



Preparation of bacterial cellulose film from rotten fruits for mulching film application

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Abstract

This research aims to reduce production capital costs and added value to natural products. The bio-mulching film was prepared by bacterial cellulose (BC) “*Acetobacter xylinum*”, extracted from three rotten fruits, grape, coconut, and pineapple under standard tests in the laboratory. The analysis from the FTIR technique confirmed to cellulose molecular vibration of BC films. XRD pattern was matched to structure crystallinity of JCPDS standard file which possessed a high percentage of crystallinity. The SEM micrographs were also revealed the 3D nanofiber network structure. The absorption capability of BC films could highly hold water in its structure. In addition, the mechanical properties of BC films came from rotten coconut, given the highest tensile strength (7.2 ± 1.1 MPa) according to nano-fiber symmetric with its dense structure. Nevertheless, the soil burial testing emphasized BC films could reduce soil temperature and increase moisture content in the soil as well. The biodegradation rate of BC films in 30 days was moderately fair. The BC film from rotten coconut had the slowest biodegradation rate (approximately 22.3 4.2%), applicable to biodegradable mulching film.

1. Introduction

Plastic mulching films are used in many applications, including plant propagation, tissue culture, field crop farming, and biotechnology [1]. Plastic mulching films can control soil temperature, scatter light, improve water use efficiency, and increase plant growth [2]. Commonly used films made of polypropylene (PP), polyethylene (PE), low-density polyethylene (LDPE), and high-density polyethylene (HDPE) do not degrade naturally because they are mostly derived from petrochemical sources [3,4]. When these plastics are used, a considerable volume of difficult-to-remove plastic garbage is produced. Although there are some biodegradable plastics derived from petrochemicals, such as polyvinyl acetate (PVA) and polylactic acid (PLA), polyhydroxy butyrate (PHA), and polybutylene succinate (PBS) [4,5], they are frequently extremely expensive for the target application. As a result, the development of bio-mulching films made from natural materials is an important technical step toward achieving future clean agricultural production.

For the above reasons, mulching films from natural materials, e.g., bacterial cellulose (BC) is an interesting concern, because they have lower costs whilst being degradable [6]. BC is produced by several types of bacteria such as “*Acetobacter xylinum*” species which are

found in rotten fruits e.g., apple, banana peel, and mangosteen [7,8]. BC structures are arranged in a 3D network structure, with more hydroxyl groups, providing a porous geometry with gas exchange [9,10]. BC has substantial crystallinity, water holding capacity, non-toxic, high resistant degradation, and higher purity, without lignin and hemicellulose [10,11]. These properties are attractive biocompatibility with its mulching film application. At present, BC is used for many applications in various fields such as the medical and pharmaceutical industries. K.A Zahan *et al.* [12] was successfully produced BC from *A. xylinum* bacterial for antimicrobial film application and improved antimicrobial properties by lauric acid (LA). The results showed that blending of BC with LA generates a good inhibition effect towards the growth of *Bacillus subtilis*, while the effect on the growth of *Escherichia coli* was absent [12]. But for agricultural applications such as mulching film has not been used because it is difficult to prepare for the film. Typically, rotten fruit that has been cultured in a lab is used to produce *A. xylinum* preparations. In contrast, the purpose of this research was to prepare *A. xylinum* and develop it into a film using rotten fruit derived from industrial waste. Therefore, this research will benefit the agricultural industry significantly. Additionally, no research has been found to develop *A. xylinum* as a mulching film, and neither has its usage been fully investigated.

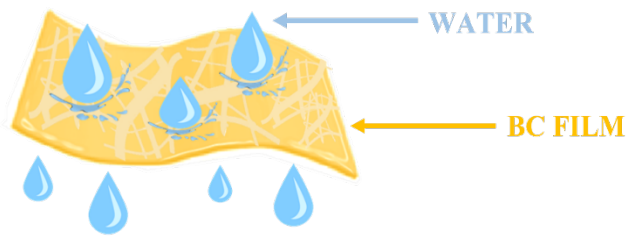


Figure 1. Schematic of the model BC film.

The main purpose of this research is to reduce waste, well prepare the BC films, added value to the mulching film by using natural materials (rotten fruits e.g., grape, coconut, and pineapple) as raw material. The optimum conditions were further investigated for the bio-mulching film for agriculture applications. Finally, the proposed model of BC film (Figure 1) was thin film, non-toxic, soil temperature control, and water release control.

2. Experimental

2.1 Materials

A. xylinum bacterial extract from rotten grape, coconut, and pineapple obtained from Able Food Marketing Co., Ltd (Bangkok, Thailand). Deionized water (DW; pH 7.12), K_2HPO_4 , $(NH_4)_2SO_4$, and $MgNO_3$ were prepared in our NANOTEC Laboratory. Glycerol (minimum assay: 99.5%, Analytical grade) was purchased from Sigma-Aldrich Pte. Ltd. (Singapore).

2.2 Synthesis of BC from *A. xylinum* bacteria with different raw materials

Bacterial cellulose used in this experiment was produced from *A. xylinum*. This was obtained from Biology Laboratory, National Nanotechnology Center, (NANOTEC). *A. xylinum* was prepared by starter media. The fermentation starter medium was composed of 25 g glucose, 2.5 g yeast extract, 2.5 g K_2HPO_4 , 2.5 g $(NH_4)_2SO_4$, and 0.05 g $MgNO_3$. in 500 mL distilled water. Then it was further treated at 20°C for 24 h. The culture medium was initially prepared by adding 3 mL. *A. xylinum* into starter media at room temperature. The prepared BC sheet was then statically cultured at 25°C for 7 days. The purified BC sheet was finally obtained from the above BC sheet after treatment with 0.1 M sodium hydroxide solution at 80°C for 20 min. and thoroughly washed with distilled water several times until its color changed to white. Bacterial cellulose was then suspended and stirred with water in the weight ratio of the BC sheet (20%w/v) for 1 h. This suspension was used for solution casting.

2.3 Preparation of BC film by solvent casting method

20 %w/v BC with different raw materials were dissolved in 100 mL deionized water. 0.5 mL glycerol was added to the BC solution as a plasticizer. It was stirred and heated at 70°C for 3 h, then cooled to room temperature for removing air bubbles. The mixture solution was further poured into a 10 cm diameter acrylic plate (30 g solution per plate) and dried in an oven at 60°C for 24 h. All composite films were characterized.

2.4 Fourier transforms infrared spectroscopy (FTIR) analysis

FT-IR spectral in the transmission mode of the BC films were recorded on a spectrometer (Perkin Elmer, USA) with a resolution of 4 cm^{-1} in a spectral range of 4000 cm^{-1} to 400 cm^{-1} using 6 scans per sample at room temperature.

2.5 Crystal structure analysis

The crystal structure of BC films was investigated by using an X-ray Diffractometer (Bruker, SRS-3400, Germany). The measurement mode at Cu-K α with the 40 kV. The samples were studied over a diffraction range of 2θ about 10° to 40° with a step size of 0.02° at room temperature. The percent of crystallinity was calculated as the following equation:

$$\% \text{ Crystallinity} = \frac{\text{Area of the crystallinity}}{\text{Total area of peaks}} \times 100 \quad (1)$$

2.6 Morphology by scanning electron microscopy (SEM)

A scanning electron microscope (JEOL, USA) was used to investigate the surface or cross-section morphologies of composite film morphologies. Samples were coated with a thin layer of gold. The image of the samples was magnified and digitally recorded. An accelerated voltage of 10 kV was used as the operation condition.

2.7 Water absorption measurement

Water absorption (W_A) investigation was carried out according to ASTM D570 standard method. Prepared samples were first dried at 100°C for 3 h and then kept in a closed container at 100% RH. The total mass of water absorbed by the sample for 30 days was determined. The percentage of water absorption was calculated as follows:

$$W_A = \frac{W_w - W_d}{W_d} \quad (2)$$

where W_w and W_d are the wet and the dried weights of the sample, respectively.

2.8 Mechanical properties

Tensile strength (TS) and elongation at break (EB) of BC films were determined, following ASTM standard method D882 - 18 (2018), using a Universal Testing Machine (Lloyd Instrument, Hampshire, UK) at room temperature, with $47 \pm 3\%$ relative humidity. Ten 10 mm \times 70 mm film samples, with an initial grip length of 50 mm were used. Each composite film was clamped and deformed under tensile loading using a 100 N load cell with a crosshead speed of 30 mm \cdot min $^{-1}$ until the samples were broken. The maximum load and the final extension at break were used to calculate TS and EB.

2.9 Soil temperature and moisture analysis

The temperature and moisture analysis automatic recording probe device was used to measure the temperature and moisture according

to the requirements for the determination of ground temperature [13]. The buried depth of the probe was 15 cm, and the data were recorded once every 24 h, with five repetitions. Finally, the daily average temperature and moisture were calculated.

2.10 UV-light barrier properties

UV-light barrier properties of BC films were measured in the UV-VIS spectra range using a spectrophotometer (UV-2600, SHIMADZU, JAPAN), settled in transmittance mode. The spectral graphs and the transmittance values obtained in the UV region allowed evaluation of the light barrier properties of the BC films in the UV region and the effect of nano-cellulose fiber on the transmittance values.

2.11 Biodegradation test

The soil biodegradation was tested for three months. According to the method described by Di Franco *et al* [14] in plastic boxes (80 cm × 15 cm × 10 cm) containing soils and composite films were cut (2 cm × 8 cm) at room temperature (27°C to 33°C) and soil humidity was maintained at 30% to 40% by a sprinkling of water. Weight loss (W_L) during soil burial was measured according to Di Franco *et al* [14]. The mass of each sample was weighed before and after degradation. The weight loss of each film sample was obtained using the following equation:

$$W_L(\%) = \frac{(M_1 - M_2)}{M_1} \times 100 \quad (3)$$

where M_1 is the pre-degraded dry weight of the film composite and M_2 is a dry weight of the sample after degradation.

2.12 Statistical analysis

Data analysis was performed with an SPSS software system (Origin Pro 8.0 Version 2020, USA). Each experiment was repeated

at least three times. The experimental data were subjected to a one-way analysis of variance (ANOVA). The mean comparisons were run by Duncan's multiple range test with the level of significance set at $p \ll 0.05$.

3. Results and discussion

3.1 The growth of BC films by fermentation biological method

The cultures of *A. xylinum* bacterial cellulose were compared to various rotten fruit extracts. It was found that the BC extract from rotten grape and coconut were more dominant to produce cellulose film fibers than BC extract from rotten pineapple. The film fibers from rotten grape and coconut are coordinated completion, smooth and uniform under medium culture. Compared to the BC extract from rotten pineapple was incomplete coordination film fibers. The film fiber formation was not found in the medium culture of the flask. It was rough but uniform. This was shown in Table 1 and Figure 2.

3.2 FT-IR analysis of BC films

FT-IR spectroscopy of BC produced from rotten grape, coconut, and pineapple extracts was characterized. The FT-IR spectra of BC film from various raw materials were similar, which demonstrated that the cellulose structure has a similar chemical structure. This was shown in Figure 3. The peak of BC films at wavenumber around 3400 cm^{-1} and 2900 cm^{-1} , characterized the stretching vibration of the O-H and C-H bonds in the polysaccharides form [15,16]. The spectra of BC films were dominant at wavenumber around 3300 cm^{-1} , characterizing the hydroxyl group in cellulose structure [15,16]. The absorption peak at wavenumber around 1300 cm^{-1} and 1100 cm^{-1} and the other signature peak of cellulose structure correspond to the C-H bending and C-O-C stretching bonds for the parts of polysaccharide form [15,16].

Table 1. The growth of BC films with increasing time by biological method.

Number of days	First Flask BC extract from rotten grape	Second Flask BC extract from rotten coconut	Third Flask BC extract from rotten pineapple
1 - 3 Days	BC does not produce cellulose fiber. Therefore, there are no fibers formation on the surface of the culture medium	BC does not produce cellulose fiber. Therefore, there are no fibers formation on the surface of the culture medium.	BC does not produce cellulose fiber. Therefore, there are no fibers formation on the surface of the culture medium.
4 - 6 Days	BC growth, which is observed by fibrous cellulose formation on the surface of the culture medium, begins at the edge of the flask first. After that the fibers bond together to form a thin film and then floats on the medium. But the fiber areas are incomplete, resulting in a non-uniform film	BC growth, which is observed by fibrous cellulose formation on the surface of the culture medium, begins at the edge of the flask first. After that the fibers bond together to form a thin film and then floats on the medium. But the fiber areas are incomplete, resulting in a non-uniform film.	The fibers of BC growth appeared. However, the resulting film was incomplete and had poor coordination due to the external centrifugal force. No bacterial cellulose fibers are formed.
7 - 9 Days	The fibers of the BC are tightly gathered to form a complete film, a uniform, and smooth film. Moreover, the film thickness increase depending on the stopping time of medium culture growth.	The fibers of the BC are tightly gathered to form a complete film, a uniform, and smooth film. Moreover, the film thickness increase depending on the stopping time of medium culture growth.	The film does not continue to grow due to centrifugal force. The fibers are not well gathering. In addition, the rough and unequal thickness areas are shown in all regions of the films.
10 Days	The thin film from BC extract was obtained.	The thin film from BC extract was obtained.	The thin film from BC extract was obtained.



Figure 2. The growth of BC films.

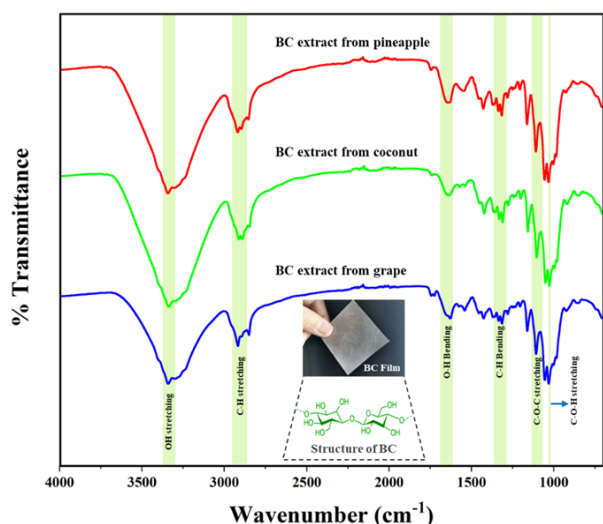


Figure 3. The FT-IR spectra of BC films obtained from different raw materials.

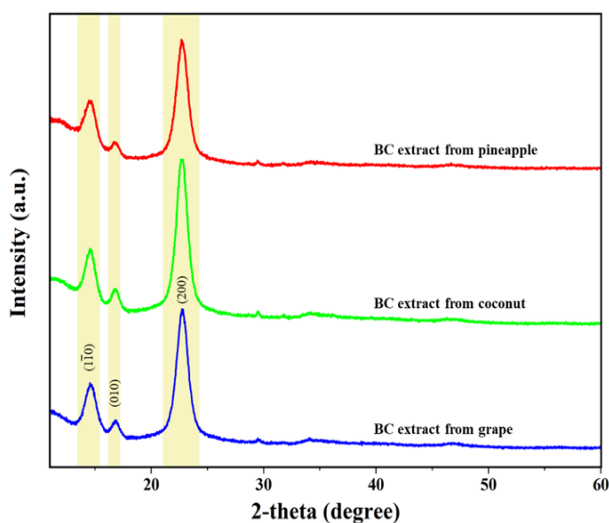


Figure 4. The XRD patterns of BC films.

Table 2. Crystallinity percentage of BC films.

Films	% Crystallinity ^a
BC extract from rotten grape	74
BC extract from rotten coconut	88
BC extract from rotten pineapple	60

^aThe percentage of crystallinity was calculated in the 15-30 range of two theta

3.3 Crystal structure analysis of BC films

BC films were analyzed by X-ray diffraction (XRD) and the results were shown in Figure 4. The cellulose structure crystallinity was also compared to the reference file [17,18]. The XRD pattern of synthesized BC films from the different raw materials indicated the sharp peaks of cellulose around 14.4°, 16.5°, and 22.7°. This was specified to the “bacterial cellulose” which has I α , I α , and I β phase index as (110), (010), and (200), respectively. These peaks for “bacterial cellulose” were corresponding to the XRD peaks (14.7°, 16.6°, and 22.5°) reported by B. Sun *et al* [19]. This is successful in the synthesis of BC films from various raw materials. In addition, XRD analysis, percent crystallinity was calculated and summarized in Table 2. The highest crystallinity percentages were identified in BC extract from rotten coconut (88%). The produced BC from rotten grape was lower (74%) and pineapple exhibited the lowest (60%) crystallinity percentage, respectively.

3.4 Morphology of BC films

The morphology of different BC films was revealed by SEM, as shown in Figure 5(a-c). The SEM images of obtained BC films had no different surface morphology appearances. All BC films were quite similar characteristics, nanofiber, random form, pure fiber, and composed of a dense network of interwoven ultrafine fiber. The results were quite similar to M. Churairat *et al* [20]. In addition, BC films had a small nano-fiber size with a compact structure, while the plant cellulose film had not shown the micro-fiber with cracks or pores, as shown in Figure 5(d).

3.5 Water absorption of BC films

Water absorption is a very important factor in the application of mulching film. Figure 6 shows the effect of absorption storage time, check after 5, 10, 15, 20, 25, and 30 days at 100%RH with different raw materials of BC films. It was found that all BC films showed an increasing trend of water absorption when storage time was increased. In addition, water absorption of different BC film materials was quite similar and had no significance. All BC films increase the water absorption because the cellulose in the BC structure has copiously hydrophilic functional groups with more -OH- [21,22] and as shown in Figure 7. Therefore, BC films extract from rotten fruits had a higher efficiency and were used to appropriate mulching film for maintaining a moist in the soil environment.

3.6 Mechanical properties of BC films

The mechanical properties of BC films were analyzed in parts of tensile strength and elongation at break, as shown in Table 3.

In this study, the tensile strength and elongation at the break of BC films could be varied to 4.9 MPa to 7.2 MPa and 52.8% to 61.8%, respectively. Moreover, this research found that BC film from rotten coconut extract had higher tensile strength than other materials. Because the BC film from rotten coconut extract is likely a dense and

symmetric nano-fiber structure than rotten grape and pineapple extract. This result corresponded to surface morphology in Figure 5(a-c). Furthermore, the mechanical properties of different BC film materials were of quite different significance after analysis by the ANOVA method.

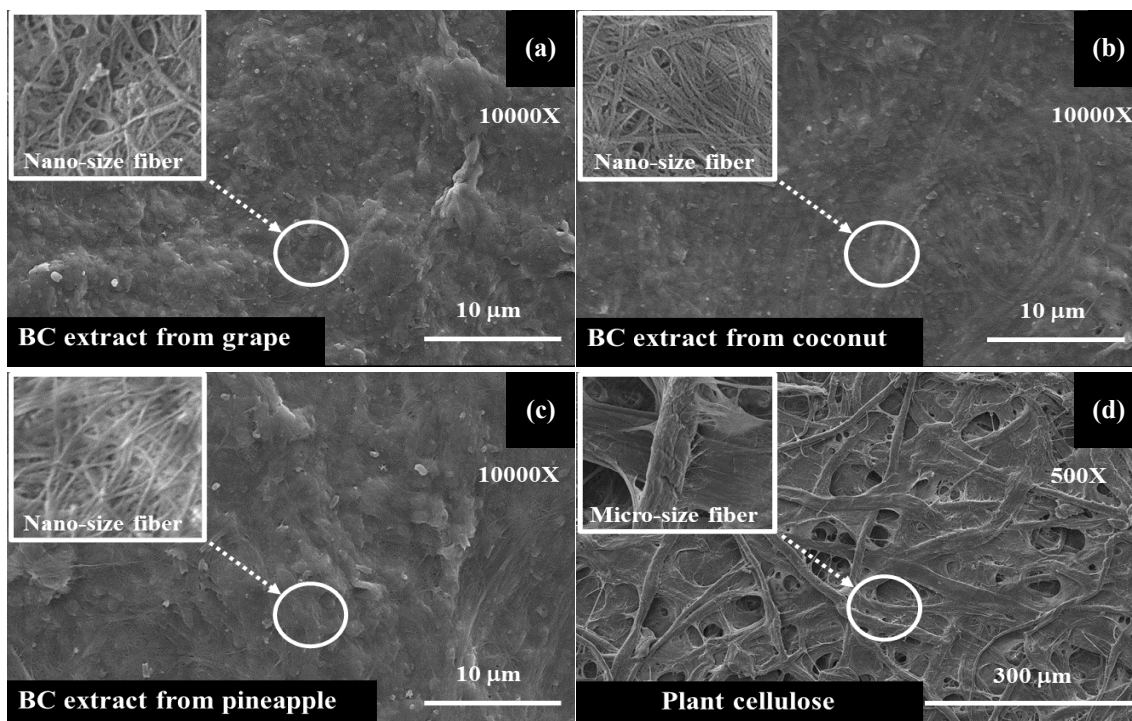


Figure 5. The surface morphology of BC films and plant cellulose film.

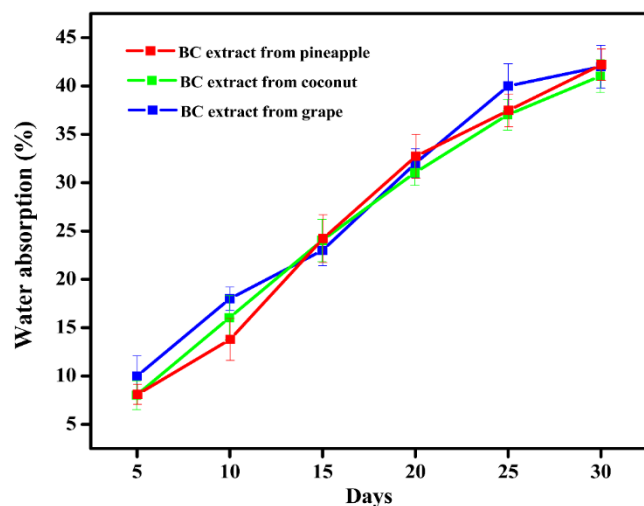


Figure 6. Water absorption of BC films.

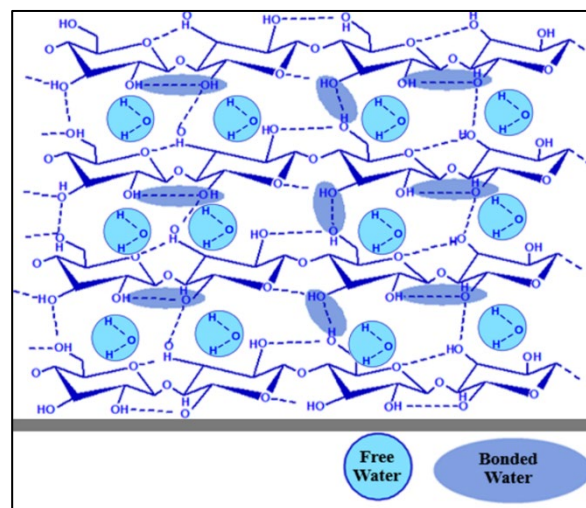


Figure 7. The intramolecular bonding of BC films.

Table 3. The mechanical properties of BC films.

Films	Tensile strength (MPa) ^a	Elongation at break (%) ^a
BC extract from rotten grape	5.3 ± 1.0	52.8 ± 15
BC extract from rotten coconut	7.2 ± 1.1	61.8 ± 13
BC extract from rotten pineapple	4.9 ± 1.4	54.4 ± 18

^a Data reported are average values ± standard deviations. Values within each column with different letters are significantly different ($p < 0.05$).

Table 4. Soil temperature and moisture of BC films.

Films	Time (days)	Soil temperature (°C) ^a	Soil moisture (%RH) ^b
BC extract from rotten grape	Initial	35.6 ± 1.5	17.1 ± 0.2
	5	34.1 ± 1.2	21.6 ± 0.8
	15	27.2 ± 1.6	29.2 ± 0.2
	30	23.7 ± 1.2	34.5 ± 0.1
BC extract from rotten coconut	Initial	35.6 ± 1.5	17.1 ± 0.2
	5	33.3 ± 1.1	23.5 ± 0.2
	15	25.3 ± 1.1	36.7 ± 0.4
	30	21.1 ± 1.4	39.8 ± 0.8
BC extract from rotten pineapple	Initial	35.6 ± 1.5	17.1 ± 0.2
	5	33.9 ± 1.8	21.8 ± 0.3
	15	26.3 ± 1.5	30.4 ± 0.6
	30	22.2 ± 1.2	36.6 ± 0.3

The soil temperature and moisture are determined at 9:00 in 15 cm depth of the soil.

^a Means of five points measurement ± standard deviations ^b Means of five points measurement ± standard deviations

3.7 Soil temperature and moisture of BC films

On testing of BC films in the laboratory, the UV light tube stands for heat and light energy. The duration time for the given light was 8 h per day. The BC films had been studied to the effect of soil temperature and moisture. The BC films revealed their capability to lower the temperature of the soil. At the 15 cm depth level from the soil surface, the average temperature was 35.6°C. The more time for BC films, the lower the soil temperature was obvious as shown in Table 4. For 15 days period, the average temperature dramatically decreased. This could explain that the BC films protected the light to films leading to lower temperatures of soil [23]. The other reason for lower soil temperature comes from the BC film structure. The BC film structure itself could behave as a UV-blocker because of nanofiber, high crystallinity with a dense structure [23,24]. When the time for mulching film was increased to 30 days, the soil temperature was gradually changed. Nevertheless, BC film extract from rotten coconut dramatically decreased soil temperature when compared to the other extracts. The BC films also increased the soil moisture when the time was increase as shown in Table 4. From the first day until 30 days, the %RH of soil moisture was increased by 34.5, 39.8, and 36.6%RH of BC films extract from rotten grape, coconut, and pineapple respectively, these results were dependent on hydroxyl groups in the structure of bacterial cellulose. It has more effect on water absorption into the soil environment. The effect of bacterial cellulose structure causes water absorption ability due to nano-fiber size, hydrophilic function, and high porosity in the structure [25]. It could store and keep water in the BC films. The lower soil temperature and higher water absorption ability are benefits of mulching film for the agricultural field. Finally, BC film extract from rotten coconut was more distinguishing than the other extracts for reducing soil temperature and absorbing moisture.

3.8 UV-light barrier of BC films

The other crucial and essential property of BC film is a UV-light barrier. This BC film could lessen UV-light on the soil surface, reduce thermal heat into the soil and decrease water evaporation or moisture out of the soil. When BC films were tested by UV spectrophotometer, the percent of transmittance of BC films in UV-A range (320 nm to

400 nm) and UV-B range (280 nm to 320 nm) was decreased (Figure 8). The BC films are composed of nano-cellulose fiber, which has high crystallinity, dense-packed structure, causing lower UV-light transmittance [26]. The UV-light barrier of rotten fruits BC films was quite different. The BC film extracts from rotten coconut and pineapple (Figure 8(b-c)) had UV-light barrier capability better than BC film extract from grape (Figure 8(a)), observed from lower percent of transmittance at initially visible light (400 nm to 500 nm). This result is corresponding to soil temperature and moisture data emphasized to BC film extract from rotten coconut and pineapple could reduce soil temperature and enhance moisture in soil better than BC film extract from a rotten grape.

3.9 Biodegradation of BC films

A Biodegradation test was conducted for soil burial. Different BC films were buried into the soil surface for 30 days. The average weight loss measurement of BC films after the soil burial test was recorded, as shown in Figure 9. After the soil burial test, the noticeable degradation rate of BC films was revealed with degradation of 25.1, 22.3, and 29.6% weight loss of BC films extract from rotten grape, coconut, and pineapple respectively. The BC films were degraded by existing microorganisms and participated with CO₂ and H₂O [27]. Furthermore, the BC film from rotten fruits had gradually slower degradability than plant cellulose film (Figure 9). Because the plant cellulose film had lignin and hemicellulose components in the structure. They could accelerate the degradation of the film in soil [28]. However, biodegradation is governed by different factors that include polymer characteristics.

The SEM images of BC films after the soil burial test are shown in Figure 10(a-c). The surface of those films had a more irregular appearance. They obviously appear some cavities and cracking from the degradation of cellulose nanofiber structure and further promote a large interconnection hole on the surface of the BC films. Moreover, the degradation effect was also much lower than the plant cellulose film, as shown in Figure 10(d). This result was corresponding to weight loss as shown in Figure 9. The above evidence support that BC films were the appropriate agriculturally mulching film because of their higher mechanical properties and gradually slow degradation.

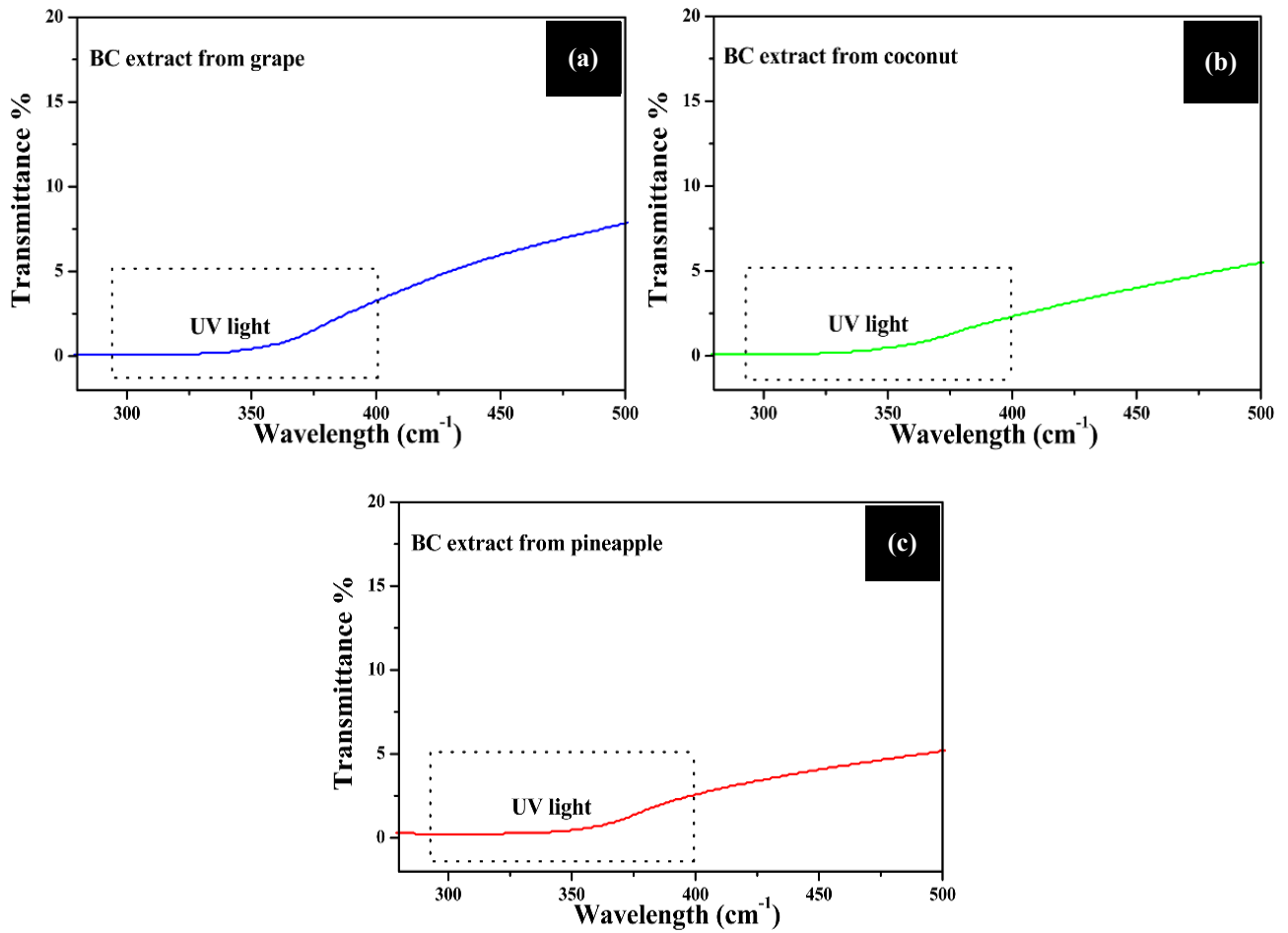


Figure 8. UV-light barrier of BC films.

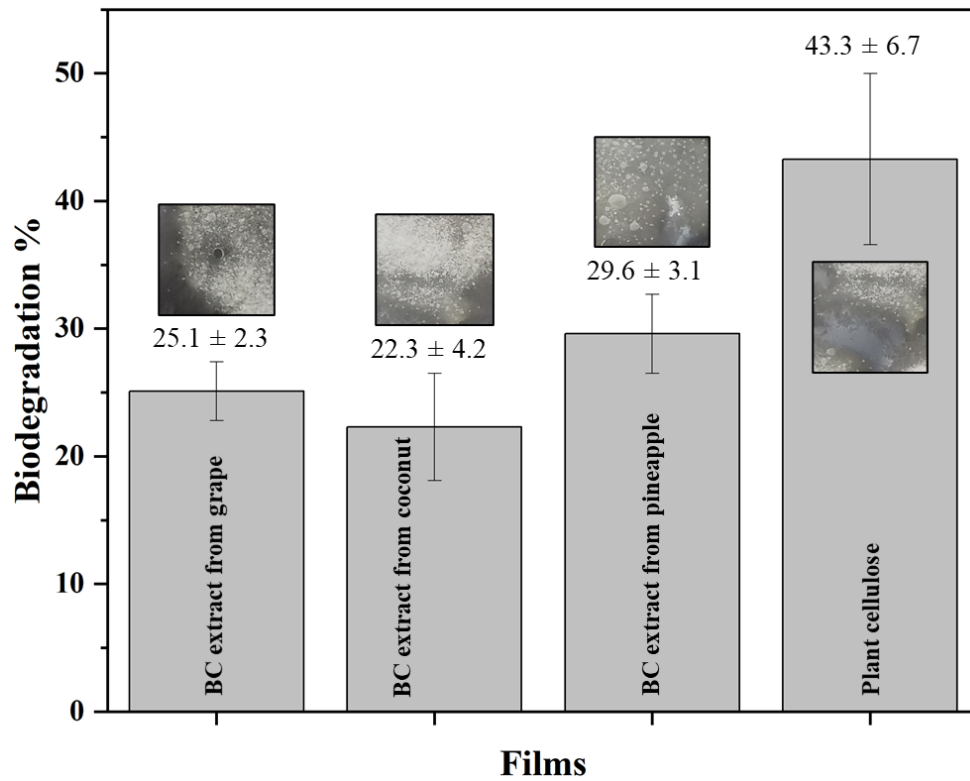


Figure 9. The average weight loss of BC films and plant cellulose film.

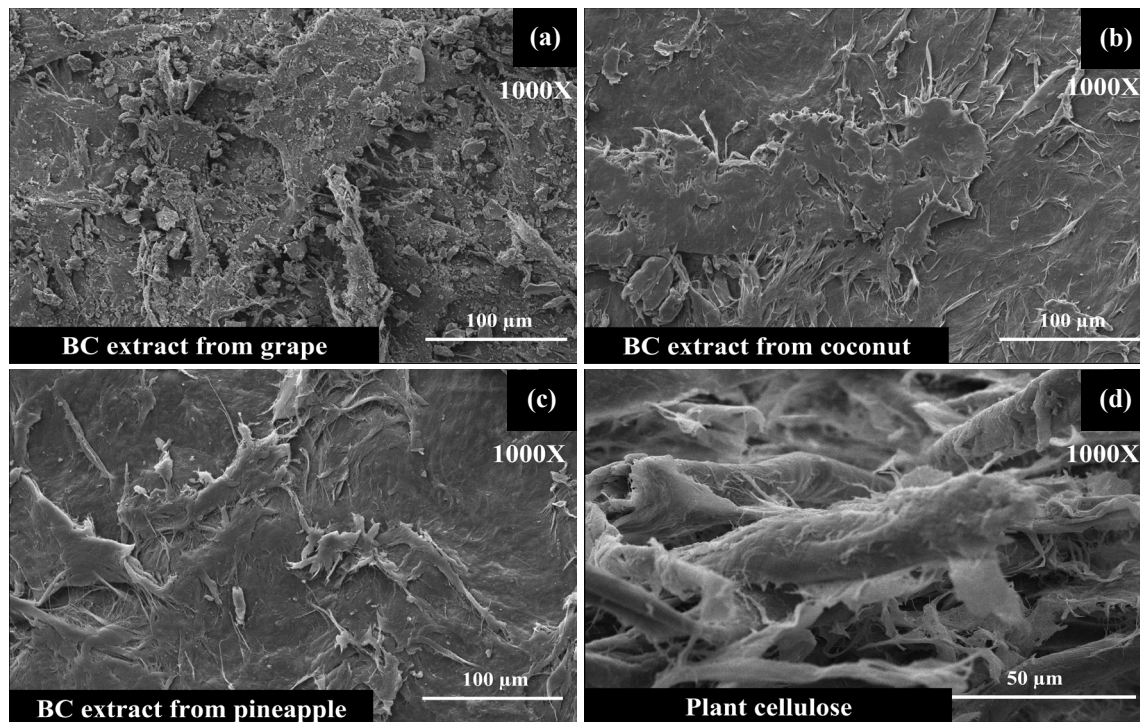


Figure 10. The surface morphology of BC films and plant cellulose film after soil burial test in 30 days.

4. Conclusions

The preparation of *A. xylinum* BC film extracted is succeeded by using the biological fermentation method. The BC film extracted from rotten coconut contains more appropriate properties than rotten grape and pineapple. Therefore, the BC film extract from rotten coconut has been matched to the requirement of agriculturally mulching film, included on good tensile strength and elongation, lower soil temperature, preserved soil moisture and gradually slow degradation BC film is an alternative selection to producing mulching films.

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