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pineapple peel waste hydrolysate
detoxification for the production of bacterial
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1 **Atmospheric cold plasma-assisted pineapple peel waste hydrolysate detoxification for the production of bacterial cellulose**

Shella Permatasari Santoso a,b,1,

5 **Shin-Ping Lin c,1, Tan-Ying Wang d,e, Yuwen Ting d, Chang-Wei Hsieh f, Roch-Chui Yu d, Artik Elisa Angkawijaya g, Felycia Edi Soetaredjo a,b, Hsien-Yi Hsu h,i, Kuan-Chen Cheng**

d,e,j,k,* a

2Chemical Engineering Department, **Widya Mandala Surabaya Catholic University, #37, Kalijudan Rd., Surabaya 60114, East Java, Indonesia** **b**
Chemical Engineering Department, **National Taiwan University of Science and Technology, #43, Sec. 4, Keelung Rd., Taipei 10607, Taiwan** **c** School of

13Food Safety, **Taipei Medical University, #250, Wuxing Street, Xinyi Dist., Taipei 11042, Taiwan**

d

13Institute of Food Science and Technology

,

1National Taiwan University, **#1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan** **e**
Institute of Biotechnology, **National Taiwan University, #1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan** **f** Department of Food Science and **Biotechnology, National Chung Hsing University, 145 Xingda Rd**

., South Dist., Taichung 40227, Taiwan **g**

2Graduate Institute of Applied Science and Technology, **National Taiwan University of Science and Technology, #43, Sec. 4, Keelung Rd., Taipei 10607, Taiwan**

h

1School of Energy and Environment & Department of Materials Science and Engineering, **City University of Hong Kong, Kowloon Tong, Hong Kong, China** **i** Shenzhen Research Institute of City University of Hong Kong, **Shenzhen 518057, China** **j** Department of

1Medical Research, **China Medical University Hospital, China Medical University, 91, Hsueh-Shih Road, Taichung 40402, Taiwan** **k** Department of Optometry, **Asia University**

, 500, Lioufeng Rd., Wufeng, Taichung 41354, Taiwan

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: Pineapple peel waste Atmospheric cold plasma Bacterial cellulose abstract Toxic compounds in pineapple peel waste hydrolysate (PPWH), namely

1formic acid, 5-hydroxymethylfurfural (HMF), and furfural

, are the major predicament in its utilization as a carbon source for bacterial cellulose (BC) fermentation. A rapid detoxification procedures using

4atmospheric cold plasma (ACP) technique were employed to reduce the toxic compounds

. ACP treatment allows the breakdown of toxic compounds without causing excessive breakdown of sugars. Herein, the performance of two available laboratory ACP reactors for PPWH detoxification was being demonstrated. ACP-reactor-1 (R1) runs on plasma

1power of 80–200 W with argon (Ar) plasma source

, while ACP-reactor-2 (R2) runs at 500–600 W with air plasma source. Treatment in R1, at 200 W for 15 min, results in 74.06%, 51.38%, and 21.81% reduction of furfural, HMF, and formic acid. Treatment in R2 at 600 W gives 45.05%, 32.59%, and 60.41% reductions of furfural, HMF, and formic acid. The BC yield from the fermentation of *Komagataeibacter xylinus* in the R1-treated PPWH, R2-treated PPWH, and untreated-PPWH is 2.82, 3.82,

11and 2.97 g/L, respectively. The results show that

ACP treatment provides a novel detoxified strategy in achieving agricultural waste hydrolysate reuse in fermentation. Furthermore, the results also imply that untreated PPWH can be an inexpensive and sustainable resource for fermentation media supplementation. © 2021 Elsevier B.V. All rights reserved. 1. Introduction Agro-industrial waste is an atypical renewable resource that has the potential to be processed into various valuable materials. The utilization and conversion of waste into value-added products is one smart solution in overcoming waste accumulation [1]. Fruit peel waste, such as pineapple peel waste (PPW), is one of the many agro-industrial wastes generated from open markets and other food-related industries [2]. The use of agro-industrial

12waste as a source of nutrients for bacterial cellulose (BC

) fermentation can *

**1 Corresponding author at: Institute of Food Science and Technology,
National Taiwan University, #1, Sec. 4, Roosevelt Rd., Taipei 10617, Taiwan**

. E-mail address: kccheng@ntu.edu.tw (K.-C. Cheng). 1 Shella Permatasari Santoso and Shin-Ping Lin are contributed equally to this work. be a great solution to relieve PPW accumulation. The major predicament that inhibits the direct-utilization of PPW in BC fermentation is the high lignocellulose content. Lignocellulosic material is mainly composed of the carbohydrate-polymers (i.e., cellulose and hemicellulose) and layers of lignin. This material is responsible for the rigidity of the biomass cell walls. These lignin layers cover and protect the cellulose and hemicellulose thus makes it inaccessible for microbial degradation [3]. Only a small number of bacterial species had been reportedly able to produce lignocellulose-degrading enzymes (e.g., cellulase, ligninase, and hemicellulases), which allow them to digest the lignocellulose and convert it into (fermentable) sugars [4]. Unfortunately, none of the BC-producing bacteria exhibited the ability to produce these enzymes [5]. In addition, the degradation of lignocellulose by lignocellulose-degrading bacteria requires a considerably-long time

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. which may not be suitable for many industrial applications [6]. To overcome this problem, acid hydrolysis had been adopted as an effective method to break down the lignocellulose into simple sugars due to its fast and straightforward process [7]. The use of acid in the hydrolysis process allows an immediate breakdown of lignocellulosic biomass into sugars through hydrogen bonding disruption of lignocellulose. However, the byproduct of this conversion (carboxylic acids and furan aldehydes) can be toxic to the bacteria and may inhibit bacterial growth and fermentation [8]. Formic acid is one of the carboxylic acids in high amounts resulting from hydrolysis, whereas furfural and 5-hydroxymethylfurfural are the two commonly produced furan aldehydes [9]. Thus, a detoxification step is necessary to reduce the toxic compounds in the hydrolysate resulting from acid hydrolysis to promote bacterial growth and fermentation. Several detoxification techniques, such as adsorption, ion-resin exchange, heat-treatment, irradiation treatment, peroxidase treatment, vacuum evaporation, and other biological treatments, can be utilized to reduce or eliminate the occurrence of toxic compounds [2,10–14]. However, most of the methods are not suitable for biomass detoxification because of the high degradation rates of essential compounds in biomass (e.g., fermentable sugars). For example, the heat, UV, microwave, and peroxidase treatment cause deformation of the physical state and degrade some of the essential compounds in biomass [15]. The use of peroxidase compound in the treatment also causes additional chemical waste generation, which can be harmful to the environment. Other techniques such as adsorption, ion-resin exchange, and biological treatment may be environmentally friendly, but they may involve a complicated and lengthy process [16]. A novel technique, namely atmospheric cold plasma (ACP), has been referred to as a breakthrough for biomass detoxification technique. ACP technique allows detoxification of biomass without any undesirable physicochemical properties change [15,17–19]. Furthermore, the ACP technique is also postulated to be potentially applied on an industrial scale due to its tunable and scalable operational parameters, including the voltage, type of feed gas, and treatment time [3]. ACP treatment has been popularly used to detoxify mycotoxins from food, where these compounds are unremovable using the conventional treatments [18,20–22]. ACP treatment works by inducing the generation of reactive species, which can inhibit microbial growth, thereby increasing the shelf-life of the food products. Owing to this work mechanism, ACP treatment can be applied to detoxify biomass from various toxic compounds [3]. Herein, ACP treatment was utilized to reduce the toxic compounds in hydrolysate from PPW prior to its use

6as the carbon source in BC fermentation. In this work, **the effect of**

ACP treatment in the compositional change of PPWH was evaluated, especially the composition of the toxic compounds and fermentable sugars in PPWH. Two ACP reactors were used for the detoxification of PPWH, where the reactors operate at different specific plasma power range and gas sources. Thus, the performance of the two ACP reactors was demonstrated separately. The detoxified-PPWH was then utilized as an inexpensive carbon source to supplement the fermentation media for BC production by *Komagateibacter xylinus* (*K. xylinus*). 2. Material

1and methods 2.1. Materials PPW **was** collected **from a local** open market in Taipei, Taiwan, as a discarded biomass waste. **Glucose and cellulose enzymes were** purchased from Sigma-Aldrich, MO, USA. **Fructose and xylose were** purchased from Himedia, Mumbai, India. **Peptone was obtained from Bioshop, Ontario, Canada. Yeast extract was obtained from Lab M, Hey-wood, UK. Citric acid, formic acid, HMF, and furfural were purchased from JT Baker Chemical Co., NJ, USA. Sodium bicarbonate was supplied by Showa Chemical Co., Tokyo, Japan. K. xylinus ATCC 23769 was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan).** **2.2. Hydrolysis of PPW** The PPW **was washed**

, crushed, and

2dried in a 60 °C oven. The dried pulp **was**

ground and sieved to obtain uniformly sized particles of 30 mesh (± 0.6 mm). The lignocellulose content in PPW was determined using the Van Soest method [23]; the lignocellulosic content is 29.4% cellulose, 23.7% lignin, and 15.4% hemicellulose. Two-stage hydrolysis was employed to maximize the sugars content in the hydrolysate. In a typical hydrolysis procedure, the dried PPW was added to the 2.5%v of H₂SO₄ solution at a solid-to-liquid ratio of 1:10. The hydrolysis was conducted at 130 °C for 60 min. The hydrolysate was then separated from the solid residue by means of centrifugation. The solid

4residue was washed and dried in a 60 °C oven for

8 h, and then subjected for the 2nd-stage hydrolysis at the same conditions. The hydrolysate was pooled together and referred to as PPWH. The composition of the sugars (i.e.,

1glucose, xylose, and fructose) and toxic

or inhibitory compounds (i.e.,

1formic acid, HMF, and furfural) in

PPWH was determined using high-pressure liquid chromatography (HPLC, JASCO, USA) equipped with Repro-Gel Ca column (Dr. Maisch GmbH, Germany). The chromatogram is provided in Supplementary data Fig. S1. 5 mM H₂SO₄ solution

1 was used as the mobile phase at a flow rate of 0.6 mL/min

. The hydrolysis rate was determined based on the amount of glucose extracted from PPWH per amount cellulose contained in dry PPW, according to Eq. (1): Hydrolysis rate $\delta\% = \frac{1}{4} \times \frac{\text{Amount of glucose in PPWH}}{100\% \times \text{Amount of cellulose in dry PPW}}$ 2.3. Detoxification of PPWH using plasma treatment Two types of ACP reactors (Industrial Technology Research Institute, Taiwan) were utilized to detoxify PPWH. Each reactor has a specific gas source and plasma power that can be achieved. Reactor 1 (R1) works at the

1 power of 80–200 W with an Ar gas plasma source

, while reactor 2 (R2) works at 500–600 W with air as the plasma source. The detailed specifications of the two reactors are provided in Supplementary data Table S1. The two reactors were used to detoxified PPWH, and the treated-PPWH

6 was used as the carbon source to supplement the fermentation media. The

effect of the plasma power of each reactor to detoxify PPWH was evaluated. The plasma power of 80, 120, 160, and 200 W was used in R1; meanwhile, plasma power of 500 and 600 W was used in R2. All plasma treatments were performed for 15 min, which is the best treatment time resulting in a minimal reduction in sugar and a moderately high reduction in inhibiting compounds (data not shown). The detoxification procedures using each reactor were carried out by putting 50 mL of PPWH in an open stainless-steel Petri dish. Subsequently, the dish was placed into the discharge chamber at 1 cm distance from the jet head, which was designated to exert gas at a flow rate of 14 mL/min. The detoxification effect from both reactors was reported, where the effect was evaluated based on the alteration in toxic compounds and fermentable sugars of PPWH. The degradation rate of each compound is calculated according to Eq. (2): Degradation rate $\delta\% = \frac{1}{4} \times \frac{C_0 - C_f}{C_0} \times 100\%$ where, C₀ and C_f are the concentration of the compound

4 before and after treatment. Table 1 Composition of

glucose (Glu), fructose (Fru), xylose (Xyl), formic acid (FA), hydroxymethylfurfural (HMF), and furfural (Fur) in the fermentation media. Mediaa Concentration (g/L) Glu Xyl Fru FA HMF Fur PPWH R1-treated and its control group ARP-PPWHb ARP-PPWH-c1 ARP-PPWH-c2 R2-treated and its control group AP-PPWHc AP-PPWH-c1 AP-PPWH-c2 16.49 8.86 11.38 6.58 11.33 6.53 11.35 6.61 15.71 8.53 15.59 8.84 15.73 8.57 14.56 0.26 10.77 0.57 10.72 0.56 10.86 – 13.99 0.28 13.88 0.26 14.06 – 0.26 0.10 0.09 – 0.22 0.21 – 0.18 0.02 0.03 – 0.02 0.03 – a Abbreviation for media naming: AP, air plasma-treated; ARP, Ar plasma-treated; c1, control media full composition; c2, control media without formic acid, HMF, and furfural. b The media was prepared mixed with the R1-treated PPWH at 200 W for 15 min. c The media was prepared mixed with the R2-treated PPWH at 600 W for 15 min. 2.4. Preparation of the culture medium Seven culture media were prepared at different concentrations of sugars and inhibitory compounds, as given in Table 1. The PPWH was derived from the non-plasma-treated PPWH. ARP-PPWH was derived from the plasma-treated PPWH using R1. ARP-PPWH-c1 and ARP-PPWH-c2 were the control media prepared by modifying the composition of HS media to mimic the composition of ARP-PPWH. Meanwhile, AP-PPWH was derived from the plasma-treated PPWH using R2. AP-PPWH-c1 and AP-PPWH-c2 were the control media prepared by

modifying the composition of HS media to mimic the composition of AP-PPWH. The pH of the culture media was adjusted to a pH value of 6.8 by the addition of 0.1 M NaOH. Then, the culture media was sterilized at 121 °C in an autoclave for 30 min. Besides the composition listed in Table 1, the media were supplemented with

81.15 g/L citric acid

85 g/L peptones, 5 g/L yeast extract, and 2.7 g/L sodium

hydrogen phosphate. 2.5. Culture of *K. xylinus* and BC production The detailed preparation of the frozen stock culture and the propagation method of *K. xylinus* can be seen elsewhere [24,25]. The BC pellicles from the propagation step were aseptically collected and pureed using a Waring Blender 7011 HS. Then, 5 mL of the puree was inoculated in 50 mL of the modified media (Table 1) and incubated at 28 °C for 9 days. After fermentation, the BC pellicles were collected and then boiled at 90 °C in 100 mL of 0.1 N NaOH solution for 30 min. The obtained translucent BC pellicles were dried using a freeze dryer, the difference in weight before and after drying was measured to determine the water content. 2.6. Cell biomass measurement The obtained BC pellicles were added into a fresh culture media, then 500 µL of cellulase from *Trichoderma reesei* (Sigma-Aldrich, USA) was added. The enzymatic degradation was allowed to proceed for 60 min at 50 °C. The obtained supernatant containing *K. xylinus* cells was centrifuged (Centrifuge-Universal 320R, Hettich Zentrifugen, Germany) at 5000 ×g at 4 °C for 10 min. Subsequently, the *K. xylinus* cells pellet was collected and washed with deionized water, then dried at 60 °C for 24 h. The dry cells were weighed and

12 recorded as the dry cell weight. 2.7. Characterization of BC pellicles The

freeze-dried BC was characterized for their dynamic weight loss using a thermogravimetric analyzer (TGA) (TA Instrument Q500, DE, USA). The morphology was observed using a scanning electron microscope (SEM) (JEOL JSM-7800F, Tokyo, Japan); the samples were first coated with thin Pt-film using a coater JEOL JEC-300FC Quick coater (Tokyo, Japan). To determine the thickness of BC pellicles, image processing software ImageJ was used. The mechanical strength was determined

1 using a texture analyzer (TXA) (TA-XT2 model, NY, USA). The

Fig. 1. Inhibitory effect of formic acid (FA), HMF, and furfural (Fur) on BC production by *K. xylinus*. The inhibitory compound was added at a specific concentration into the normal HS medium. Fig. 2. The composition of sugars (a) and inhibitory compounds (b) in PPWH after 15 min ACP treatment using R1.

1 Different letters on the error bars represent a significant difference ($P < 0.05$) relative to the control

at 0 watt plasma power. functional groups were determined using Fourier-transform infrared spectroscopy (FTIR) spectroscopy analysis using Shimadzu IRTracer- 100 with KBr background. The crystallinity was determined based on X-ray diffraction analysis (XRD) using X'Pert PRO X-ray diffractometer, Philips,

Netherlands, with Ni-filtered Cu K α (1.540562 Å) radiation.

3. Results and discussion

3.1. Effect of the inhibitory compounds on BC production

The inhibitory effects of formic acid (FA), 5-hydroxymethylfurfural (HMF), and furfural (Fur) on the BC production by *K. xylinus* were studied using the one-factor-at-a-time (OFAT) method. Fig. 1a–c shows the de-moting effect of these three inhibitors on BC yield and the percent reduction on BC yield. The quantitative data are provided in Supplementary data Tables S2 and S3. It can be noted that the presence of these compounds, even in small amounts, had a hugely detrimental impact on the formation of BC. The presence of 0.1%wt of Fur results in 72.1% BC yield reduction; meanwhile, the same concentration FA gave a 29.2% reduction. HMF shows more significant inhibitory activity to the BC formation, where the presence of this compound at concentrations 2.7 times smaller than Fur and FA resulted in a 43.3% reduction. This result is in well-convergence with a previous report describing the inhibitory effect of HMF on some enzyme functions, which caused a prolonged lag phase of the bacteria [3,26]. Disparate from HMF inhibitory effect, Fur is reported to affect bacterial cell viability due to its toxic effect [5].

3.2. Evaluation of the ACP treatment in the detoxification of PPWH

3.2.1. Effect of plasma power

ACP R1 works in the plasma power range from 80 to 200 W with an Ar gas source. The effect of different plasma power on the composition of inhibitory compounds and sugars in PPWH are presented in Fig. 2; the detailed quantitative values are presented in Supplementary data Table S4. As shown in Fig. 2b, the FA, Fur, and HMF are significantly decreased by treatment using a high plasma power. However, a significant decrease in the sugars content was also observed after high plasma power treatment (Fig. 3a). Separate plasma treatments to the PPWH were conducted using ACP R2, which is equipped with air gas plasma and power of 500 W and 600 W. Interestingly, although ACP R2 has much higher plasma power, it has less impact on the sugars content in PPWH compared to ACP R1. As presented in Fig. 3b, no significant reduction of sugars occurs after plasma treatment using R2 at 500 and 600 W. Interestingly, as shown in Fig. 3a, the inhibitory compounds were significantly reduced after treatment using the highest plasma power of R2. The degradation rate of each investigated compound in PPWH after treatment using R1 and R2 are presented in Table 2. It can be observed, both for R1 and R2, that a significant decrease in inhibitory compounds occurs at higher plasma power. This is since an increased plasma power allows faster plasma mobility and consequently promotes the interaction between the plasma and the inhibitory compounds, thus accelerating their degradation [3]. Furthermore, the inhibitory compounds are more susceptible to degradation since they have smaller molecular sizes than the sugars [17,18,27]. Based on the analysis of the Fig. 3. The composition of sugars (a) and inhibitory compounds (b) in PPWH after 15 min ACP treatment using R2.

1 Different letters on the error bars represent a significant difference ($P < 0.05$) relative to the control

at 0 watt plasma power. Table 2

4 Degradation rate of each compound in PPWH after 15 min plasma treatment

using R1 and R2 at different plasma power.

Power (W)	Degradation rate (%) Glu	Xyl	Fru	FA	HMF	Fur
Plasma treatment in R1 80	20.03 \pm 0.01	20.29 \pm 0.14	22.24 \pm 0.13	24.51 \pm 0.18		
Plasma treatment in R2 500	8.46 \pm 0.12	8.46 \pm 0.22	17.87 \pm 0.09	18.99 \pm 0.18	1.38 \pm 0.01	-2.18 \pm 0.02a
600	0.48 \pm 0.01	-2.28 \pm 0.05a	32.19 \pm 0.30	32.48 \pm 0.10	34.75 \pm 0.21	37.55 \pm 0.17
	5.06 \pm 0.02	5.12 \pm 0.02	37.61 \pm 0.01	39.92 \pm 0.03	34.75 \pm 0.01	31.55 \pm 0.02
	59.47 \pm 0.12	32.59 \pm 0.05	0.87 \pm 0.01	4.66 \pm 0.01	62.10 \pm 0.00	51.38 \pm 0.01
	26.97 \pm 0.11	32.58 \pm 0.31	1.23 \pm 0.01	-0.06 \pm 0.00a	76.91 \pm 0.11	74.06 \pm 0.20
	41.80 \pm 0.11	45.04 \pm 0.22	a			

The negative value shows that the compound was formed instead of degraded.

degradation rate (Table 2), it can also be noted that ACP-treatment of PPWH using R1 shows a higher sugar loss than the treatment using R2 —Even though the plasma power in R1 is much lower than R2. One possible scenario for this result is the difference in the type of gas used as the plasma source. As mentioned in

several studies, treatment using Ar plasma can induce a higher ROS formation than treatment using air-plasma [28,29]. The increased formation of ROS promotes the degradation of the compounds contained in PPWH. Furthermore, in the plasma treatment using R1, the high loss of sugars also followed by the significant increase of FA, which can be accounted for the degradation reaction of the sugars. Based on the degradation rate (Table 2) evaluation, the best detoxification effect was produced using plasma treatment at 200 W for R1 and 600 W for R2. 3.3. BC production from fermentation of *K. xylinus* in PPWH detoxified media To investigate the performance of the untreated and plasma-treated PPWH, these hydrolysates were supplemented to the growth media of *K. xylinus*. The composition of the media can be seen in Table 1. Fig. 4a Fig. 4. a, b) BC yield (g/L) and cell biomass (g/L) after 9-days fermentation in R1-treated media and R2-treated media along with their control groups, respectively. c, d) BC yield in g/g of glucose after 9-days fermentation in R1-treated media and R2-treated media along with their control groups, respectively.

1 Different letters on the error bars represent a significant difference ($P < 0.05$) relative to the

BC yield and cell growth in PPWH media. Table 3 Comparison of BC yield produced from the fermentation of several bacterial strain in different media. Producing strain Fermentation media BC yield (g/L) Ref. *Acetobacter xylinum* *Gluconacetobacter xylinus* *Acetobacter aceti* *Komagataibacter xylinus* *Komactobacter intermedius* *Komagataibacter xylinus* Treated corn stalk Litchi extract Optimized HS CPPEb Optimized HS AP-PPWH ARP-PPWH PPWH 2.86 2.53 1.73 5.7 3.91 3.82 2.82 2.97 [35] [14] [36] [11] [25] This work a *Acetobacter xylinum* and *Gluconacetobacter xylinus* are the former names of *K. xylinus*. b CPPE: citrus peel and pomace enzymolysis. and b shows the amount of BC yield and cell biomass growth after 9- days of fermentation of *K. xylinus* in PPWH and the plasma-treated PPWH containing media, along with the controls. A high BC yield can be obtained from the fermentation of *K. xylinus* in the plasma-treated PPWH compare to their control groups. The highest BC yield of 3.82 g/L is obtained from the fermentation of *K. xylinus* in the media containing R1-treated PPWH (ARP-PPWH). The second highest yield of 2.97 g/L is obtained from the fermentation in media containing untreated PPWH. Meanwhile, the fermentation in media containing R2- treated PPWH (AP-PPWH) resulted in a BC yield of 2.82 g/L. The BC yield obtained from media containing AP-PPWH may seem lower than the BC yield obtained from PPWH in terms of g BC per L of media. However, when considering the BC yield in terms of g BC per g of Glu, the BC yield obtained from the AP-PPWH-containing media is much higher than the PPWH-containing media. As shown in Fig. 4d, BC yield of 0.24 g/g Glu is obtained after fermentation in AP- PPWH-containing media; meanwhile, 0.18 g BC/g Glu is obtained from PPWH-containing media. Interestingly, the fermentation in untreated PPWH also resulted in a significantly high BC yield; it implies that the PPWH alone can serve

6 as the carbon source for BC fermentation. Investigation of

cell biomass concentration of *K. xylinus* after 9-days fermentation (Fig. 4) indicates that the plasma-treated PPWH can significantly promote the growth of BC-producing bacteria. It also can be noted from Fig. 4 that the formation of BC and the cell growth in PPWH supplemented media was higher than that of in ARP-PPWH-c2 and AP-PPWH-c2, where all of the media contained a specific concentration of the inhibitory compounds. This phenomenon can be attributed

5 to the presence of other complex components in

PPWH (i.e., plant phytochemicals) that may be promoted the growth of *K. xylinus* [30]. As postulated in other studies, organic compounds and some trace elements in fruit juices can facilitate the fermentation of BC [31–34]. The BC produced by *K. xylinus* in the Ar and air-plasma treated media has a comparable yield to

BC produced from several other strains (Table 3). It is worth noting that the BC produced in fermentation using ACP plasma-treated media has a higher yield than several reported strains. Specifically, *A. xylinum*, which able to produce 2.86 g/L of BC using the acetic acid pre-hydrolysis liquor of corn stalk; *G. xylinus*, which able to produce 2.53 g/L of BC using litchi extract; and *A. aceti*, which only able to yield 1.73 g/L of BC after fermentation using optimized HS media [14,35,36]. The BC yield produced by *K. xylinus* (in this study) is still lower than several reported studies [11]. Even so, the utilization of PPW as the low-cost carbon source in BC fermentation can be a great solution in overcoming the high accumulation rate of this waste.

3.4. Characterization of the BC product As shown in Fig. 5a, all BC samples share similar FTIR characteristic peaks. The peak corresponds to the O\H stretching vibration detected at the wavenumber 3358–3374 cm⁻¹. The C\H stretching of CH₂ and CH₃ peak is detected at wavenumber 2858–2936 cm⁻¹. Next, the peak corresponds

9to the H-O-H bending of the absorbed water

is observed at a wavenumber range of 1644–1682 cm⁻¹. The peak displaying the CH₂ symmetric bending is observed at a wavenumber range of Fig. 5. Characterization on BC produced from the fermentation of *K. xylinus* in different media: (a) FTIR spectra and (b) XRD pattern. Fig. 6. The fiber morphology BC from *K. xylinus* fermented in different media. a) PPWH, b) AP-PPWH, c) ARP-PPWH, d) AP-PPWH-c1, e) ARP-PPWH-c1, f) AP-PPWH-c2, g) ARP-PPWH-c2. 1378–1474 cm⁻¹, commonly convoluted with the peak corresponding to O\H bending vibration. The C\O bending spectra are observed at a wavenumber range of 1040–1070 cm⁻¹. The FTIR spectra of BC samples are in good accordance with that of the reported literature [24]. XRD patterns and the corresponding crystal planes of the BC are presented in Fig. 5b. The typical pattern associated with the cellulose peaks is observed in all BC samples, which indicates the purity of the obtained BC [37]. Overall, the peak corresponds to cellulose I α at the plane (110) is detected at a 2-Theta ranging from 13.1 to 14.2°, which is accompanied by the appearance of shoulder peak at near 16°. The respective peak associated with the cellulose I β , plane (200), is detected in the range of 22.1–22.4°. The plane (004) reflection appears obviously for BC fermented in PPWH, AP-PPWH, and ARP-PPWH at 33.4, 34.2, and 33.4°, respectively. This plane corresponds to the non-equatorial reflection of cellulose I α [38]. Table 4 Measured properties of BC produced from the fermentation of *K. xylinus* in different media.

Fermentation media	Properties of BC	Water content (%)	Mechanical strength	Crystallinity (%)		
Decomposition temp. (°C)	Tensile stress (MPa)	Tensile strain (%)	PPWH	ARP-PPWH	ARP-PPWH-c1	ARP-PPWH-c2
98.91 ± 0.08a	98.68 ± 0.06a	97.82 ± 0.24b	98.86 ± 0.16a	98.70 ± 0.03a	98.76 ± 0.13a	98.73 ± 0.01a
4.0 ± 1.07c	5.21 ± 1.48c	43.83 ± 14.59a	46.14 ± 13.15a	6.04 ± 0.94c	47.25 ± 9.11a	33.55 ± 1.54b
0.48 ± 0.32c	1.05 ± 0.24bc	0.59 ± 0.16bc	0.95 ± 0.73bc	0.52 ± 0.09bc	1.02 ± 0.18b	2.47 ± 0.92a
72.93	73.46	83.53	84.40	70.54	85.18	86.11
258.1	311.3	342.7	316.5	264.5	335.5	338.7

Different superscript letters

1indicate a significant difference (P < 0.05). The morphology of the

BC produced in PPWH and plasma-treated PPWH containing media (Fig. 6a–c) are only slightly different from the control (Fig. 6d–g). All of the samples show similar fibrous morphology, suggesting that the BC structure

11mostly depends on culture condition rather than feedstock sources

[1,12]. The most-dense BC fibers distribution is observed from the fermentation using the ARP-PPWH medium, followed by AP-PPWH and PPWH. The resultant BC fibers have a diameter ranging from 10 to 70 nm (inset Fig. 6), with an average width of 20 to 24 nm. Other properties of the BC were also studied (Table

4). The crystallinity of the BC samples was determined based on the amorphous and crystalline area ratio, as detected in the XRD pattern. The BC obtained from the fermentation of *K. xylinus* using PPWH containing media has a lower crystallinity than the control groups. The decreased crystallinity of BC produced from PPWH containing media can be due to polysaccharides content in PPW, which induces aggregation of cellulose chains into microfibrils [10,39]. All BC samples possess equally high water content, indicating their excellent water retention ability. The thermostability of BC was assessed based on decomposition temperature, as measured using TGA. It was noted that the BC obtained via fermentation using PPWH containing media have lower thermal stability. This is due to the larger amorphous moiety of BC, which is more susceptible to thermal breakdown [10]. The mechanical strength of BC is expressed as tensile strength and tensile strain; both values depict the capability of the BC fiber to resist and elongate without cracking after given external forces. The BC produced from fermentation in PPWH containing media is susceptible to deformation than BC produced from their control groups. This can be attributed to the higher amorphous moiety of the BC from PPWH containing media, resulting in lower mechanical strength.

4. Conclusion Detoxification of PPWH using atmospheric cold plasma (ACP) treatment with argon and air gas source has been demonstrated. Both treatments result in an adequate reduction of the toxic compounds (i.e., formic acid, HMF, and furfural) in PPWH. The argon plasma, which works at the plasma power of 80–200 W, has a more significant degradation effect on the sugars content in PPWH than air plasma, which operates at a much higher plasma power of 500–600 W. The use of ACP-treated PPWH, as well as the untreated PPWH, as the carbon source for BC production can result in a high yield of BC. The BC fermented in the ACP-treated PPWH and untreated PPWH do not show meaningful characteristics change, which indicates that the use of those bioresources does not cause a decrease in the quality of the BC. This also suggests that both treated and untreated PPWH can serve as a low-cost and sustainable carbon source to supplement the fermentation media.

CRedit authorship contribution statement Shella Permatasari Santoso: Data curation,

10 **Writing – Reviewing and editing** Shin-Ping Lin: **Data curation, Writing – Reviewing and editing**

Tan-Ying Wang: Formal analysis, Writing – Original draft Yuwen Ting: Project administration, Supervision Chang-Wei Hsieh: Data analysis; Validation Roch-Chui Yu: Project administration Artik Elisa Angkawijaya: Writing – Original draft,

10 **Writing – Reviewing and editing** Felycia Edi Soetaredjo: **Writing – Reviewing and editing**

Hsien-Yi Hsu: Writing – Reviewing and editing Kuan-Chen Cheng: Supervision, Conceptualization, Resources, Project

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7 **Appendix A. Supplementary data** Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijbiomac.2021.01.169>. **References**

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