.5. The lower absorption peaks were suggested to result from breaking intra- and inter-hydrogen bonds in biocellulose. The O-H bonds cleavage damaged its structure, including distance enlargement among biocellulose layers and the formation of fractured layers. Consequently, it affected biocellulose characteristics, i.e. tensile strength and elongation at break (Suryanto *et al.*, 2019). The study exhibited reduced mechanical properties because of a high NaOH treatment. BC1 exhibited lower mechanical properties than BC0.5. A previous study reported that NaOH treatment to biocellulose produced by *Acetobacter xylinum* affected its nanofibril network and sheet-like structures (Suryanto *et al.*, 2019). It caused a reduction of tensile strength and elongation at the break of biocellulose. BC0.5 had the highest mechanical properties, but BC0.25 exhibited the lowest value. A lower concentration of NaOH treatment for BC0.25 was suggested, making it still contain impurities, such as remaining organic compounds of medium ingredients, nucleic acids, proteins, or other substances resulting from bacteria metabolism during fermentation, which interfered with its mechanical strength.

Based on morphological images of SEM, it was known that BC0.5 had more fibre with smaller pore sizes than BC0.25 and BC1. It was suggested that regular arrangement and a greater number of fibres caused a higher mechanical strength of BC0.5. SEM image of BC0.25 described that there were still impurities embedded in biocellulose matrix, causing its fibre to not be visible, even though it used the same magnification as BC0.5 and BC1. The figure was similar to a previous study reporting that NaOH solution removed impurities and trapped microbes in the nanofibre structure of cellulose, which increased layer porosity and wettability (Meftahi *et al.*, 2015).

Other properties of biocellulose, i.e. swelling degree and moisture content, showed similar results. BC1 exhibited the highest values of 131.24±0.70% and 6.44±0.14%, respectively. NaOH treatment caused morphological structure alteration, which increased biocellulose ability in water or moisture absorption through its wide pores size and fracture layers (Suryanto *et al.*, 2019). Furthermore, biocellulose purification using NaOH increased the degree of swelling due to the enhancement of fibre diameter (Al-Shamary & Al-Darwash, 2013). However, the swelling degree of BC0.25 and BC0.5 was more than 100% after 24 h immersion. BC0.5 showed the best water vapour transmission (350.98±0.62 g/m^2), which is suggested for its fibre structure density.

Conclusion

Compared to other biocelluloses with different NaOH treatments, BC0.5 showed the best properties. The lower NaOH treatment of BC0.25 caused the removal of impurities and trapped bacteria, which was ineffective. Otherwise, the higher NaOH concentration in the biocellulose purification of BC1 resulted in lower mechanical strength, although it had a high degree of swelling, water vapour transmission, and moisture content. The result was consistent with the analysis of FTIR spectra and SEM images for all samples.

Acknowledgement

The authors thank the Faculty of Pharmacy, Universitas Airlangga, and the Faculty of Pharmacy, University of Jember, for supporting the research.

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