Chiang Mai J. Sci. 2020; 47(1) : 16-27
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Bacterial Cellulose Production by *Komagataeibacter nataicola* TISTR 2661 by Agro-waste as a Carbon Source

Churairat Moukamnerd*, Keeratiya Ounmuang, Natthakan Konboa and Chayatip Insomphun

Faculty of Agro-Industry, Chiang Mai University, Chiang Mai, 50100, Thailand.

*Author for correspondence; e-mail: churairat.m@cmu.ac.th

Received: 24 August 2019
Revised: 5 October 2019
Accepted: 8 November 2019

Abstract

To reduce the cost of obtaining bacterial cellulose (BC) and increase the value of fruit processing waste, the use waste from banana peel (BP) and passion fruit peel (PFP) as a carbon source for the production of bacterial cellulose (BC) by *Komagataeibacter nataicola* TISTR 2661 was developed. BP and PFP were digested with commercial saccharifying enzymes and the sugar content of these aqueous extracts reached 0.57±0.01 g/g- substrate (35.20±1.60 g/L) and 0.31±0.00 g/g- substrate (15.05±0.07 g/L), respectively. After 9 days of incubation, the BC production of BP and PFP aqueous extracts *K. nataicola* TISTR 2661 were 0.89±0.04 g/L and 0.31±0.07 g/L, respectively. To enhance the BC production, the aqueous extracts of BP and PFP were supplemented with $MgSO_4$, KH_2PO_4 and $(NH_4)_2HPO_4$. The addition of KH_2PO_4 1 g/L increased the BC production yield from BP aqueous extracts by 13.85±1.18%, which is higher than the yield from Hestrin & Schramn (HS) medium of 6.13±1.50%. The BC membrane was characterised by scanning electron microscope (SEM) and fourier-transform infrared spectroscopy (FTIR), which demonstrated that the aqueous extracts from BP and PFP could be used as carbon sources for the production of BC with low value residues.

Keywords: waste utilization, agricultural residues, waste management, biocellulose

1. Introduction

Bacterial cellulose or biocellulose (BC) is a biopolymer that can be produced by bacteria such as *Gluconacetobacter, Sarcina* and *Agrobacterium* [1]. Among these genera, *Komagataeibacter* (formerly *Gluconacetobacter*) species is well-known as BC producers, which have been used for industrial BC production scale [2]. BC has advantages over cellulose derived from plant sources since it does not contain lignin and hemicellulose. BC displays unique properties such as higher purity, crystallinity, water absorption and tensile strength, a low degree of polymerisation and a stronger biological adaptability than natural plant cellulose [3]. Therefore, it could be exploited in numerous important sectors such as the food, electronic (electrical conductivity, redox flow battery, etc.), and biomedical (nanofilm, antimicrobial would dressing, etc.) sectors [4]. The production cost of BC is very high due to the expensive culture media, which requires the addition of glucose as a carbon source and other nutrient sources [5]. To reduce the production cost of BC, inexpensive carbon sources such as cashew tree residues [6], dry olive mill residue [7], fruit juice [8], waste

water from the candy processing industry [9], rice bark [10], and tobacco waste [11] have been investigated.

The value of fruit exports increases every year in Thailand. The growth has focused on the fruit processing industries, which significantly contribute to the Thai economy. In 2009, 87.4 tons of processed banana (chips, flours, dried pulps, and jam) was exported and there is a trend to increase every year [12] Significant quantities of banana peel, equivalent to 40% of the total weight of fresh bananas, are available and contain starch (39.3% dry weight), potassium, and calcium [13]. Similarly, more than 8.1 tons of passion fruit was produced in 2016 [14]. Approximately 60% of the by-product of passion fruit juice processing is peel, which is separated from the pulp by making the fruits explode, and the peel contains high levels of cellulose and other nutrient such as calcium, sodium, and phosphorus. Due to the increasing demand of the banana and passion fruit industries, a lot of waste is produced which could cause environmental problems unless it is converted into a useful product or properly disposed of. However, there is little information on their use since the applications of these peels depend on their chemical compositions. Some reports in the literature deal with different practical applications of banana peels, e.g. in the production of alcohol [15], methane [16], food for livestock [17], or adsorbents for water purification [18].

To increase the value of banana peels and passion fruit peels, the potential utilisation of these residues as carbon sources for BC production in static conditions was evaluated. The use of these raw materials could reduce the production cost of BC and increase the value of residue from fruit processing industries. The results obtained in this study have significance for the future production of BC. This might lead to a major new industry, when large-scale BC fermentation using renewable resources becomes reality.

2. MATERIALS AND METHODS

2.1 Culture Media and Conditions

The organism used was a wild type strain of *Komagataeibacter nataicola* TISTR 2661, which was obtained from the Division of Agro-Industry Biotechnology, Faculty of Agro-Industry, Chiang Mai University, Thailand. The cell suspension was stored at -80°C in 15% glycerol. To activate the culture, 1 mL of stored suspension was added into 9 mL of Hestrin & Schramn (HS) medium (all in g/L in de-ionised water: glucose: 2, peptone: 0.5, yeast extract: 0.5, Na_2HPO_4: 0.27, and citric acid: 0.15) and cultivated at 30°C and 150 rpm for 24 hours. Then, the activated culture suspension was transferred to 90 mL of HS medium and cultivated at 30°C and 150 rpm for 72 hours. The absorbance of the inoculum should be between 0.50 and 0.60 for a bacterial content of 2.70×10^7 CFU/mL.

2.2 Materials and Preparation

The peels from the banana *Musa sapientum* Linn. (banana peels, BP) were collected from the market in Chiang Mai, Thailand. The peels from the passion fruit *Passiflora laurifolia* Linn. (passion fruit peels, PFP) were kindly donated by the Royal project foundation, Thailand. The BP and PFP were cut into 1×1 cm pieces and dried at 80°C for 24 h and grounded in a blender into powder (using a mesh with a pore size of 1 mm). The materials were kept in sealed bag at room temperature (30 ± 3°C).

2.3 Digested of BP and PFP

BP samples (5 g) were digested with 0.01 mL/g substrate of commercial amyloglucosidase (GA, 260,000 U/mL) and 0.20 mL/g substrate of commercial cellulase enzyme which included endoglucanase activity (2,200–2,800 carboxymethyl cellulose U/g) and beta-glucosidase activity (450–775 p-Nitrophenyl-β-D-galactoside U/g) in 100 mL of citric buffer at pH 5 following the manufacturer's instructions. PFP was digested with 0.20 mL/g substrate of commercial cellulase

enzyme, the same amount as used for BP. The mixtures were incubated at 50°C and 150 rpm for 48 hours. Then, they were centrifuged at 4000×*g* for 15 min to remove sediments. The aqueous extracts were stored at -20°C for future experiments.

2.4 BC Production using BP and PFP Aqueous Extracts

The prepared seed inoculum (10 mL of inoculum contained 10^7 CFU/mL) was transferred into an Erlenmeyer flask (250 mL) containing 90 mL of BP or PFP aqueous extract as described above. The initial pH was adjusted to pH 5 using 0.01 M NaOH. Three replicates of each condition were incubated statically at 30°C for 9 days.

2.5 BC production using BP and PFP Aqueous Extracts with Nutrient Supplementation

The BP and PFP aqueous extracts were separately supplemented with $(NH_4)_2HPO_4$, KH_2PO_4, and $MgSO_4$. The aqueous extracts would have at 1, 5, and 10 g/L of each salt, respectively. All the experiments were carried out in triplicates and the operational conditions were the same as those described above.

2.6 BC Purification

After incubation, the BC membranes were collected and rinsed with running water, treated with 0.5 M NaOH at 90°C for 30 h to remove bacteria, and then washed with deionised water several times to completely remove any alkaline residues. The purified cellulose was dried at 105°C for 12 h and weighed until a constant weight was achieved.

2.7 Chemical Analysis

Samples were collected every 12 h for 9 days of fermentation. Types of sugar derived from BP and PFP aqueous extracts were analyzed by compendium of method for food analysis (2003). Total reducing sugar was determined by 3, 5-dinitrosalicylic acid (DNS) assays [19]. The pH of the medium was also measured over the course of fermentation.

2.8 Fermentation Kinetics

The efficiency of the BC production was evaluated after 9 days of cultivation and the substrate conversion ratio (S_c), substrate consumption rate (R_s), BC production (P), BC production rate (R_p), BC production yield ($Y_{p/s}$), and water holding activity (WHC) were calculated as follows:

- Substrate conversion ratio;

$$S_c(\%) = \frac{S_i - S_f}{S_i} \times 100$$

- BC production;

$$P(g/L) = \frac{m_{BC}}{V}$$

- BC production rate;

$$R_p\ (g/L \cdot day) = \frac{m_{BC}}{V \times t}$$

- BC production yield;

$$Y_{P/S}\ (\%) = \frac{m_{BC}/V}{S_i - S_f} \times 100$$

- Water holding activity;

$$WHC = \frac{Mass\ of\ water\ removed\ during\ drying\ (g)}{Dry\ weight\ of\ cellulose\ (g)}$$

Where S_i is the initial concentration of substrate (g/L), S_f is the residual concentration of substrate (g/L), m_{BC} is the amount of BC produced (g), V is the reaction volume (L), and t is the duration of the reaction (d)

2.9 Characterisation of BC Membranes Scanning Electron Microscopy (SEM)

For scanning electron microscopy observations, the oven dried BC membrane was mounted on a copper stub using double adhesive carbon

conductive tape and coated with gold. The SEM photographs were obtained using a low vacuum scanning electron microscope (JSM-5910LV, JEOL, Japan) at 15 kV with 10,000× magnification.

2.10 Fourier-Transform Infrared Spectroscopy (FTIR)

The oven dried BC membrane was cut into 1×1 cm pieces and subjected to a Nicolet FTIR-6700 spectrometer (ThermoFisher Scientific, USA) in attenuated total reflection (ATR) with a diamond crystal. The absorbance mode FTIR data were recorded with 4 cm^{-1} resolution in the wave number region of the range 4000–400 cm^{-1} range.

2.11 X-ray Diffraction (XRD)

XRD pattern of dried BC membranes were measured with a Rigaku SmartLab X-ray diffractometer equipped with Cu Kα radiation at 40 kV and 30 mA. Data were collected in reflection mode in the 5-60° 2θ-range, at a speed of 0.06°/sec.

2.12 Statistical Analysis

Each treatment was conducted in triplicate. The obtained data were statistically analysed by a one-way analysis of variance (ANOVA) using the SPSS programme (version 23). A Duncan's multiple range test with the option of homogeneous groups ($p<0.05$) was used to determine the significance between samples.

3. Results and Discussion

3.1 BC Production and Kinetics

The BC production by *K. nataicola* TISTR 2661, isolated from nada de coco, was investigated in static culture using BP and PFP as carbon sources. The main component of BP and PFP is carbohydrate, which composed 58.28% and 73.97% (dry weight) of them, respectively (Table 1). The residues were digested with saccharifying enzymes instead of pre-treatment by acid to avoid the formation of by-products such as aliphatic acids, furan aldehydes, and phenolic compounds, which could inhibit the metabolism of *Acetobacter* sp. and consequently BC production [7, 20]. The type of selected enzyme depends on the chemical composition of the residues. BP was digested by commercial cellulase and amyloglucosidase due to its high starch content to achieve a total reducing sugar content in the aqueous extracts of 0.57±0.01 g/g substrate or 35.20±1.60 g/L. PFP was digested with commercial cellulase, and the total reducing sugar content in these aqueous extracts was 0.31 g/g substrate or 15.05±0.07 g/L. The main carbon sources present in the BP and PFP aqueous extracts were fructose and glucose, which are fermentable sugars by *Acetobacter* sp. (Table 2.) [21]. The production of reducing sugar from PFP was relatively low compared to the amount of analysed carbohydrate, due to the complex structure of the lignin and hemicellulose contained in the peel which inhibits the digestion

Table 1. The composition of banana peel powder and passion fruit peel powder.

Parameters (% dry weight)	Banana peel powder	Passion fruits peel powder
Moisture	4.48	10.89
Carbohydrate	58.28	73.97
Ash	15.05	9.06
Fat	14.65	2.44
Protein (%Nx6.25)	7.54	7.41
Energy (kcal)	395.13	347.48

Table 2. The concentration of the main carbon sources present in the BP and PFP aqueous extracts.

Main carbon sources (g/L)	Banana peel (BP)	Passion fruits peel (PFP)
Fructose	2.60	2.50
Glucose	7.80	11.10
Total sugar	10.40	13.50

of sugar by the enzyme. Naturally, lignin has much more complex structure than cellulose, since it possesses a phenolic hydroxyl group, carbonyl group, aliphatic hydroxyl group, methoxyl group, benzyl alcohol group, and some terminal aldehyde groups [22].

The BP and PFP aqueous extracts were then autoclaved and tested as carbon sources in BC production by *K. nataicola* TISTR 2661 without any additional nutrients. The BC production of the BP and PFP aqueous extracts was 0.89±0.04 g/L and 0.31±0.07 g/L, respectively, which is relatively low compared to HS medium (2.73±0.67, $p<0.05$). While the final sugars of HS, BP and PFP were reduced to 6.11±0.10, 26.53±0.82 and 10.30±1.11 g/L, respectively. The amount of BC production from BP was comparable to other studies that used the residues as the sole carbon source, as shown in Table 3. Revin et al. [23] reported that high BC production was achieved using thin stillage and whey which contain carbohydrate, salt, protein, and mineral and organic compounds. Only high-sugar fruit juice is insufficient to increase the rate of bio-cellulose production [24].

Table 3. Comparison of BC production from different residues as the sole carbon source.

Carbon source	Total sugar (g/L)	BC production (g*/L)	Incubation period (days)	References
Thin stillage	NA	6.19±0.12	3	[21]
Whey	NA	5.45±0.09	3	[21]
Dry olive mill residue	11.91	0.85±0.04	4	[6]
Orange juice	73	2.10±0.20	14	[20]
Pineapple juice	89	0.60±0.10	14	[20]
Apple juice	85	0.20±0.10	14	[20]
Japanese pear juice	62	0.60±0.10	14	[20]
Grape juice	103	0.30±0.10	14	[20]
Banana peels	35	0.89±0.04	9	This study
Passion fruit peels	15	0.31±0.07	9	This study

*Based on dry matter, NA = not available

The kinetics of BC production in HS medium, BP, and PFP were compared in Table 4. The kinetic parameters showed a difference between the substrates of HS medium, BP, and PFP. The BC production rate (R_p) of HS medium (0.30±0.07 g/L·day) was approximately three times higher than that of BP medium (0.10±0.00 g/L·day). The substrate conversion ratio (S_c) of HS medium was also higher than those of BP and PFP. Surprisingly, the production yield ($Y_{p/s}$) of BP (6.14±0.34%) and PFP (7.40±1.02%) were similar to that of HS medium (6.13±1.57%). The results indicated that most of the sugars from BP and PFP that were consumed by *K. nataicola* TISTR 2661 were used for BC production rather than to promote cell growth. The low substrate consumption rate of BP and PFP might be caused by the inhibitory effect of the phenolic compounds released during the enzymatic digestion [20] or the lack of some essential supplements [7].

Table 4. Parameters of fermentation kinetics of *K. nataicola* TISTR 2661 on BC production.

Parameters	Fermentation media		
	HS	BP	PFP
S_c (%)	54.40±1.56	25.67±2.09	28.92±7.08
P (g/L)	2.73±0.67	0.89±0.04	0.31±0.07
R_p (g/L.day)	0.30±0.07	0.10±0.00	0.04±0.01
$Y_{p/s}$ (%)	6.13±1.57	6.14±0.34	7.40±1.02

3.2 Effects of Various Nutrient Supplements on BC Production

In order to enhance the production of BC, the BP and PFP aqueous extracts were supplemented with $MgSO_4$ (Mg), KH_2PO_4 (P), and $(NH_4)_2HPO_4$ (N), so that the aqueous extracts would have 1, 5, and 10 g/L of supplementary nutrients, respectively (Figure 1). The results (Figure 1B) showed that PFP aqueous extracts supplemented with KH_2PO_4 increased BC production threefold from 0.31±0.07 g/L to 0.93±0.10 g/L by the addition of 1 g/L of KH_2PO_4, however, the production decreased with a higher concentration of KH_2PO_4. Moreover, it was found that the addition of $(NH_4)_2HPO_4$ at 5 and 10 g/L to both BP and PFP did not increase BC production but resulted in the consumption of sugar instead, as shown in Figure 1C and 1D. The decrease in the amount of BC produced when the concentration of $(NH_4)_2HPO_4$ increased could be explained by the growth of cells, which has previously been reported for the addition of nitrogen and phosphate into medium of *Gluconacetobacter xylinus* which decreased the efficiency of BC production [7, 25]. The addition of $MgSO_4$ (1, 5, and 10 g/L) to aqueous extracts showed no change in BC production, even though Mg is a macronutrient required for cellular composition and inner enzymatic reactions. The results could be explained by the report by Son et al. who demonstrated that the amount of BC produced seemed to be higher with increasing concentrations of $MgSO_4$, however, at more than 0.08% of $MgSO_4$, no additional BC was produced [25]. There are some reports revealed that further than supplemented with nutrient, added of other carbon source (e.g. ethanol) could effective enhance yield of BC [26].

Considering the BC production yield (Figure 1E and 1F) from BP and PFP aqueous extracts, it was found that when no nutrients were added, the BC production yield was equivalent to that of HS medium. The addition of 1 g/L

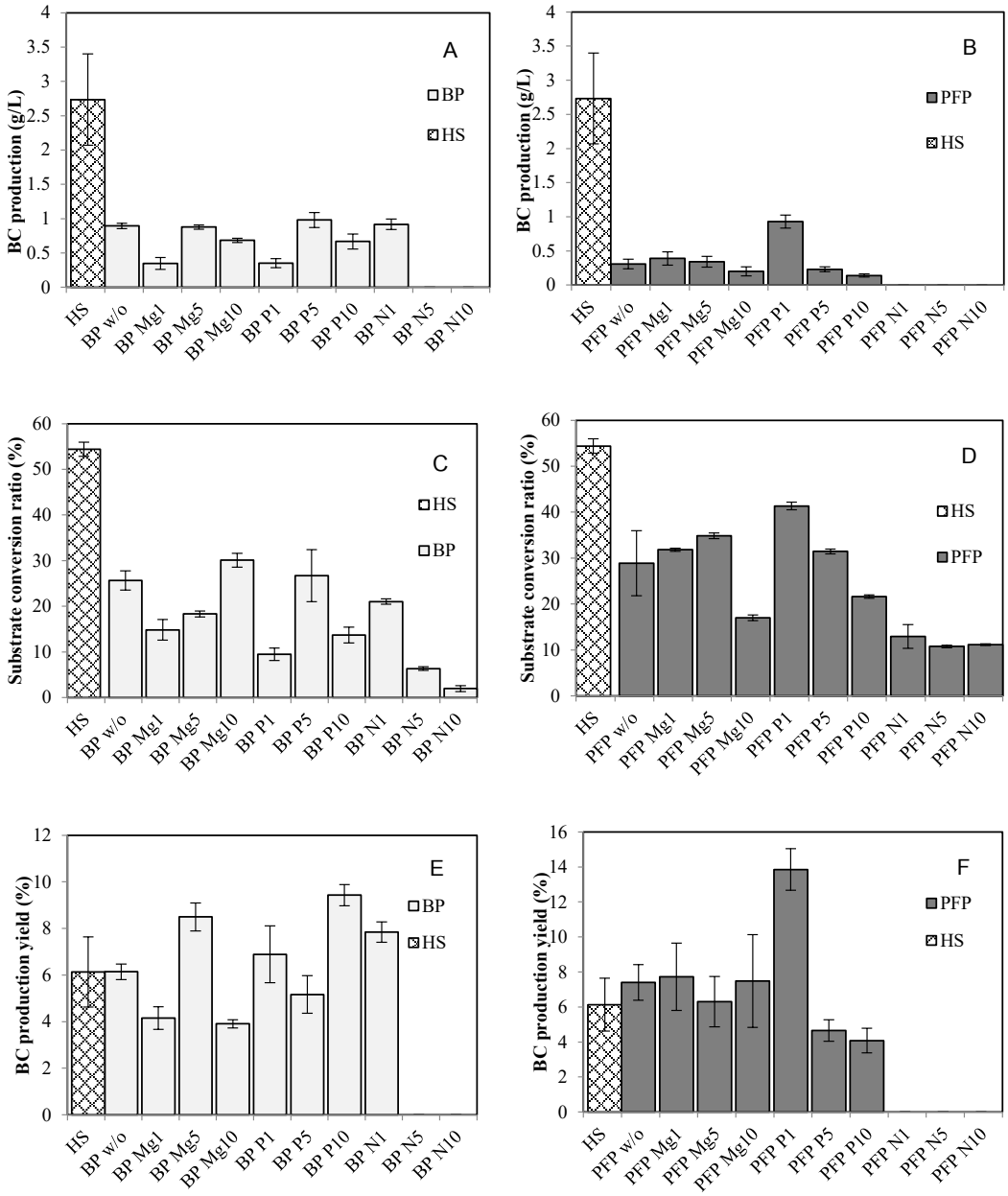

Figure 1. BC production (A and B), substrate conversion ratio (C and D), and BC production yield (E and F) from HS medium, banana peel (BP), and passion fruits peel (PFP) aqueous extracts with and without supplementation with $MgSO_4$ (Mg), KH_2PO_4 (P), and $(NH_4)_2HPO_4$ (N) at 1, 5, and 10 g/L for BC production after 9 days of static cultivation.

KH_2PO_4 increased the BC production yield from PFP aqueous extracts by 13.85±1.18%, which is higher than for the HS medium which was 6.13±1.50%. This suggests that this condition is optimal since almost all the sugar has been converted to BC.

3.3 Characterisation of BC Membranes

The water holding capacity (WHC) represents the weight of water held per unit weight of cellulose nanofibrils forming a reticulated structure [27]. The BC pellicles exhibited a high retention of water (Figure 2) due to the BC composed of ultrafine nanofibrils in an ultrafine network. The WHC of BC produced by PFP without any supplementation (313.97±71.66%) and BP with 1 g/L of KH_2PO_4 (274.93±58.94%) showed twofold higher WHC values than that BC obtained from HS medium (118.00±14.76%). Even the WHC in other conditions by BP and PFP aqueous extracts were comparable to that of BC obtained from HS medium, except the BC from the PFP aqueous extracts supplemented with 1 g/L of KH_2PO_4 which was relatively low compared to the others. This result might be due to the water molecules that are physically caught in the reticulated nanofibrils of the BC. When the BC nanofibrils are loosely arranged, there is more vacant space to trap more water molecules and thus the WHC is high [27]. The BC obtained from the PFP aqueous extracts supplemented with 1 g/L of phosphate showed a more crowed cellulose network with less free space to trap water than BC from HS medium (Figure 3A and 3C). This could explain the decreased WHC of BC from BP and PFP aqueous extracts. Detailed morphology characterization of different BC samples was carried using SEM. However, the analysis performed did not show significant different among the nanofibers obtained from the different carbon source. All the samples displayed nanofiber bundles and random assembly with 20-77 nm [6, 28].

FTIR spectroscopy of BC produced in HS medium, BP, and PFP was carried out to detect an effect of the composition of the culture media on the profile of the cellulose polymer. The FTIR spectra were similar for the different media, which indicates that the cellulose polymer has a similar chemical structure (Figure 4). The peak in the wave number region 3600–2900 cm^{-1} was characterised

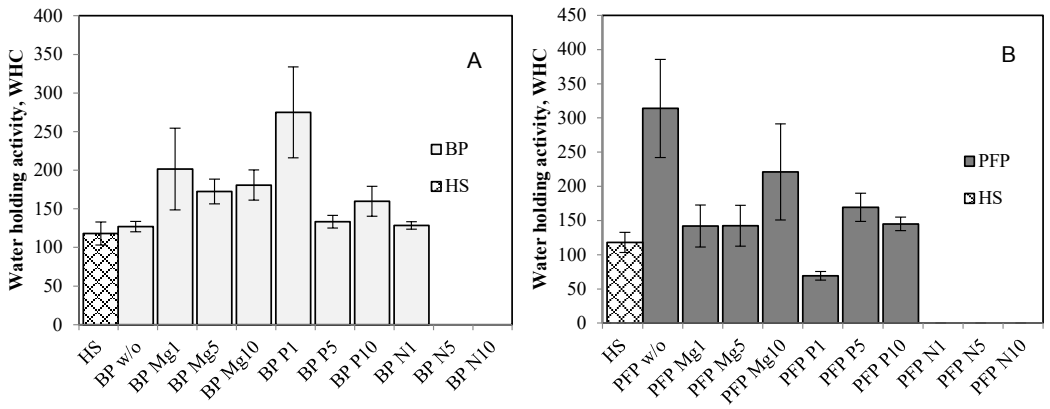

Figure 2. Water holding capacity (WHC) of BC produced from HS medium; banana peels, BP (A); and passion fruit peel, PFP (B), aqueous extracts with and without supplementation with $MgSO_4$ (Mg), KH_2PO_4 (P) and $(NH_4)_2HPO_4$ (N) at 1, 5, and 10 g/L for BC production after 9 days of static cultivation.

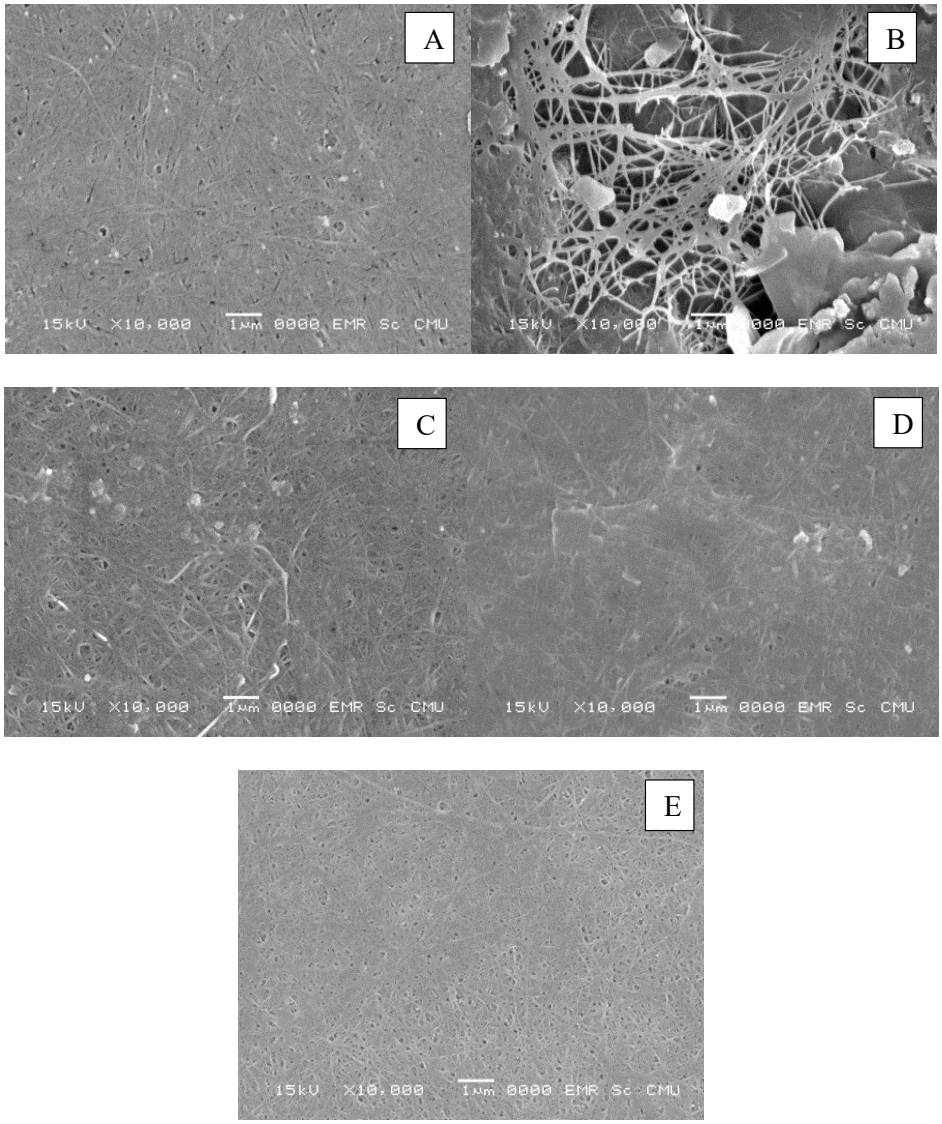

Figure 3. SEM images of bacterial cellulose produced under different conditions. HS medium (A), PFP with $MgSO_4$ (B), PFP with KH_2PO_4 (C), BP with $(NH_4)_2HPO_4$ (D), and BP with KH_2PO_4 (E) (all images with 10,000× magnification).

for the stretching vibration of the O-H and C-H bonds in the polysaccharides [23, 29]. The spectra of BC with strong bands at around 3300 cm^{-1} were characteristic of the hydroxyl group in cellulose [5]. An absorption peak around 1100 cm^{-1}, another signature band of cellulose, corresponds to the C-O-C and C-O bonds of the monomer units of the polysaccharide [7]. Figure 5 showed the XRD patterns of BC films produced from HS, BP and PFP medium. The range of main peaks for BC film were around 14.5-15.3°, 16.0-17.0° and 22.0-23.0°. These peaks were corresponded to XRD for pure cellulose which showed the peaks at 14.7°, 16.6° and 22.5° by Ford et al. [30]. Therefore, this set of diffraction planes corresponds to the crystalline native cellulose I. The influence

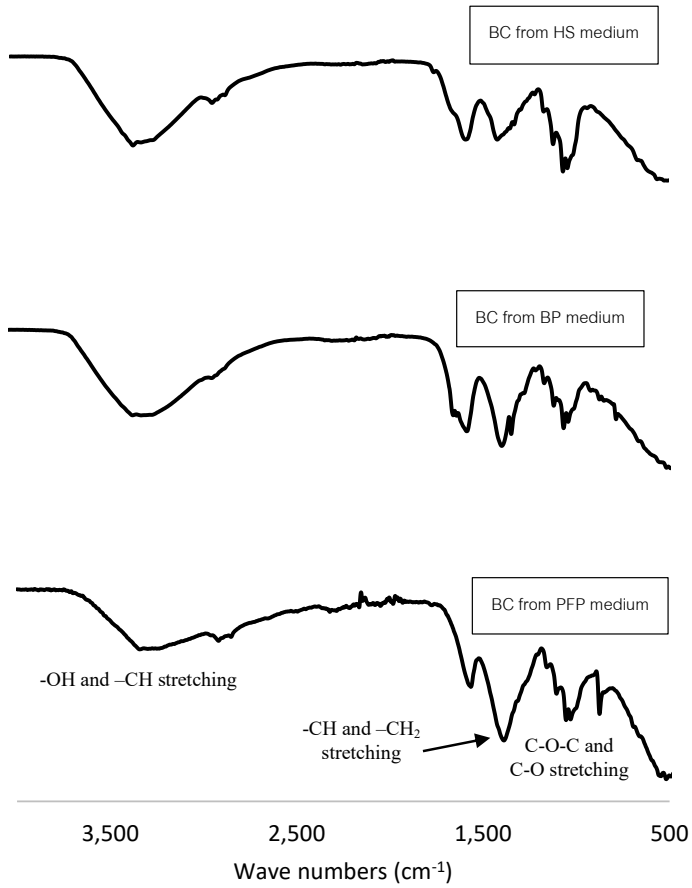

Figure 4. FTIR spectra of BC membranes obtained with HS medium, BP medium, and PFP medium.

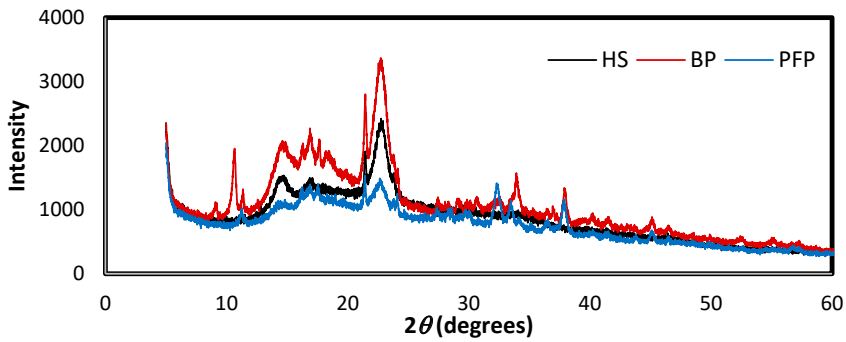

Figure 5. The XRD spectrum of BC membranes obtained with HS medium, BP medium, and PFP medium.

of substrate on the XRD patterns demonstrated that the BC network formed of each substrate revealed various intensity [10].

4. Conclusions

The present study demonstrated that banana peels and passion fruit peels digested with enzymes could be used as carbon sources for the production of bacterial cellulose. Even though BC was achieved in lower yields than those from the HS medium, the results are encouraging since the study was dealing with inexpensive residues. Therefore, our work is promising for upcoming valorisation studies of these residues by producing a biomaterial with unique properties such as bacterial cellulose.

Acknowledgements

The authors gratefully acknowledge the Faculty of Agro-Industry, Chiang Mai University, for facilities support and Mr. Sathian Boonkum for microbial culture collection.

References

[1] Wang J., Tavakoli J. and Tang Y., *Carbohyd. Polym.,* 2019; **219:** 63-76. DOI 10.1016/j.carbpol.2019.05.008.

[2] Naloka K., Yukphan P., Matsushita K. and Theeragool G., *Chiang Mai J. Sci.* 2018; **45(4)**; 1620-1622

[3] Dayal M.S., Goswami N., Sahai A., Jain V., Mathur G. and Mathur A., *Carbohyd. Polym.,* 2013; **94(1):** 12-16. DOI 10.1016/j.carbpol.2013.01.018.

[4] Esa F., Tasirin S.M. and Rahman N.A., *Agric. Agric. Sci. Proc.*, 2014; **2:** 113-119. DOI 10.1016/j.aaspro.2014.11.017.

[5] Carreira P., Mendes J.A.S., Trovatti E., Serafim L.S., Freire C.S.R. and Silvestre A.J.D., *Bioresour. Technol.*, 2011; **102(15) :** 7354-7360. DOI 10.1016/j.biortech.2011.04.081

[6] Pacheco G., Nogueira C.R., Meneguin A.B., Trovatti E., Silva M.C.C. and Machado R.T.A., *Ind. Crop. Prod.* 2017; **107:** 13-19. DOI 10.1016/j.indcrop.2017.05.026.

[7] Gomes F.P., Silva N.H.C.S., Trovatti E., Serafim L.S., Duarte M.F. and Silvestre A.J.D., *Biomass Bioenerg.,* 2013; **55:** 205-211. DOI 10.1016/j.biombioe.2013.02.004.

[8] Kongruang S., *Appl. Biochem. Biotechnol.,* 2008; **148(1):** 245-256. DOI 10.1007/s12010-007-81196.

[9] Li Z., Wang L., Hua J., Jia S., Zhang J. and Liu H., *Carbohyd. Polym.,* 2015; **120**: 115-119. DOI 10.1016/j.carbpol.2014.11.061.

[10] Goelzer F.D.E., Faria-Tischer P.C.S., Vitorino J.C., Sierakowski M.R. and Tischer C.A., *Mater. Sci. Eng. C-Mater.*, 2009; **29(2):** 546-551. DOI 10.1016/j.msec.2008.10.013.

[11] Ye J., Zheng S., Zhang Z., Yang F., Ma K. and Feng Y., *Bioresour. Technol.,* 2019; **274:** 518-524. DOI 10.1016/j.biortech.2018.12.028.

[12] OTOP 2019, Processing of Banana; Available at: http://www.sptn.dss.go.th/otopinfo/index.php/en/2014-10-09-08-12-02/2017-07-04-03-19-03/139-2017-07-04-04-00-08?showall=1&limitstart=

[13] Happi Emaga T., Andrianaivo R.H., Wathelet B., Tchango J.T. and Paquot M., *Food Chem.*, 2007; **103(2):** 590-600. DOI 10.1016/j.foodchem.2006.09.006.

[14] DOAE, Passion 2559; Available at: http://production.doae.go.th/

[15] Gebregergs A., Gebresemati M. and Sahu O., *Pac. Sci. Rev. A: Nat. Sci. Eng.,* 2016; **18(1):** 22-29. DOI 10.1016/j.psra.2016.06.002.

[16] Nathoa C., Sirisukpoca U. and Pisutpaisal N., *Enrgy. Proced.,* 2014; **50:** 702-710. DOI 10.1016/j.egypro.2014.06.086.

[17] Katongole C.B., Bakeeva A., Passoth V. and Lindberg J.E., *Livest. Sci.,* 2017; **199:** 14-21. DOI 10.1016/j.livsci.2017.03.002.

[18] Ali A., *Environ. Nanotechnol. Monit. Manage.,* 2017; **7:** 57-63. DOI 10.1016/j.enmm.2016.12.004.

[19] Miller G., *Anal. Chem.*, 1959; **31**, 426. DOI 10.1021/ac60147a030.

[20] Zhang S., Winestrand S., Guo X., Chen L., Hong F. and Jönsson L.J., *Microb. Cell Fact.,* 2014; **13(62):** 1-10. DOI 10.1186/1475-2859-13-62.

[21] Molina-Ramírez C., Castro M., Osorio M., Torres-Taborda M., Gómez B. and Zuluaga R., *Materials (Basel, Switzerland),* 2017; **10(6):** 639. DOI 10.3390/ma10060639.

[22] Wijaya C.J., Saputra S.N., Soetaredjo F.E., Putro J.N., Lin C.X. and Kurniawan A., *Carbohyd. Polym.,* 2017; **175:** 370-376. DOI 10.1016/j.carbpol.2017.08.004.

[23] Revin V., Liyaskina E., Nazarkina M., Bogatyreva A. and Shchankin M., *Braz. J. Microbiol.*, 2018; **49:** 151-159. DOI 10.1016/j.bjm.2017.12.012.

[24] Kurosumi A., Sasaki C., Yamashita Y. and Nakamura Y., *Carbohyd. Polym.,* 2009; **76(2):** 333-335. DOI 10.1016/j.carbpol.2008.11.009.

[25] Son H.J., Kim H.G., Kim K.K., Kim H.S., Kim Y.G. and Lee S.J., *Bioresource Technol.* 2003; **86(3):** 215-219. DOI 10.1016/S0960-8524(02)00176-1.

[26] Sharma C. and Bhardwaj N.K., *Int. J. Biol. Macromol.*, 2019; **132**; 166-177. DOI 10.1016/j.ijbiomac.2019.03.202.

[27] Wu J.M. and Liu R.H., *J. Biosci. Bioeng.* 2013; **115(3):** 284-290. DOI 10.1016/j.jbiosc.2012.09.014.

[28] Hospodarova V., Singovszka E. and Stevulova N., *Am. J. Anal. Chem.*, 2018; **9(6):** 303-310. DOI 10.4236/ajac.2018.96023.

[29] Sheykhnazari S., Tabarsa T., Ashori A., Shakeri A. and Golalipour M., *Carbohyd. Polym.*, 2011; **86(3):** 1187-1091. DOI 10.1016/j.carbpol.2011.06.011.

[30] Ford E.N.J., Mendon S.K., Thames S.F., and Rawlins J.W., *J. Eng. Fiber. Fabr.*, 2010. **5(1)**: 10-20. Doi 10.1177/155892501000500102.