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Production of nano bacterial cellulose from waste water of candied jujube-processing industry using *Acetobacter xylinum*

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ABSTRACT

The work is aimed to investigate the suitability of waste water of candied jujube-processing industry for the production of bacterial cellulose (BC) by *Gluconacetobacter xylinum CGMCC No.2955* and to study the structure properties of bacterial cellulose membranes. After acid pretreatment, the glucose of hydrolysate was higher than that of waste water of candied jujube. The volumetric yield of bacterial cellulose in hydrolysate was 2.25 g/L, which was 1.5-folds of that in waste water of candied jujube. The structures indicated that the fiber size distribution was 3–14 nm in those media with an average diameter being around 5.9 nm. The crystallinity index of BC from pretreatment medium was lower than that of without pretreatment medium and BCs from various media had similar chemical binding. Ammonium citrate was a key factor for improving production yield and the crystallinity index of BC.
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1. Introduction

Production of high value-added materials from waste residual originating from renewable feedstock is an interesting possibility (Cavka et al., 2013). One such material is bacterial cellulose (BC), which can be produced by some microorganisms and has been widely accepted as a multifunctional nano-biomaterial (Haigler, White, Brown & Cooper, 1982; Huang et al., 2014; Kose, Sunagawa, Yoshida & Tajima, 2013; Nakagaito, Iwamoto & Yano, 2005). It is composed of the homopolyer of β-1,4-linked D-glucose attached with hydrogen bonds, which appears to be similar to plant cellulose (Brown, 1886; Huang et al., 2014). However, BC has exceptional physicochemical properties, such as an ultrafine reticulated structure, high crystallinity, high tensile strength, high hydrophilicity, and biocompatibility (Backdahl et al., 2006; Hsieh, Yano, Nogi & Eichhorn, 2008; Iguchi, Yamanaka & Budhiono, 2000; Kose et al., 2013; Lin et al., 2013; Rouabhia et al., 2014; Svensson et al., 2005; Yamanaka et al., 1989). Therefore, BC has been suggested in a variety of applications, such as the production of health food, cosmetics, pharmaceutical and biomedical products, reinforcement of high-quality papers, diaphragms for electro-acoustic transducers, paint additives, coatings, reinforcement for optically transparent films, and proton-conducting membranes of fuel cells (Backdahl et al., 2006; Cavka et al., 2013; Iguchi et al., 2000; Klemm, Schumann, Udhardt & Marsch, 2001; Lin et al., 2013; Nakagaito, Iwamoto & Yano, 2005; Svensson et al., 2005; Wan et al., 2007; Yamanaka et al., 1989). The future potential for BC is far beyond the existing applications, especially if it can be produced in large amounts from an inexpensive feedstock, and it may include areas such as specialty textiles, advanced functional materials, and packaging (Cavka et al., 2013).

Production of BC from agricultural and industrial waste residues, which include food wastes (Moon, Park, Chun & Kim, 2006), wheat straw (Chen, Hong, Yang & Han, 2013; Hong, Zhu, Yang & Yang, 2011), fruit wastes (Brown, 1886; Castro et al., 2011; Kongruang, 2008), waste glycerol (Kose et al., 2013) and cotton-based waste textiles (Hong et al., 2012), has previously been demonstrated. An advantage of using agricultural or industrial waste residual as feedstock not only provides a cheap way to produce BC but also works as a basin for environment cleaning.

In 2012, the total amount of jujube was 5.88 million ton/year in China. Large amount of waste water of candied jujube-processing industry (WWCJ) was generated during the process of producing candied jujube at Cangzhou city in China. The WWCJ contained various saccharides, amino acids, vitamins and other nutrients (Lin, Che, Guo & Wang, 2014). Therefore, using the WWCJ as a nutrition

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source for the production of BC is of considerable importance for materials productions and environment protection. Even though, to the best of our knowledge, there are no reports on the use of the WWCJ as nutrient for the production of BC.

The objectives of this study were to investigate the feasibility of waste water of candied jujube-processing industry for the production of BC. In addition, we investigated the properties and structure of these BC. The study would benefit for environment protection and for the production of high value-added products.

2. Material and methods

2.1. Strain and culture medium

Gluconacetobacter xylinum CGMCC No.2955 was screened in the Key Laboratory of Industrial Microbiology, Ministry of Education, Tianjin University of Science and Technology, and stored in China General Microbiological Culture Collection Center (Zhu et al., 2010). The seed medium contained (g/L): glucose 20, peptone 5, yeast powder 5, ammonium citrate 1, disodium hydrogen phosphate 5, agar 20, and pH 6.0(Zhu et al., 2010). The 1# WWCJ media (g/L) containing ammonium citrate 1, sodium dihydrogen phosphate 5 and calcium carbonate 0.05 were diluted two and four folds, respectively. The 2# WWCJ without ammonium media (g/L) containing sodium dihydrogen phosphate 5 and calcium carbonate 0.05 were diluted two and four folds, respectively. The 3# WWCJ hydrolysate media (g/L) containing ammonium citrate 1, sodium dihydrogen phosphate 5 and calcium carbonate 0.05 were diluted two and four folds, respectively.

2.2. Pretreatment of WWCJ

The WWCJ media used in this study were kindly provided by Ouyakuang food Co., Ltd., Hebei, China. The WWCJ was diluted with water to a concentration of 200 g/L, and the pH of the WWCJ was adjusted to 1 by addition of 9 M sulfuric acid. Hydrolysis was performed at 60, 70, 80 and 90 °C (Li, Han, Ji, Wang & Tan, 2010).

2.3. Production of bacterial cellulose

Gluconacetobacter xylinum CGMCC No.2955 was cultivated in 500 mL flasks with 100 mL of seed medium for 24 h at 30 °C, 130 rpm. The seed was inoculated into fermentation medium and then incubated at 30 °C under static culture conditions for 6 days (Wu et al., 2010). The BC pellicles were harvested and cleaned by immersion in 0.1 M NaOH solution at 100 °C for 1 h. The BC membranes were washed with deionized water and sterilized by autoclaving (121 °C for 15 min). Membranes were then frozen at −80 °C and lyophilized. After that, the BC was weighed for calculation of the yield. The specimens were kept in a desiccator before analysis. The thickness of such membranes was estimated to 100 – 200 μm (Rouabhia et al., 2014; Tang, Jia, Jia & Yang, 2010).

2.4. Analyzes methods

2.4.1. Scanning electron microscopy

The BC was lyophilized and mounted on SEM grids, and gold particles were evaporated to the sample surfaces. The specimens were observed with a scanning electron microscopy at an accelerating voltage of 10 kV (S-4800 model, Hitachi Corporation, Japan) (Kose et al., 2013).

2.4.2. Wide angle X-ray diffractometry

The thick samples of BC were prepared according to the method from Keshk (Keshk, 2014). The reflectogram of the samples was recorded at room temperature with Rigaku Print 2200 V series using Ni-filtered CuKα radition (λ = 0.154 nm) from 4 to 40° (2θ angle). The operating voltage and current were 40 kV and 40 mA, respectively. Crystallinity was calculated from the reflected intensity data using Segal, CM, 1959 method according to Eq. (A) (Keshk, 2014).

$$(C.I.) = \frac{I_{020} - I_{am}}{I_{020}}$$

I_{020} was the maximum intensity of the lattice diffraction and I_{am} was the intensity at 2θ = 18°.

2.4.3. Fourier transform infrared spectroscopy

BC membranes were analyzed on a BRUKER TENSOR-37 spectrophotometer (BRUKER Corporation, Germany). For each analysis, the scan range was 4 000–400 cm^{-1} with a resolution of 4 cm^{-1}(Rouabhia et al., 2014).

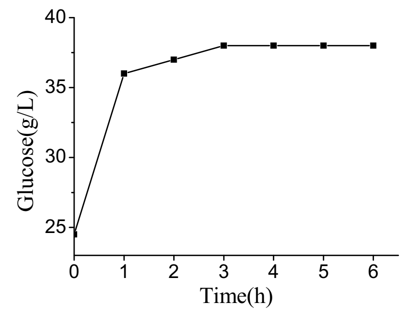

Fig. 1. The curve of glucose concentration during pretreatment of WWCJ (The waste water of candied jujube-processing industry (WWCJ) was diluted with water to a concentration of 200 g/L, and the pH of WWCJ was adjusted to 1 by the addition of 9 M sulfuric acid. Hydrolysis was performed at 80 °C).

3. Results and discussion

3.1. Production of BC using WWCJ

Waste water of candied jujube-processing industry (WWCJ) is an abundantly available type of agro-industrial wastes that is produced during the processing of candied jujube in many jujube producing areas in China. The process of candied jujube indicated that WWCJ was mainly consisted of glucose, glucan and very low levels of other carbohydrates. As far as we know, there were no reports on WWCJ being used for the production of BC.

According to previous report (Song, Li, Seo, Kim & Kim, 2009), the pretreatment of starchy wastes would increase the production of BC. To investigate the efficiency of hydrolysis for WWCJ, the processes of acid-hydrolysis at 70, 80 and 90 °C were tested (Li et al., 2010). The hydrolysis temperature of 80 °C was chosen to hydrolyze WWCJ considering the efficiency and the cost of energy

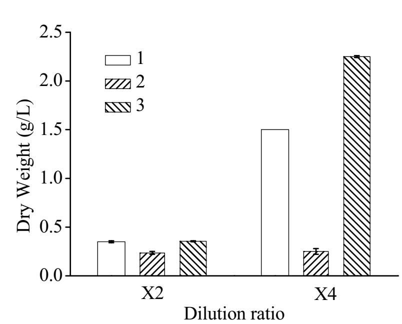

Fig. 2. Yields of bacterial cellulose after six days of culture of *G. xylinus* with cultures of (1) WWCJ media, (2) WWCJ without ammonium citrate and (3) WWCJ hydrolysate media.

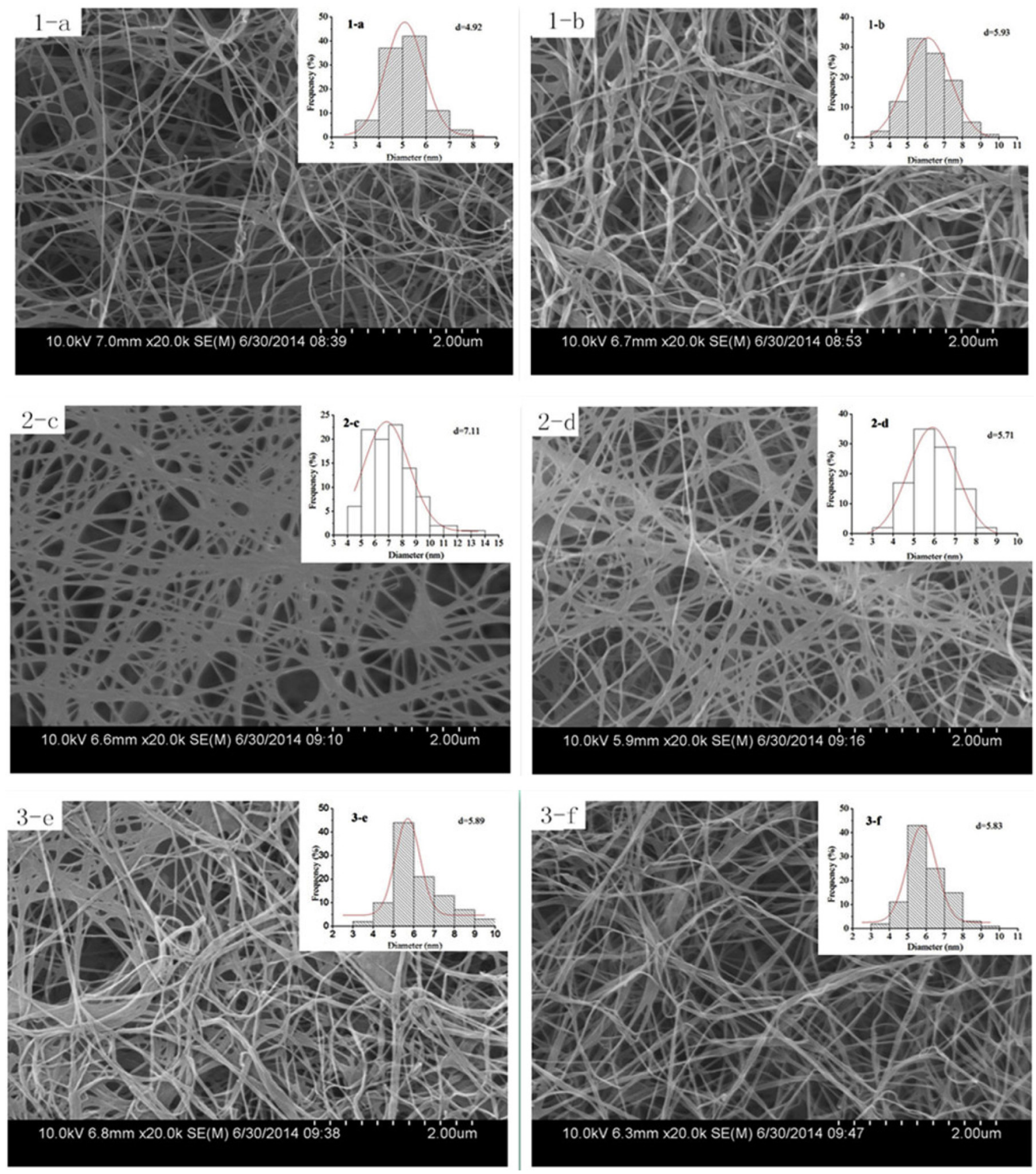

Fig. 3. SEM of freeze-dried BC from cultures of (1) WWCJ media, (2) WWCJ without ammonium citrate media, (3) the pretreatment WWCJ media. (The BC was harvested after 6 days of culture. The figures of fiber diameter show results from calculations of the diameter of fibers from (1) WWCJ media, (2) WWCJ without ammonium citrate media, (3) the pretreatment WWCJ media.).

consumption. After 3 h hydrolysis, the glucose concentration in hydrolysate was 38 g/L which was almost constant during the following hydrolysis (Fig. 1). This concentration was increased 58% compared with that of WWCJ before hydrolysis.

The first batch of experiments in which *G. xylinum* was grown on WWCJ showed that it was advantageous to dilute the hydrolysate in order to facilitate BC production (Fig. 2). The bacterium grew well in all diluted media. The results indicated that similar amounts of glucose were consumed, namely around 20 g/L of glucose during 6 days of cultivation. The pH value of the media decreased during the cultivations, from around pH 2 to around pH 3.

The results indicated that ammonium citrate was a key factor for production of BC in those media. The volumetric yield of BC (DW) in a two-fold dilution of the WWCJ with ammonium citrate was 1.5 g/L (Fig. 2), which was increased five folds compared to that of the WWCJ without ammonium citrate (0.25 g/L). The volumetric yield of BC (DW) in a two-fold dilution of the WWCJ and hydrolysate were 1.5 g/L and 2.25 g/L, respectively (Fig. 2). The results indicated that the amount of favorite carbon source was increased by acid-hydrolysis. When the hydrolysate and WWCJ were diluted four folds, the volumetric yields of BC were both decreased to 0.35 g/L, which meant that other carbon source would be utilized by *G. xylinum* after the favorite carbon source was consumed.

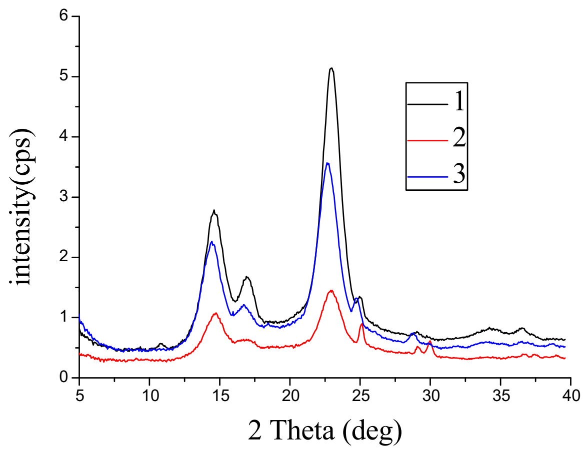

Fig. 4. X-ray diffraction diagrams of BCs produced by (1) WWCJ medium, (2) WWCJ without ammonium citrate medium, (3) the pretreatment WWCJ medium.

Table 1
Reflective angle, d-spacing and crystallinity index of BC productivity from different media.

Sam	2θ			
	Cr I	1#/d_{1-10}	2#/d_{110}	3#/d_{200}
1#	80.92	14.600(6.06)	16.900(5.24)	22.950(3.87)
2#	64.89	14.750(6.00)	16.700(5.30)	22.900(3.88)
3#	75.42	14.450(6.12)	16.700(5.30)	22.650(3.92)

After evaluating the initial results, two-fold dilution was selected for further experiments.

3.2. SEM

Scanning electron microscope (SEM) displayed the structure of the cellulose membranes at a magnification of 20,000 times (Fig. 3). The Image-Pro Plus software was used to measure the diameter size of BC. The fracture surface morphology of the BC pellicles from different media is essentially the same and the histograms (Fig. 3) based on the SEM images illustrate a small average diameter and a relatively narrow diameter distribution for the nanofibers of the BC pellicles. The fiber distribution data indicate that BC samples produced from difference media exhibited the same interweaving with similar fiber sizes (3–14 nm). The special structure implied that those BCs might be potential material for special uses. The average diameter was around 5.9 nm, which was almost the same as those of BC produced from other waste feedstock (Kose et al., 2013; Zhang, 2013). Therefore, from static culture, the diameter of BCs mostly depends on culture condition rather than feedstock sources.

3.3. XRD

XRD patterns of various samples obtained in this study were shown in Fig. 4. The upper curve is a typical XRD pattern of BC. As is shown in Fig. 4, three major characteristic peaks standing for crystal plane <−1 1 0>, <1 1 0>, <2 0 0> were observed in XRD results of BC samples, which suggested that both BC samples were in cellulose I-β form. Moreover, the d-spacing of BC membranes from various media were different (Table 1). So, the discernment in crystallinity index due to different media could be explained by the discernment of hydrogen bonding between the cellulose chains, which was almost the same as those of BC produced from other waste feedstock (Kose et al., 2013; Zhang, 2013). The crystallinity of BC produced from pretreatment WWCJ medium (75.42%) was higher than that from WWCJ without ammonium citrate (64.89%), but it was lower than that from WWCJ medium (80.92%) under the condition of 100 g/L.

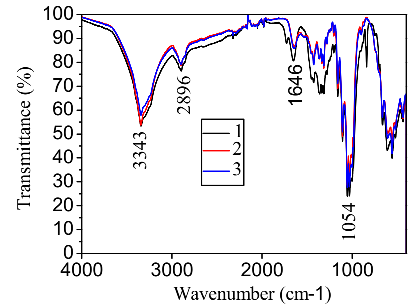

Fig. 5. FTIR spectra of BCs produced by (1) WWCJ medium, (2) WWCJ without ammonium citrate medium, (3) the pretreatment WWCJ medium.

3.4. FTIR

FTIR technique was used to obtain the specific functional groups or chemical bonds in BC samples. The FTIR spectra of the BC membranes were measured with the wave number of 4000–400 cm^{-1} as is shown in Fig. 5. The various BC samples showed several important peaks of BC at 1054 (stretching of C—O), 1646 (stretching of C=O), 2896 (stretching of CH) and 3343 (stretching of C=O) (Brown, 1886; Lin et al., 2014). The results confirmed that three BC exhibited similar chemical binding.

4. Conclusions

The results of this study show that there is a great potential in utilizing waste water of candied jujube-processing industry for the production of bacterial cellulose. WWCJ can be used as a low cost and abundant raw material which can be easy hydrolyzed to glucose by acid pretreatment.

Hydrolysis at 80 °C for 3 h increased the glucose content by 58% compared with that of the original WWCJ. Using *Gluconacetobacter xylinum CGMCC No.2955* and pretreatment of waste jujube juice medium can achieve a higher BC productivity (0.375 g/L/d) in 6 days' cultivation. The structure studies indicated that the fiber size distribution was 3–14 nm in those media with an average diameter being around 5.9 nm. The crystallinity index of BC from pretreatment medium was lower than that of without pretreatment medium and BCs from various media had similar chemical binding. Ammonium citrate was a key factor for improving the production of BC in those media and crystallinity index of BC. Thus, utility of WWCJ as feedstock for the production of BC is not only economical but also environment friendly. Further studies on enhancing BC productivity in waste jujube juice system and materials properties analysis will be the next challenge from application aspects.

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