http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.335.344

Research Article
Characterization of Bacterial Cellulose Produced by *Acetobacter xylinum* Strain LKN6 Using Sago Liquid Waste as Nutrient Source

[1]Nur Arfa Yanti, [1]Sitti Wirdhana Ahmad, [2]Nurhayani H. Muhiddin, [3]La Ode Ahmad Nur Ramadhan, [1]Suriana and [1]Taufik Walhidayah

[1]Department of Biology, Faculty of Mathematics and Natural Science, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia
[2]Faculty of Mathematics and Natural Science, Makassar State University, Makassar, South Sulawesi, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Science, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia

Abstract

Background and Objective: Bacterial Cellulose (BC) is an exopolysaccharide produced by bacteria with unique structural and mechanical properties and is highly pure compared to plant cellulose. This study aimed to produce novel bacterial cellulose using sago liquid waste substrate and evaluate its characteristics as a potential bioplastic. **Materials and Methods:** Production of BC by static batch fermentation was studied in sago liquid waste substrate using Acetobacter xylinum LKN6. The BC structure was analyzed by Scanning Electron Microscopy (SEM) and Fourier Transform infrared spectroscopy (FT-IR). Mechanical properties were measured include tensile strength, elongation at break, elasticity (Young's modulus) and Water Holding Capacity (WHC). **Results:** The BC yield from sago liquid waste as a nutrients source was achieved 12.37 g L^{-1} and the highest BC yield 14.52 g L^{-1} in sago liquid waste medium with a sugar concentration of 10% (w/v) after 14 days fermentation period. The existence of bacterial cellulose is proven by FT-IR spectroscopy analysis based on the appearance of absorbance peaks, which are C-C bonding, C-O bonding, C-OH bonding and C-O-C bonding and represents the fingerprints of pure cellulose. The mechanical properties of BC from sago liquid waste were showed a tensile strength of 44.2-87.3 MPa, elongation at break of 4.8-5.8%, Young's Modulus of 0.86-1.64 GPa and water holding capacity of 85.9-98.6 g g^{-1}. **Conclusion:** The results suggest that sago liquid waste has great potential to use as a nutrient source in the production of bacterial cellulose and BC's prospect as the bioplastic.

Key words: Bacterial cellulose, sago industry by-product, sago liquid waste, mechanical properties, bioplastic

Citation: Yanti, N.A., S.W. Ahmad, N.H. Muhiddin, L.O.A.N. Ramadhan, Suriana and T. Walhidayah, 2021. Characterization of bacterial cellulose produced by *Acetobacter xylinum* strain LKN6 using sago liquid waste as nutrient source. Pak. J. Biol. Sci., 24: 335-344.

Corresponding Author: Nur Arfa Yanti, Department of Biology, Faculty of Mathematics and Natural Science, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The Bacterial Cellulose (BC) is a secondary metabolite that is synthesized extracellularly by bacteria. It has the same molecular formula as plant cellulose; however, its physicochemical properties are different. The BC is characterized as a high purity cellulose polymer since free from hemicellulose and lignin, has a unique strength, an ultra-fine structure and biodegradable. Besides, the isolation and purification of this BC are also simple[1]. These properties allow BC to be used as a substitute for wood cellulose in various areas, including the textile industry, plastics, paper, food, pharmaceutical, cosmetics, tissue engineering and dentistry and predominantly in the medical field[1,2].

Many studies have focused on agricultural waste and an industrial by-product as a potential medium to find a new economic culture medium for industrial-scale production of BC[3,4]. The utilization of wastes as fermentation media could improve the cost-competitiveness of BC production. The industrial waste could be utilized as a substrate for producing a good quality BC because the residues still contain carbon and nitrogen[2,5]. Some of them have been proven as beneficial carbon sources for BC production, such as waste beer yeast[6], dry oil mill residue[3], by-product streams from the biodiesel industry and waste streams from confectionery industries[4], fruits waste[7], coconut water and pineapple waste[8], crude glycerol from biodiesel production processes, grape bagasse[9], wastewater of candied jujube[10] and Acetone Butanol Ethanol (ABE) fermentation wastewater[11]. On the other hand, the use of such products serves the corresponding industry by decreasing the environmental problems associated with the disposal of waste[3].

Sago liquid waste is a sago industrial by-products derived from sago pith and starch deposition. In Indonesia, especially Southeast Sulawesi, the sago liquid waste is usually discharged into the rivers, resulted in severe contamination of the rivers. Based on the study by Phang *et al.*[12], sago liquid waste contains a very high carbon to nitrogen ratio (105:0.12) and it is acidic, so it has been made more suitable for fermentation acetic acid bacteria such as Acetobacter xylinum[13]. Acetobacter xylinum can convert sugar in the medium into a cellulose gel commonly known as bacterial cellulose[14]. Yanti *et al.*[15] have been evaluated the inoculum size and incubation period for the production of BC using sago liquid waste as a substrate. However, its characteristics are unknown. This study evaluates bacterial cellulose production from sago liquid waste. Furthermore, the bacterial cellulose's mechanical and morphological properties produced from sago liquid waste were determined.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biology, Microbiology Laboratory, Halu Oleo University, Indonesia from March-September, 2018.

Bacteria and culture media: *Acetobacter xylinum* strain LKN6 used in this study was obtained from pineapple waste[13]. A starter medium was made using coconut water and media pH was adjusted using glacial acetic acid to 4.5-5 and sterilized. The bacterial cellulose production media was made using sago liquid waste, with the treatment of sugar concentration, namely without added sugar/sugar-free (0%) and 5, 10 and 15% (w/v), which is added to the medium.

Production of bacterial cellulose: All fermentations were conducted under sterile conditions in a glass containers with the following dimension: diameter (d) = 6 cm and height (h) = 12 cm and surface area (a) = 56.4 cm^2, with 100 mL working volume at room temperature (28-30°C). The inoculum volume was 10% (v/v) and the broth's pH value was adjusted to 5.0 using glacial acetic acid. The flasks were incubated statically until the end of the fermentation (14 days). The BC production using sago liquid waste as fermentation media and the effect of different sugar concentrations was evaluated. Each treatment was conducted in triplicate.

Determination of the yield of bacterial cellulose production: The yield of BC was determined based on the thickness of the cellulose pellicle and BC concentration. The bacterial cellulose produced during the fermentation was measured at the end of each run. The bacterial cellulose was typically washed with water to remove any residual sugar. The pellicle was then boiled in 2% NaOH solution for 1 hr. The cellulose was washed with deionized water until the remaining base was removed. The thickness of the bacterial cellulose pellicle was measured with a hand-held micrometer screw gauge at five different positions and values were averaged. The dry weight was measured after drying the pellicle for three days at 45°C. The dried bacterial cellulose samples were weighed and values were converted to g L^{-1} of the medium to determine BC concentration.

Characterization of bacterial cellulose samples: Bacterial cellulose samples obtained in the sago liquid waste media were characterized in the water holding capacity and

mechanical properties. BC morphology characterization was carried out using Scanning Electron Microscopy (SEM) and conformation characteristic was analyzed by FT-IR spectrometer.

Water holding capacity: The water holding capacity of the samples was measured using the method described by Tsouko *et al.*[4]. The water holding capacity was calculated using the following Eq.[4]:

$$\text{Water holding capacity} = \frac{\text{Mass of water removed during drying (g)}}{\text{Dry weight of bacterial cellulose (g)}}$$

Mechanical characterization: The mechanical properties of BC were determined by ultimate tensile strength, elongation at break and Young's modulus. The mechanical properties were measured using Universal Testing Machine based on ASTM D638. The thickness of each specimen to the nearest 0.025 mm. Tensile strength and Elongation at break were determined directly from the stress-strain curves and Young's Modulus was calculated as the slope of an initial linear portion of this curve[16].

Scanning electron microscopy (SEM): The surface and morphology of BCs were studied by Scanning Electron Microscopy (Tescan Vega III Easyprobe, Brno, Czech Republic). The BC pellicle dried samples were coated with gold and examined at an accelerated voltage of 5 kV and magnification of 20 k.

Fourier transformed infrared (FT-IR) spectroscopy: The cellulose pellicles, harvested on the fourteenth days of fermentation were rinsed 3 times with distilled water, cut into slices and were dried. The cellulose pellicle dried samples were recorded using FTIR Spectra 2000 (Perkin Elmer, Waltham, Massachusetts, US) at room temperature. The spectra were recorded between 4000-400 cm^{-1} wavelength.

RESULTS

Bacterial cellulose production in sago liquid waste: Production of BC was evaluated based on the thickness of cellulose pellicles and the yield of BC formed in sago liquid waste media. BC pellicle was formed on the surface of the media. The formation of the BC pellicle is shown in Fig. 1.

The time profile of BC production is given in Fig. 2, showed a similar pattern of thickness and yield of BC.

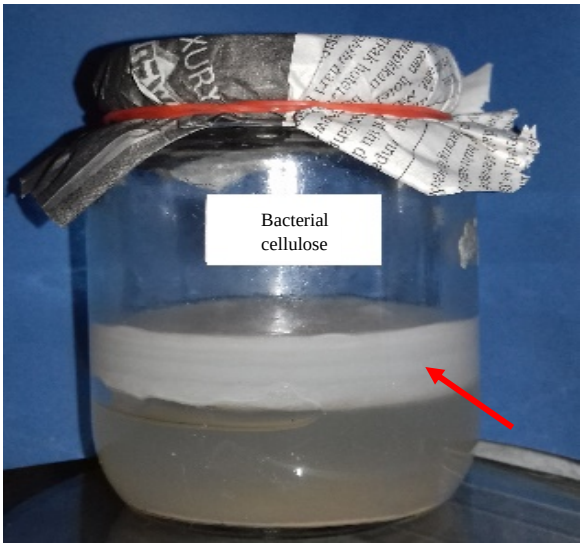

Fig. 1: Formation of bacterial cellulose (BC) pellicle on the surface of sago liquid waste medium after 14th days fermentation
The arrow showed the BC pellicle

The data in Fig. 2 shows that BC pellicle formed at 2nd-day fermentation and reaches a maximum yield by the 14th day, after that, the production of BC is static. The result of Fig. 2 also shows that the pellicle BC thickness increased in parallel with the concentration of BC production under static conditions.

Formation of bacterial cellulose from sago liquid waste by *Acetobacter xylinum* LKN6: SEM analysis of the formation of bacterial cellulose from sago liquid waste after 3, 5, 7 and 14 days fermentation, was performed on bacterial cellulose dried sheets (Fig. 3). The data in Fig. 3 showed that the viable cell of *A. xylinum* LKN6 was embedded in the bacterial cellulose pellicles.

The formation of bacterial cellulose began with the synthesis of precellulosic polymer molecules in cells, which are then released to the cell surface and formed a layer of polysaccharides that surround the cell wall (Fig. 3a). Cellulose is released from cells in the form of protofibrils that form inter-cell interwoven and several protofibrils combine to form microfibrils (Fig. 3b) until finally, cellulose fibrils form fibrilar bands that make up the cellulose pellicle and bacterial cells trapped inside it (Fig. 3c-d).

Evaluation of different sugar concentration for bacterial cellulose production: In general, the wastewater contains high carbon sources but is dominated by polysaccharides, so

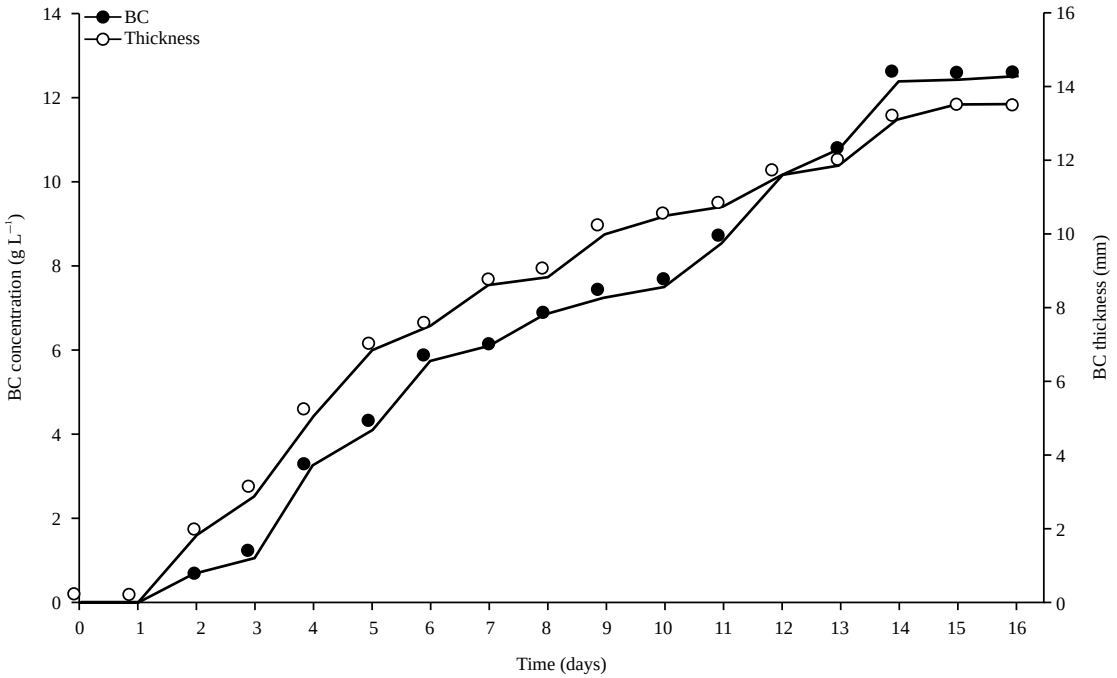

Fig. 2: Time profile of BC production in sago liquid waste media

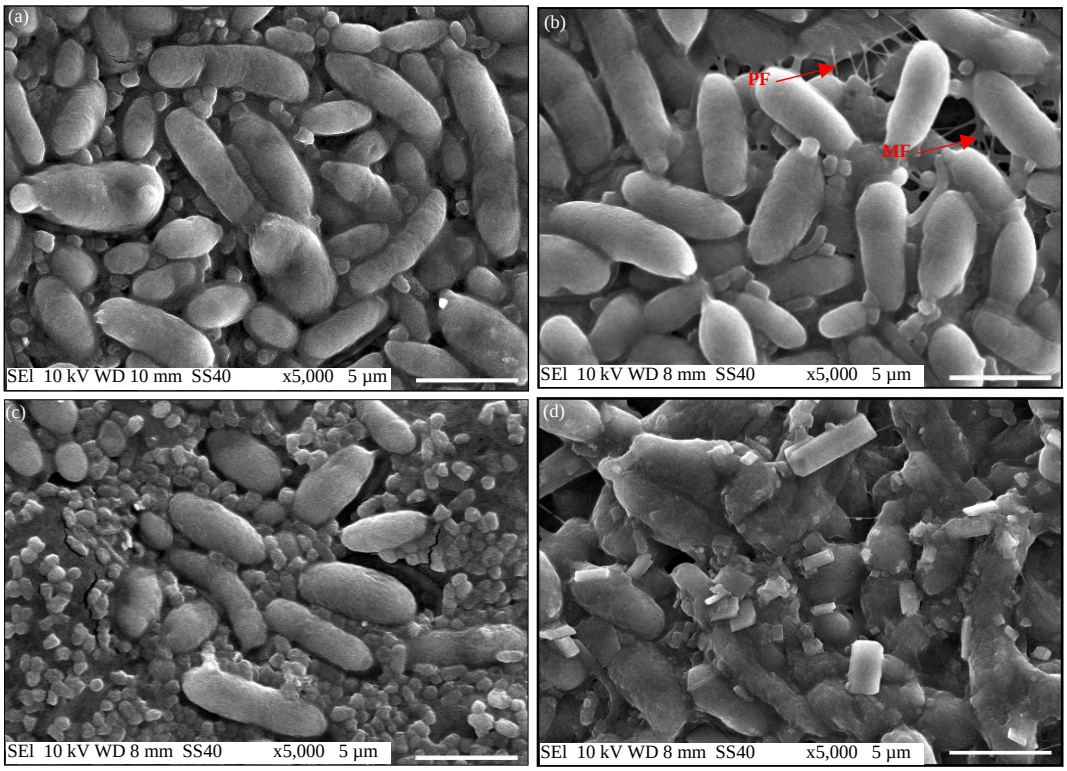

Fig. 3(a-d): Formation of bacterial cellulose by *Acetobacter xylinum* LKN6 using sago liquid waste media at (a) 3 days, (b) 5 days, (c) 7 days and (d) 14 days

PF: Protofibril, MF: Microfibril

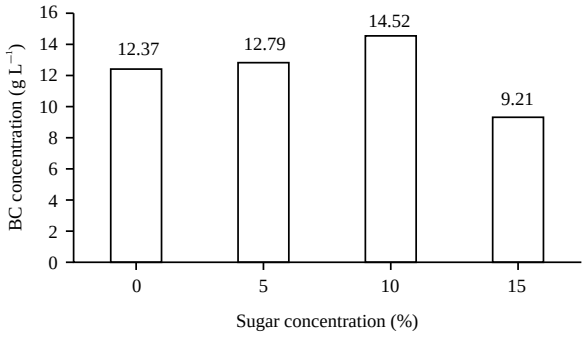

Fig. 4: Bacterial cellulose production achieved when various sugar concentration in sago liquid waste medium for 14 days of fermentation

that the production of bacterial cellulose using wastewater as a substrate was needed the addition of commercial sugar (sucrose) as the simple carbon source. Hence, the subsequent experiments focused on the evaluation of the sugar concentration on sago liquid waste substrate for BC production. The result in Fig. 4 showed that the highest BC yield was obtained on the sago liquid waste substrate with 10% sugar added (14.52 g L^{-1}), while the lowest was obtained on the substrate with a sugar concentration of 15% (9.21 g L^{-1}).

The result of Table 1 presents the BC production achieved in literature-cited publications when various wastewaters were used. In this study, the BC concentration achieved was in the range of 9.2-14.5 g L^{-1}. The highest BC concentration of 14.5 g L^{-1} was achieved when the bacterial strain *Acetobacter xylinum* LKN6 was cultivated on sago liquid waste with an added sugar concentration of 10% (w/v). In this study, also known that BC can be produced from sago liquid waste without adding sugar with BC concentration as much as 12.37 g L^{-1}. Comparison of BC production from sago liquid waste with BC from various waste according to literature in Table 1 showed that BC production from sago liquid waste is lower than BC from coconut water but higher than BC production from a few wastes. This result indicates that sago liquid waste is potential as a substrate for BC production.

Characteristics of bacterial cellulose from sago liquid waste

Morphology characteristics of bacterial cellulose by SEM: Observation of the bacterial cellulose dried sheet in Fig. 5 showed the bacterial cellulose formed ribbons of fibrils cellulose. The ribbons appear as layers of cross cellulose ribbons arranged randomly.

The result in Fig. 5a-d showed that in all treatments, the BC membranes comprised a network of nanoribbons; however, differences in the size of fibril and porosity were observed. BC membrane from sago liquid waste with sugar concentration 10% (Fig. 5c), was achieved a greater microfibril and smoother compared to the treatments of sugar concentration 0% (Fig. 5a), 5% (Fig. 5b) and 15% (Fig. 5d). According to the surface morphology in Fig. 5b, it can be seen that BC from 5% sugar showed a relatively less dense and more porous structure. On the other hand, BC from sago liquid waste media with 10% sugar (Fig. 5c) seems to be formed by a more dense and multi-layered fibril network.

FT-IR of bacterial cellulose: Fourier transform infrared (FT-IR) spectra of the BC sample was taken to detect any peak shift that could be attributed to BC produced in sago liquid waste substrate and compared with that of pure cellulose powder (micro granular cellulose powder from SIGMA) of high purity grade reagents. The FTIR spectra of bacterial cellulose (BC) and pure cellulose showed in Fig. 6.

The FT-IR spectra' band position for pure cellulose and BC sample was in the same range and did not show any significant difference in Fig. 6. For the pure cellulose spectrum, distinguish peaks of 3388 cm^{-1} and shouldering around 3700-3200 cm^{-1} indicates O-H stretching, 3000-2850 cm^{-1} indicates C-H stretching, 1450-1375 cm^{-1} indicates bending C-H, 1300-1000 cm^{-1} indicates C-O-C stretching and 1035-1060 cm^{-1} indicates C-O stretching. The analysis of bacterial cellulose produced from sago liquid waste on Fig. 6 showed peaks at 3412, 2924, 1427, 1114 and 1058 cm^{-1}, that correspond to O-H stretching, C-H stretching, C-H bending, C-O-C stretching and C-O stretching, respectively and represent the fingerprints of pure cellulose, thus confirming the purity of the cellulose produced.

Characteristics of mechanical and physical of Bacterial Cellulose: In the following sections, the properties of the mechanical and physical BC produced from sago liquid waste with different sugar concentrations were determined in Table 2.

Most of the studies concerning the mechanical properties of BC are carried out using hydrated pellicles to determine their potential application as a plastic raw material. In the present study, the mechanical properties of bacterial cellulose films were evaluated are tensile strength, elongation at break and Young's modulus. Tensile strength is the maximum tensile stress sustained by the sample during the tension test.

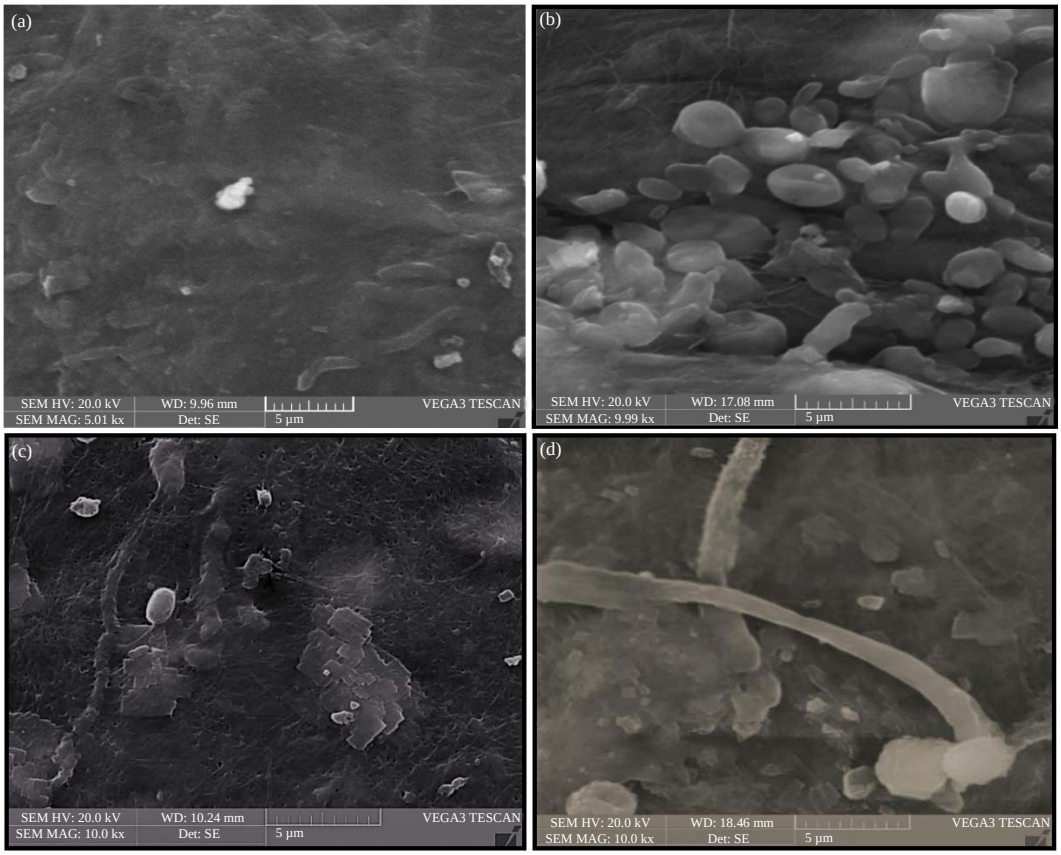

Fig. 5(a-d): SEM images of bacterial cellulose (BC) dried sheets were produced by *A. xylinum* LKN6 from sago liquid waste with different sugar concentrations, (a) BC with sugar-free (0%) (BC_0), (b) BC with sugar 5% (BC_5), (c) BC with sugar 10% (BC_{10}) and (d) BC with sugar 15% (BC_{15})
All micrographs correspond to 20k magnification

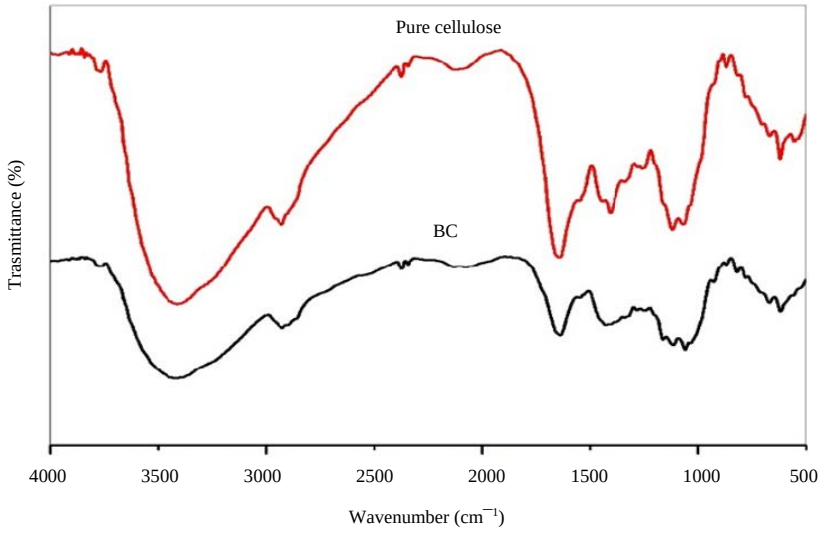

Fig. 6: FTIR spectra of pure cellulose and bacterial cellulose (BC) from sago liquid waste

Table 1: Comparison of BC production using sago liquid waste and BC production using various wastes based on literature-cited publications

Bacterial strains	Fermentation mode	Carbon source	BC (g L^{-1})	References
Acetobacter xylinum LKN6	Static batch fermentations	Sago liquid waste	9.2-14.5	The present study
Komagataeibacter sucrofermentans DSM 15973	Static batch fermentations	confectionery industry waste	13	Tsouko *et al.*[4]
Gluconacetobacter hansenii CGMCC 3917	Static batch fermentations	Waste beer yeast hydrolysates	7	Lin *et al.*[6]
Acetobacter xylinum	Static batch fermentations	Coconut water	50	Lestari *et al.*[8]
Gluconacetobacter xylinum CGMCC 2955	Static batch fermentations	Wastewater of candied jujube processing industry	2.2	Li *et al.*[10]
Gluconacetobacter xylinus CH001	Static batch fermentations	Acetone-butanol-ethanol fermentation wastewater	1.3	Huang *et al.*[11]
Gluconacetobacter xylinus ATCC 23770	Static batch fermentations	Waste fiber sludge	11	Chavka *et al.*[17]
Gluconacetobacter medellinensis	Static batch fermentations	Pineapple waste and sugar cane juice	4	Algar *et al.*[18]
Gluconacetobacter xylinus ATCC 10245	Static batch fermentations	Beet molasses	2	Keshk *et al.*[19]

Table 2: Mechanical and physical properties of bacterial cellulose fiber from sago liquid waste with different sugar concentration

Properties	BC_0	BC_5	BC_{10}	BC_{15}
Tensile strength (σ)] (MPa)	48.4	51.7	87.3	44.2
Elongation at break (ε) (%)	4.8	5.8	5.3	5.1
Young's modulus (E) (GPa)	1.01	0.89	1.64	0.86
Water holding capacity (WHC) (g water/g dry weight BC)	98.6	94.8	85.9	92.5

Elongation at break is the ratio between changed length and initial length after breakage of the test specimen, while Young's modulus a measure of a solid's stiffness or resistance to elastic deformation under load. These parameters help to determine the flexibility and stretchability of films. The desired flexibility of a biopolymer depends on its intended application. According to the obtained results in Table 2, tensile strength, elongation at break and Young's modulus ranged from 44.2-87.3 MPa, 4.8-5.8% and 0.86-1.64 GPa, respectively. BC_{10} showed the highest tensile strength (87.3 MPa) followed by BC_5 (51.7 MPa), BC_0 (48.4 MPa) and BC_{15} (44.2 MPa) (Table 2).

The water holding capacity (WHC) is considered one of the most important physical characteristics of bacterial cellulose pellicles regarding the bioplastic application of BC as food dressing material. The WHC of the different BC produced by different sugar concentrations in sago liquid waste media was shown in Table 2. The WHC of BC from sago liquid waste with sugar-free (BC_0) is the highest (98.6 g g^{-1}) of all BC samples followed by BC with a concentration of sugar 5% (BC_5) (94.8 g g^{-1}), BC with 15% sugar (BC_{15}) (92.5 g g^{-1}) and BC with 10% sugar (BC_{10}) (85.9 g g^{-1}).

DISCUSSION

In the present study, sago liquid waste can be used as a source of nutrients for BC production. The BC production from sago liquid waste can increase with the addition of sugar (commercial sucrose) as a simple carbon source in the medium. However, BC production decreased if sugar was added as much as 15% (Fig. 4). This result was supported by Esa *et al.*[1] which stated that the utilization of simple carbon sources such as sucrose into medium fermentation is commonly found in high-yielding BC, however, the excess of carbon source in the medium ultimately decreases the production of BC. The result indicated that the addition of commercial sucrose into sago liquid waste substrate can enhance BC production. Tsouka *et al.*[4] also reported that sucrose is the best carbon source to use on BC production compared with glucose, fructose, xylose and lactose. The production of bacterial cellulose under static conditions is shown by the forming of a pellicle on the medium surface (Fig. 1) and the yield depends on the carbon source concentration moderately[14]. The increasing growth time will increase the formation of the BC pellicle[20].

The formation of bacterial cellulose was described by Esa *et al.*[1] and Villareal-Soto *et al.*[21]. Esa *et al.*[1] stated that the bacterial cellulose formed ribbons of fibrils cellulose with the ribbons' dimensions are 3-4 nm thick and 70-80 nm wide. The compact cellulose network structure consisted of a random assembly of fibrils[22,23]. The cellulose chains and their assembly outside the cells and these discrete structures of the lipopolysaccharide layer are presumed to be extrusion sites for precellulosic polymers[1,14]. The precellulosic polymer molecules synthesized in the interior of the bacterial cell are spun out of the cellulose export components to form a protofibril of approx. About 2-4 nm diameter and the protofibrils are bundled in the form of a ribbon-shaped microfibril of approximately 80.4 nm[1]. The development of the BC along with hydrogen and C-H bonding will continue through all fermentation, its synthesis will reach its limit when all bacteria are trapped and the oxygen supply insufficient[1,24,25]. The addition of sugar on media affected the cellulose fibrils arrangement at the microscopic level and it could influence the structure of the cellulose membrane. Jagannath *et al.*[26] also reported that the added sugar affected the arrangement of cellulose fibril.

Bacterial cellulose from sago liquid waste has high-level purity. FTIR curve of BC from sago liquid waste showed a pattern similar to the pure cellulose and represent the fingerprints of the pure cellulose component. The curve of peaks of bacterial cellulose obtained from sago liquid waste also have similar shapes with the shape of the curve of BC had been produced from waste beer fermentation by *Gluconacetobacter hansenii*[27] and nata de coco was synthesized by *Acetobacter xylinum*[25]. BC from sago liquid waste is very potential to be used as a source of cellulose for various industrial applications because of its high purity[28].

The mechanical properties of BC from sago liquid waste are influenced by the sugar concentration in the production medium. The result indicated that the increased concentration of sugar in the production medium can improve the mechanical properties of BC. However, excess sugar content in the media can reduce its mechanical properties. Betlej *et al.*[29] also reported that an increase in the amount of sucrose in the growth medium causes the strength of the biopolymer to increase and the best mechanical properties were obtained for bacterial cellulose from cultures with the addition of 10% sucrose. The range of BC tensile strength obtained in this study is almost the same as the BC tensile strength produced from several types of substrates. George *et al.*[30] reported a tensile strength of 43.68 MPa of the BC produced by *Acetobacter xylinum* using a medium rich in sucrose and yeast extract. In another study, the tensile strength of BC was produced by *Gluconacetobacter hansenii* reached 76.7 MPa when cultivated in a medium consisting of glucose, yeast extract and peptone[22], while the tensile strength of BC produced by *Acetobacter xylinum* grown on coconut water medium was reported to be 84.1 MPa[31].

Differences in morphology between the BC samples are related to their mechanical properties, as can be observed by Scanning Electron Microscopy (SEM) micrographs (Fig. 5). For example, BC from sago liquid waste with 10% sugar (BC_{10}) has the highest tensile strength compared to the other BC, is characterized by a more organized fibril network and a tighter braid of fibrils compared to the other BC samples. Furthermore, several factors affect the mechanical properties of bacterial cellulose films, such as the incubation period, failure stress under uniaxial or biaxial tension[32,33], the drying technique applied[34], the pressure applied to the pellicle before drying[35] and the concentration of sugar in the medium for BC production[29]. The mechanical properties of BC are very supportive of BC applications as a plastic raw material[36-38]. The mechanical properties of the BC pellicles produced using sago liquid waste medium indicate possible applications of this biomaterial as bioplastics. The tensile strength of BC from sago liquid waste ranged from 44-87 MPa, including in the tensile strength range of polypropylene (conventional plastic), i.e., 24.7-302 MPa[39] and higher than the tensile strength of low-density polyethylene (16-18 MPa)[40]. This indicates that the BC of sago liquid waste could potentially be used as a raw material of plastic.

The morphology of the BC surface also is related to the Water Holding Capacity (WHC). The thinner and longer fibrils BC, the greater the amount of water trapped in the matrix of BC, so WHC was also high. As revealed from SEM morphology in Fig. 5, BC was produced from sago liquid waste with 10% sugar (BC_{10}), its fibrils are thicker in size as compared to other BC fibrils. Therefore, the WHC for BC_{10} (BC with 10% sugar) is lower than the other BC. The fibrils of BC_0, BC_5 and BC_{15} are thinner than BC_{10} (Fig. 5) and having a large surface area, thus holding more water as compared to the BC_{10} sample. Shezad *et al.*[35] reported that BC samples with thinner and longer fibrils have high water holding capacity. Furthermore, the high WHC of BC may be due to thinner and more crowded fibrils[27]. The WHC of the BCs produced in this study are in good agreement with literature-cited publications[35] and sometimes even lower[4].

The results of this study indicate that bacterial cellulose produced from sago liquid waste potential to be applied as plastic. However, further depth study is needed to improve the mechanical characteristics of bacterial cellulose for use as a plastic.

CONCLUSION

The present study showed that sago liquid waste could be used as the source of the sole nutrients for the production of bacterial cellulose. The bacterial cellulose from sago liquid waste can produce a high yield when added with sugar as much as 10% (w/v) in the medium. The properties of BC from sago liquid waste have the potential to be applied as plastics as it has high mechanical strength and purity.

SIGNIFICANCE STATEMENT

This study discovers the bacterial cellulose was produced using sago liquid waste that potential to use as plastic. This study will help the researcher to uncover the formation of bacterial cellulose from sago liquid waste and its

physical-mechanical properties. Therefore, a new finding on this bacterial cellulose may be exploited to develop a bioplastic.

ACKNOWLEDGMENTS

The authors were very grateful to the Ministry of Research, Technology and Higher Education, Government of Indonesia, for financially supported by applied research grant 2018 with grant number 486/UN29.20/PPM/2018.

REFERENCES

1. Esa, F., S.M. Tasirin and N.A. Rahman, 2014. Overview of bacterial cellulose production and application. Agric. Sci. Procedia, 2: 113-119.
2. Zhao, H., J. Li and K. Zhu, 2018. Bacterial cellulose production from waste products and fermentation conditions optimization. IOP Conf. Ser.: Mater. Sci. Eng., Vol. 394. 10.1088/1757-899X/394/2/022041.
3. Gomes, F.P., N.H.C.S. Silva, E. Trovatti and L.S. Serafim, 2013. Production of bacterial cellulose by *Gluconacetobacter sacchari* using dry olive mill residue. Biomass. Bioenerg., 55: 205-211.
4. Tsouko, E., C. Kourmentza, D. Ladakis, N. Kopsahelis and J. Mandala *et al*., 2015. Bacterial cellulose production from industrial waste and by-product streams. Int. J. Mol. Sci., 16: 14832-14849.
5. Kongruang, S., 2008. Bacterial cellulose production by *Acetobacter xylinum* strains from agricultural waste products. Appl. Biochem. Biotechnol., 148: 245-256.
6. Lin, D., P. Lopez-Sanchez, R. Li and Z. Li, 2014. Production of bacterial cellulose by *Gluconacetobacter hansenii* CGMCC 3917 using only waste beer yeast as nutrient source. Bioresour. Technol., 151: 113-119.
7. Kosseva, M.R., M. Li, J. Zhang, Y. He and N.A. Tjutju, 2017. Study on bacterial cellulose production from fruit juices. Proc. Int. Conf. BioSci. Biotechnol., 2 : 36-42.
8. Lestari, P., N. Elfrida, A. Suryani and Y. Suryadi, 2014. Study on the production of bacterial cellulose from *Acetobacter xylinum* using agro-waste. Jordan J. Biol. Sci., 7: 75-80.
9. Vazquez, A., M.L. Foresti, P. Cerrutti and M. Galvagno, 2013. Bacterial cellulose from simple and low cost production media by *Gluconacetobacter xylinus*. J. Polym. Environ., 21: 545-554.
10. Li, Z., L. Wang, J. Hua, S. Jia, J. Zhang and H. Liu, 2015. Production of nanobacterial cellulose from waste water of candied jujube-processing industry using *Acetobacter xylinum*. Carbohydr. Polym., 120: 115-119.
11. Huang, C., X.Y. Yang, L. Xiong, H.J. Guo and B. Luo *et al*., 2015. Evaluating the possibility of using acetone-butanol-ethanol (ABE) fermentation wastewater for bacterial cellulose production by *Gluconacetobacter xylinus*. Lett. Appl. Microbiol., 60: 491-496.
12. Phang, S.M., M.S. Miah, B.G. Yeoh and M.A. Hashim, 2000. Spirulina cultivation in digested sago factory wastewater. J. Applied Phycol., 12: 395-400.
13. Yanti, N.A., S.W. Ahmad, S. Ambardini, N.H. Muhiddin and L.O.I. Sulaiman, 2017. Screening of acetic acid bacteria from pineapple waste for bacterial cellulose production using sago liquid waste. Biosaintifika, 9: 387-393.
14. Gorgieva, S. and G. Trcak, 2019. Bacterial cellulose: Production, modification and perspectives in biomedical applications. Nanomaterials, 9: 1352-1372.
15. Yanti, N.A., S.W. Ahmad and N.H. Muhiddin, 2018. Evaluation of inoculum size and fermentation period for bacterial cellulose production from sago liquid waste. J. Phys.: Conf. Ser., 10.1088/1742-6596/1116/5/052076.
16. Raheem, Z., 2019. Standard test method for tensile properties of plastics 1. http://www.dept.aoe.vt.edu/~aborgolt/aoe3054/manual/expt5/D638.38935.pdf.
17. Chavka, A., X. Guo, S.J. Tang, S. Winestrand, L.J. Johnson and F. Hong, 2013. Production of bacterial cellulose and enzyme from waste fiber sludge. Biotechnol. Biofuels., Vol. 6. 10.1186/1754-6834-6-25.
18. Algar, I., S.C.M. Fernandes, G. Mondragon, C. Castro and C. Garcia-Astrain *et al*., 2015. Pineapple agroindustrial residues for the production of high value bacterial cellulose with different morphologies. J. Appl. Polym. Sci., Vol. 132. 10.1002/app.41237.
19. Keshk, S.M.A.S., T.M.A. Razek and K. Sameshima, 2006. Bacterial cellulose production from beet molasses. Afr. J. Biotechnol., 5: 1519-1523.
20. Sheykhnazaria, S., T. Tabarsaa, A. Ashorib, A. Shakeric and M. Golalipourd, 2011. Bacterial synthesized cellulose nanofibers; effects of growth times and culture mediums on the structural characteristics. Carbohydr. Polym., 86: 1187-1191.
21. Villarreal-Soto, S.A., S. Beaufort, J. Bouajila, J.P. Souchard and P. Taillandier, 2018. Understanding kombucha tea fermentation: A review. J. Food. Sci., 83: 580-588.
22. Costa, A.F.S., F.C.G. Almeida, G.M. Vinhas and L.A. Sarubbo, 2017. Production of bacterial cellulose by *Gluconacetobacter hansenii* using corn steep liquor as nutrient sources. Front Microbiol., Vol. 8. 10.3389/fmicb.2017.02027.
23. Norouzian, D., A. Farhangi, S. Tolooei, Z. Saffari and M.R. Mehrabi *et al*., 2011. Study of nano-fiber cellulose production by *Glucanacetobacter xylinum* ATCC 10245. Pak. J. Biol. Sci., 14: 780-784.

24. Borzani, W. and S.J. Souza, 1995. Mechanism of the film thickness increasing during the bacterial production of cellulose on non-agitated liquid media. Biotechnol. Let., 17: 1271-1272.
25. Halib, N., M.C. Iqbal, M. Amin and I. Ahmad, 2012. Physicochemical properties and characterization of Nata de Coco from local food industries as a source of cellulose. Sains Malays, 41: 205-211.
26. Jagannath, A., A. Kalaiselvan, S.S. Manjunatha, P. Raju and A. Bawa, 2008. The effect of pH, sucrose and ammonium sulphate concentrations on the production of bacterial cellulose (*Nata-de-coco*) by *Acetobacter xylinum*. World J. Microbiol. Biotechnol., Vol. 24. 10.1007/s11274-008-9781-8.
27. Petroudy, S.D., 2017. Physical and Mechanical Properties of Natural Fibers. In: Advanced High Strength Natural Fibre Composites in Construction, Fan, M. and F. Fu (Eds.). Woodhead Publishing, Cambridge, UK., pp: 59-83.
28. Marchessault, R.H. and P.R. Sundararajan, 1983. Cellulose, In: The Polysaccharides, Aspinal, G.O., (Ed.)., Elsevier Ltd., New York, pp: 11-95.
29. Betlej, I., R. Salerno-Kochan, K. Krajewski, J. Zawadzki and P. Boruszewski, 2020. The influence of culture medium components on the physical and mechanical properties of cellulose synthesized by kombucha microorganisms. BioResources, 15: 3125-3135.
30. George, J., K.V. Ramana, S.N. Sabapathy and A.S. Bawa, 2005. Physico-mechanical properties of chemically treated bacterial (*Acetobacter xylinum*) cellulose membrane. World J. Microb. Biot., 21: 1323-1327.
31. Indrarti, L., Indriyati, A. Syampurwadi and S. Pujiastuti, 2016. Physical and mechanical properties of modified bacterial cellulose composite films. AIP Conf. Proc., Vol. 1711. 10.1063/1.4941633.
32. Mc Kenna, B.A., D. Mikkelsen, J.B. Wehr, M.J. Gidley and N.W. Menzies, 2009. Mechanical and structural properties of native and alkali-treated bacterial cellulose produced by *Gluconacetobacter xylinus* strain ATCC 53524. Cellulose, 16: 1047-1055.
33. Pogorelova, N., E. Rogachev, I. Digel, S. Chernigova and D. Nardin, 2020. Bacterial cellulose nanocomposites: Morphology and mechanical properties. Materials, Vol. 13. 10.3390/ma13122849.
34. Clasen, C., B. Sultanova, T. Wilhelms, P. Heisig and W.M. Kulicke, 2006. Effects of different drying processes on the material properties of bacterial cellulose membranes. Macromol. Symp., 244: 48-58.
35. Hsieh, Y.C.. H. Yano, M. Nogi and S.J. Eichhorn, 2008. An estimation of the Young's modulus of bacterial cellulose filaments. Cellulose, 15: 507-513.
36. Shehzad, O., S. Khan, T. Khan and J.K. Park, 2010. Physicochemical and mechanical properties characterization of bacterial cellulose produced with an excellent productivity in static conditions using simple fed-batch cultivation strategy. Carbohydr. Polym., 82: 173-180.
37. Sukara, E. and R. Meliawati, 2014. Potential values of bacterial cellulose for industrial applications. Jurnal Sellulosa, 4 : 7-14.
38. Azeredo, H.M.C., H. Barud, C.S. Farinas, V.M. Vasconcellos and A.M. Claro, 2019. Bacterial cellulose as a raw material for food and food packaging applications. Front. Sustain. Food Syst., 3 : 1 -14.
39. Krochta, J.M. and C.M. Johnston, 1997. Edible and biodegradable polymers film: Challenges and opportunities. Food Technol., 51: 61-74.
40. Shit, S.C. and P.M. Shah, 2014. Edible polymers: Challenges and opportunities. J. Polymers, Vol. 2014. 10.1155/2014/427259.