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The Mechanical Strength and Morphology of Bacterial Cellulose Films: The Effect of NaOH Concentration

H Suryanto[1*], M Muhajir[2], T A Sutrisno[2], Mudjiono[2], N Zakia[3], U Yanuhar[4]

[1] Department of Mechanical Engineering, Faculty of Engineering, Universitas Negeri Malang, Indonesia
[2] Master Program of Mechanical Engineering, Graduate School, Universitas Negeri Malang, Indonesia
[3] Department of Chemical, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Indonesia
[4] Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang, Indonesia

*Corresponding author's email: heru.suryanto.ft@um.ac.id

Abstract. Bacterial cellulose (BC) is a natural material with attractive physical properties produced by *Acetobacter xylinum*. Commonly, BC is subjected to a chemical treatment prior to use. Therefore, this study aimed to investigate the effect of chemical treatment using alkali (NaOH) with the various concentrations on the mechanical strength of the resulting BC films. The research method included BC film production using a pineapple peel extract as the main culture material. The resultant BC film was treated with NaOH solution with concentrations of 0%, 1%, 5%, and 10% and then dried in the oven. The mechanical strength of each sample was evaluated by a tensile test, and the film morphology was observed by scanning electron microscopy (SEM). The results showed that the tensile strength of the BC film before the treatment was 208 MPa and decreased to 165 MPa after the treatment with the highest concentration of NaOH because some BC component was dissolved in NaOH solution causing bulk fractured layers. The NaOH concentrations of 1%, 5%, and 10% resulted in the strains of BC films by 2.96%, 3.64%, and 3.00%, respectively. The chemical treatment of NaOH caused BC films to swell and damage to the network and layers in BC film.

Keywords: Bacterial cellulose film, NaOH treatment, mechanical strength, tensile test, morphology

1. Introduction

Cellulose is a natural polymer derived from various sources and found in great abundance. This material attracts a considerable interest because of its biodegradability, mechanical strength, low density, worldwide availability, and comparable to synthetic fibers [1]. Cellulose, as the most abundant renewable natural biopolymer, contains a linear homopolysaccharide comprising D-glucopyranose units connected by 1,4-glycosidic bonds [2]. Currently, bacterial cellulose (BC) has been developed for the specialty papers, healthy foods, biomedical wound care products, biodegradable food packaging materials, high-end acoustic diaphragms, and many other industrial applications [3].

Cellulose can be obtained from animals, plants, and bacteria. A crucial feature of cellulose-producing bacteria is their ability to generate the extracellular cellulose as a fiber network with extremely fine, pure, and random structure that has high mechanical strength, high water absorption capacity, and crystallinity. The resulting cellulose network is called the pellicle. Despite being free of hemicellulose and lignin, BC film still contains impurities and remaining culture medium and hence the importance of NaOH treatment for removing the remnants of bacterial cells and impurities in the pellicle [4]. The low concentration of NaOH used for the treatment did not influence the mechanical strength of the BC film [3]. Also, at high concentrations, NaOH could exert a significant effect on mechanical strength and elastic modulus [5].

BC film has received considerable attention and been extensively studied as a novel type of scaffold material because it has fine fiber network, high tensile strength, good biocompatibility, and high water holding capacity [6]. BC film has an elastic modulus range from 15 to 35 GPa, the tensile strength range from 200 to 300 MPa, and elongation range from 1.5 to 2.0% [7]. BC had been applied in a various applications [8] such as bio-based packaging [9], tissue scaffolding [10], blood vessel grafts [11], wound dressings [12], implants [13], electro-active paper sensor [14], and smart sensor [15]. Due to its potential applications in biomedical and other fields, sterilized BC film is highly required; it is usually achieved by boiling in high concentrations of NaOH solution [3]. This NaOH treatment is a harsh solution and applied to initiate the degradation of cellulose through the alkalization process which influences the mechanical strength of BC film. Since the unique mechanical strength of BC film is indispensable, any unfavorable effects on mechanical strength should be prevented. Therefore, this study aimed to characterize the morphology and mechanical strength of BC film derived from pineapple peel extract after treated by NaOH solution.

2. Methods

2.1 Materials

The raw material in this research was the Pineapple peel waste extract as the basic cultivation medium of bacteria to produce BC fiber. The chemical compounds, i.e. CH_3COOH), $(NH_4)_2SO4$ and NaOH, were purchased from the UD Sumber Ilmiah, Malang, Indonesia. The strain of *A. xylinum* was used for producing BC fiber.

2.2 Synthesis of BC Pellicle

BC pellicle was prepared by referring to the procedure of previous research [16]. The medium for culturing the bacteria contains 2-liter extract of pineapple peel waste, 0.5% solution of $(NH_4)_2SO_4$, 10% sugar, and 5% CH3COOH) (v/v) at a pH 4.5, then the *A. xylinum* by 10% (v/v) was added into it. The incubation time of bacterial was 10 days under static conditions at 30 °C. The BC pellicle produced by this process floated on the upper of the culture medium. Pellicles were collected and rinsed using some water to remove the residual medium components and the other impurities.

2.3 NaOH Treatment

The untreated bacteria cellulose pellicles were sieved to reduce water content. The BC pellicles in the wet state with a dimension of 5 x 170 x 110 mm3 were soaked in a solution of 150 ml NaOH with concentrations of 0%, 1%, 5%, and 10% for 24 hours at ambient temperature and then rinsed with water until the pH about 7. BC pellicle then dried in the oven at temperature 70 °C for 24h to produce BC film.

2.4 Tensile Testing of BC Film

The tensile strength was determined according to the ASTM D638-V [17] by taking an average of five measurements for each sample treatment. The tensile testing was performed using a fiber tensile test machine (Techno Lab, Indonesia) with a maximum load of 50 N. Samples of BC films (0.033 mm in thickness, measured using the digital calipers (Mitutoyo, Japan)) were cut following the ASTM D638-V using a cutter and mounted between tensile testing grips. The crosshead rate was set at 3 mm/min for each sample.

2.5 Morphology of BC Film

The morphology of BC film structure was scanned by the SEM (FEI, Inspect-S50 type) with the voltage of 10.00 kV. The coated film with a 10 nm gold layer applied to the sample prior to the SEM analysis through sputtering method (sputter coater, SC7-620 Emitech) [18].

3. Results and Discussion

3.1 Mechanical Strength

The tensile test was conducted on BC film without NaOH treatment and with NaOH treatment for 24-hour immersion. Figure 1 shows the representative stress-strain curves of BC film before and after NaOH treatment. After immersion in NaOH with concentration of 0%, 1%, 5%, and 10%, BC film had the tensile stress of 208, 201, 182, and 165 MPa with breaking strain of 3.08, 2.96, 3.64, and 3.00%, respectively. Overall, the BC film characteristic did not show linear stress-strain at low deformations. BC films with strains range of 3 to 3.5% indicate a brittle fracture. This finding is in line with previous research of BC film washed, and NaOH boiled which exhibited strains of 10-20% and low deformation [3]. All samples showed a linear stress/strain relationship with a a slightly curved at a strain of about 0.5%, suggesting some damage to the fiber network during deformation such as bond breaking. This type of curve may also take place due to the twisting of BC fibrils [19]. After maximum tensile stress achieved, the sample was broken so the curve was decline vertically. The utilization of high NaOH concentrations could exert a significant effect on the mechanical strength of the BC film [5]. Also, non-linear BC network could affect the mechanical strength of BC film [19].

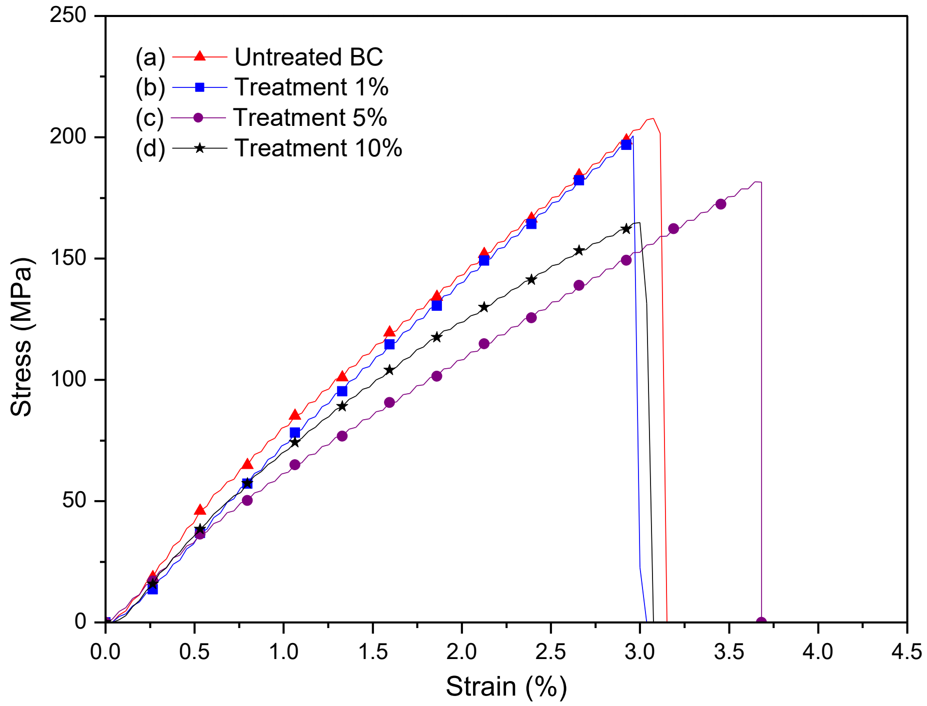

Figure 1. Stress-strain curves of BC film

3.2 Morphology of BC Film

BC fiber film has a different structure compared than plant fiber. Plant fiber contains cellulose that bounded with hemicellulose, lignin, and other chemicals occurring naturally. The BC fiber is relatively high purity so unbound to other chemical compounds [20]. Thus the purification applied differs from that in plant cellulose as it aims to remove only the remaining organic compound as a food for microbes in the medium. Also, nucleic acids and proteins, either from yeast extract or generated by microbes during the incubation process, result in impurities. Figure 2 demonstrates the BC pellicles prior to and after treatment of NaOH with concentrations of 1%, 5%, and 10%. The contrasting colors of samples immersed in different concentrations of NaOH solutions are shown in Figure 2a-d.

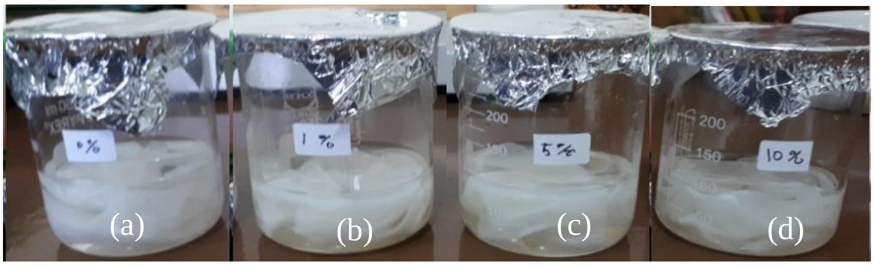

Figure 2. Pellicles of BC: untreated BC (a), BC treated by NaOH with concentration 1% (b); 5% (c); and 10% (d)

The SEM figure provids important visual information on BC films before and after NaOH treatment (sees Figure 3 and Figure 4). The surface structure of BC film was characterized by a three-dimensional fibrous extremely fine network of randomly arranged nanofibrils and hydrogen bonds present in BC fibril units (see Figure 3) [21][22]. The film surface consisted of many intertwined strings creating a network-like structure, comprising overlapping fibrils that formed a layer of randomly oriented BC ribbons. The ultrafine ribbons of BC having a length of 1 to 9 µm, build a dense reticulation structure with extensive hydrogen bonds as structure stabilizer. The degree of polymerization value ranges from 2,000 to 6,000 and can reach 20,000 [23].

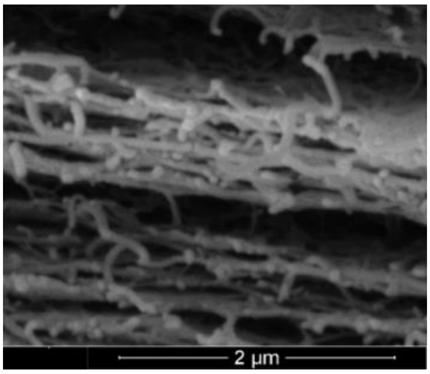

Figure 3. 3-D ultrafine network of BC

Figure 4a shows that BC film without NaOH treatment had a uniform fracture and a homogeneous and dense network. Figure 4b shows the fracture of BC treated by 1% NaOH, consisting of open layers of cellulose. Figure 4c shows a greater fracture in the layers of the BC treated with 5% NaOH. As shown in Figure 4d, 10% NaOH-treated BC film has layers with the largest fracture. Overall all samples of BC had a multi-layered structure formed due to culture fermentation. This results are similar to BC film produced by *Gluconacetobacter hansenii* [24][25]. Also, the NaOH treatment of BC pellicles could affect the nanofibril network and the sheet-like structures as shown in Figure 4.

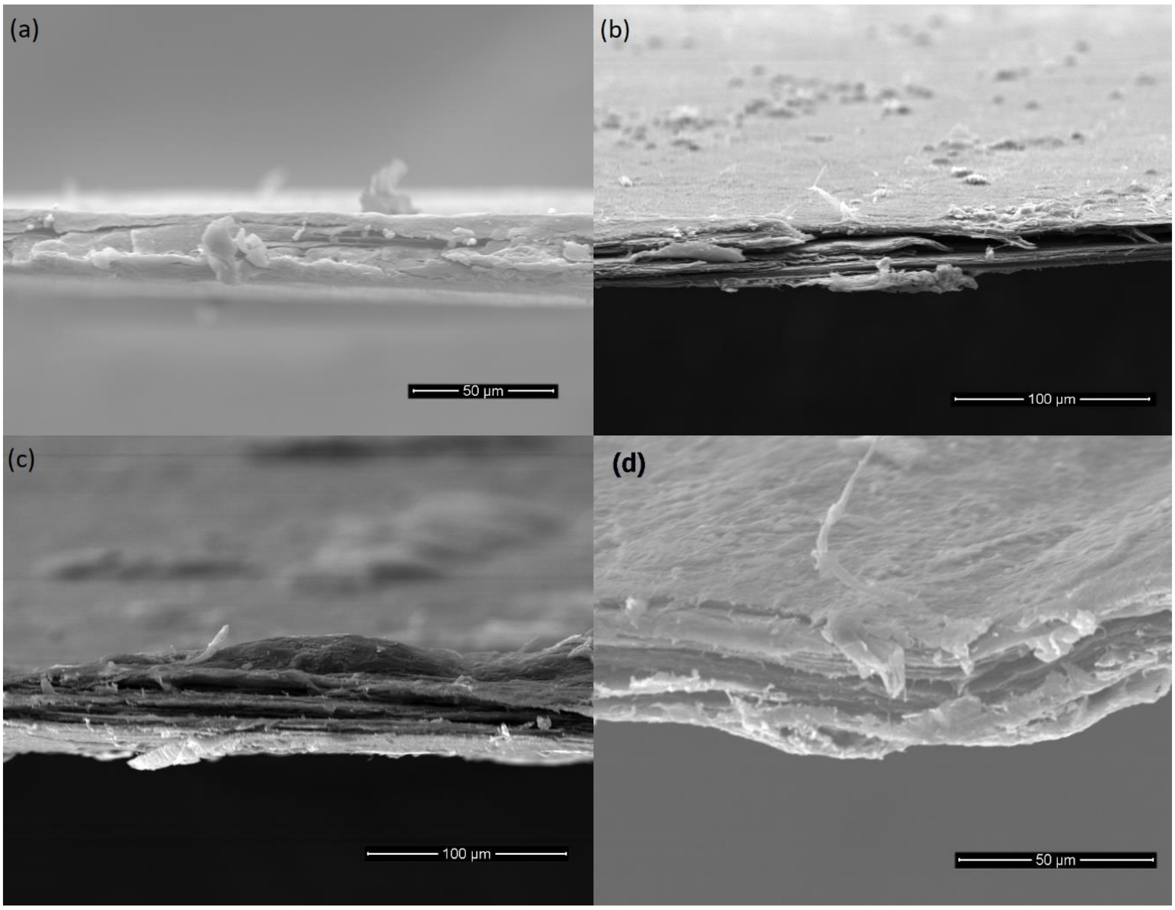

Figure 4. Fracture surface of BC film after tensile test: untreated BC film (a), BC treated by NaOH with concentration 1% (b); 5% (c); and 10% (d)

The structure of BC layers before and after the NaOH treatment with various concentrations of 1%, 5%, and 10% showed differences in the distance between layers of cellulose fibers (see Figure 4a-d). The untreated BC film had dense layers with the shortest distance of 1.88 µm (see Figure 4a). The BC film treated with 1% NaOH had bulk fractured layers with a distance of 1.23 µm (see Figure 4b). The 5% NaOH-treated BC showed layers with the largest opening with a distance of 2 µm (see Figure 4c). The BC subjected to 10% NaOH treatment experienced an increase in layer distance, i.e. 4.67 µm (see Figure 4d). NaOH treatment causes a swelling of BC film. Therefore, NaOH treatment at high concentrations affects the hydrogen bonds present in the BC. It can cleave the intra- and inter-hydrogen bonds in cellulose so damaging the BC film structure [26].

4. Conclusion

NaOH treatment at concentrations up to 10% could exert a significant effect on the mechanical properties of BC film. The higher concentration of NaOH causes the decrease in mechanical properties of BC film and it makes the morphology of BC film swells. These findings are hoped to provide worthwhile information to those studying the utilization of BC film for new applications.

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References

[1] Kawee N, Lam N T and Sukyai P 2018 Homogenous isolation of individualized bacterial nanofibrillated cellulose by high pressure homogenization *Carbohydr. Polym.* **179** 394–401
[2] Yan H, Chen X, Song H, Li J, Feng Y, Shi Z, Wang X and Lin Q 2017 Synthesis of bacterial cellulose and bacterial cellulose nanocrystals for their applications in the stabilization of olive oil pickering emulsion *Food Hydrocoll.* **72** 127–35
[3] McKenna B A, Mikkelsen D, Wehr J B, Gidley M J and Menzies N W 2009 Mechanical and structural properties of native and alkali-treated bacterial cellulose produced by Gluconacetobacter xylinus strain ATCC 53524 *Cellulose* **16** 1047–55
[4] Tsalagkas D, Lagaňa R, Poljanšek I, Oven P and Csoka L 2016 Fabrication of bacterial cellulose thin films self-assembled from sonochemically prepared nanofibrils and its characterization *Ultrason. Sonochem.* **28** 136–43
[5] Nishi Y, Uryu M, Yamanaka S, Watanabe K, Kitamura N, Iguchi M and Mitsuhashi S 1990 The structure and mechanical properties of sheets prepared from bacterial cellulose - Part 2 Improvement of the mechanical properties of sheets and their applicability to diaphragms of electroacoustic transducers *J. Mater. Sci.* **25** 2997–3001
[6] Tang W, Jia S, Jia Y and Yang H 2010 The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane *World J. Microbiol. Biotechnol.* **26** 125–31
[7] Gatenholm P and Klemm D 2010 Bacterial Nanocellulose as a Renewable Material for Biomedical Applications *MRS Bull.* **35** 208–13
[8] Shi Z, Zhang Y, Phillips G O and Yang G 2014 Utilization of bacterial cellulose in food *Food Hydrocoll.* **35** 539–45
[9] Iguchi M, Yamanaka S and Budhiono A 2000 Bacterial cellulose - a masterpiece of nature's arts *J. Mater. Sci.* **35** 261–70
[10] Andersson J, Stenhamre H, Bäckdahl H and Gatenholm P 2010 Behavior of human chondrocytes in engineered porous bacterial cellulose scaffolds *J. Biomed. Mater. Res. - Part A* **94** 1124–32
[11] Klemm D, Schumann D, Udhardt U and Marsch S 2001 Bacterial synthesized cellulose - Artificial blood vessels for microsurgery *Prog. Polym. Sci.* **26** 1561–603
[12] Sindhu K A, Prasanth R and Kumar V Medical Applications of Cellulose and Its Derivatives : Present and Future
[13] Cherian B M, Leão A L, de Souza S F, de Olyveira G M, Costa L M M, Brandão C V S and Narine S S 2013 Bacterial Nanocellulose for Medical Implants *Advances in Natural Polymers* ed S Thomas, P Visakh and A Mathew (Springer Berlin Heidelberg) pp 337–59
[14] Abas Z, Kim H S, Kim J and Kim J-H 2014 Cellulose Electro-Active Paper: From Discovery to Technology Applications *Front. Mater.* **1** 17
[15] Rull-Barrull J, d'Halluin M, Le Grognec E and Felpin F-X 2016 Chemically-modified cellulose paper as smart sensor device for colorimetric and optical detection of hydrogen sulfate in water *Chem. Commun.* **52** 2525–8
[16] Retegi A, Gabilondo N, Peña C, Zuluaga R, Castro C, Gañan P, de la Caba K and Mondragon I 2010 Bacterial cellulose films with controlled microstructure-mechanical property relationships *Cellulose* **17** 661–9
[17] Jonoobi M, Harun J, Mathew A P and Oksman K 2010 Mechanical properties of cellulose nanofiber (CNF) reinforced polylactic acid (PLA) prepared by twin screw extrusion *Compos. Sci. Technol.* **70** 1742–7
[18] Sari N. H, Wardana, I N G, Irawan Y S, Siswanto E 2016 Physical and Acoustical Properties of Corn Husk Fiber Panels *Advances in Acoustics and Vibration* **10** 1155
[19] Hsieh Y C, Yano H, Nogi M and Eichhorn S J 2008 An estimation of the Young's modulus of bacterial cellulose filaments *Cellulose* **15** 507–13

[20] Gea S, Reynolds C T, Roohpour N, Wirjosentono B, Soykeabkaew N, Bilotti E and Peijs T 2011 Bioresource Technology Investigation into the structural, morphological, mechanical and thermal behaviour of bacterial cellulose after a two-step purification process *Bioresour. Technol.* **102** 9105–10
[21] Esa F, Tasirin S M and Rahman N A 2014 Overview of Bacterial Cellulose Production and Application *Agric. Agric. Sci. Procedia* **2** 113–9
[22] Vitta S and Thiruvengadam V 2012 Multifunctional bacterial cellulose and nanoparticle-embedded composites *Curr. Sci.* **102** 1398–405
[23] Suryanto H 2017 Analisis struktur serat selulosa dari bakteri *Prosiding SNTT 2017 – Politeknik Negeri Malang* vol 3pp 17–22
[24] Henning A L and Catchmark J M 2017 The impact of antibiotics on bacterial cellulose in vivo *Cellulose* **24** 1261–85
[25] Lee S-H, Lim Y-M, Jeong S I, An S-J, Kang S-S, Jeong C-M and Huh J-B 2015 The effect of bacterial cellulose membrane compared with collagen membrane on guided bone regeneration *J. Adv. Prosthodont.* **7** 484

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