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## Materials Today: Proceedings

journal homepage: [www.elsevier.com/locate/matpr](http://www.elsevier.com/locate/matpr)

## Application of bacterial cellulose film as a biodegradable and antimicrobial packaging material

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### ARTICLE INFO

#### Article history:

Received 16 October 2019

Received in revised form 4 January 2020

Accepted 8 January 2020

Available online xxxx

#### Keywords:

Bacterial cellulose  
Acetobacter xylinum  
Packaging  
Biodegradable  
Antimicrobial  
Biopolymer

### ABSTRACT

Nowadays, conventional petroleum-based plastic had become the third-largest solid waste volume in municipal solid waste. Although recycling plastic was aimed to minimize its quantity, the recycling process still releases toxic materials into the environment. Thus, the best solution is to use biodegradable plastic. Additionally, due to the health concern of consumers, especially over the safety of food, the packaging industry had shown greater interest in the use of bio-preservatives for antimicrobial (AM) packaging. This study aimed to develop a new biodegradable and AM packaging material from bacterial cellulose (BC) film. BC is a natural biopolymer synthesized by bacteria. Here, BC was produced through a static fermentation of *Acetobacter xylinum* 0416 in the Hestrin-Schramm medium, while the AM packaging was developed using a soaking method. The BC film produced was incorporated with lauric acid (LA) at different concentration (2, 4 and 6% w/v) and different soaking time (4, 14 and 24 hr). The characteristics and functionalities of the incorporated BC-LA film were analyzed for the AM activity and biodegradation test. The results depicted that incorporation of BC film with LA provides a good inhibition effect towards the growth of *Bacillus subtilis*, while the effect on the growth of *Escherichia coli* was absent. Additionally, the pure BC film also goes through more than 50% of degradation in 3 days and 100% of degradation starting from day 7 in the soil. Further investigation had determined that the responsible degrading microbes were *Bacillus* sp. and *Rhizopus* sp. As overall, this new packaging material was successfully developed and showed wide potential applications to be used in food, pharmaceutical and medical field.

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Selection and Peer-review under responsibility of the scientific committee of the 4th International Conference on Green Chemical Engineering and Technology: Materials Science.

### 1. Introduction

Plastic manufacturer plays as one of the major roles in many sectors such as clinical, food, and pharmaceutical, especially in packaging application, while a person uses an average of 350 plastic bags per year. Unfortunately, the conventional plastic derived from petroleum resources cannot be decomposed and degraded naturally or biologically using bacteria or fungi, which subsequently stays longer in the environment and causes harmful effects to the earth [1]. The conventional petroleum-based plastic took

around 400–500 years to be degraded by itself. Although recycling plastic was aimed to minimize its quantity, the recycling process still releases heavy and toxic materials into the environment. Because of that, the best solution is to use biodegradable plastic. Additionally, due to the health concern of consumers especially over the safety of food, the packaging industry had shown greater interest in the use of bio-preservatives for antimicrobial (AM) packaging [2].

The AM packaging is one of the applications of active packaging system which define as a packaging system that can kill or inhibit spoilage and pathogenic microorganisms that are contaminating food [3]. It prevents surface growth of pathogenic microbes in food by the use of AM agents and also allows controlled release of the

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<https://doi.org/10.1016/j.matpr.2020.01.201>

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Selection and Peer-review under responsibility of the scientific committee of the 4th International Conference on Green Chemical Engineering and Technology: Materials Science.

agents into food surface during storage and distribution [2,4]. When the packaging system acquires AM activity, the packaging system could limit or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms [5]. The AM packaging system can be achieved by incorporating active substances into the packing-based material through various techniques including soaking, spraying, coating and crosslinking method [5].

The production of biodegradable packaging derived from biopolymer-based material is not totally aimed to replace the conventional plastic, but it is one of the alternatives to reduce the solid waste volume in municipal solid waste facilities (MSW) [6]. The origin of biopolymer-based materials comes from various types of natural renewable resources such as polysaccharides, proteins, cellulose, and lipids. The use of biopolymer-based materials is also convenient due to the lower environmental impact as compared to conventional plastic materials. However, these natural biopolymers also possess poor mechanical and water vapor barrier properties due to its hydrophilic nature [4,6]. Interestingly, the properties of biopolymer could be improved by using modification techniques during its formation process and further enhanced its applications by the incorporation of AM agents.

Thus, this study aimed to develop a new biodegradable and AM packaging material from bacterial cellulose (BC) film. BC is a natural biopolymer synthesized by bacteria. The most effective producers of BC are the *Acetobacter xylium*. BC has similar chemical properties with plant-based cellulose. As compared with plant-based cellulose, BC exhibit the absent of lignin and hemicelluloses (extremely pure), an ultra-fine network structure, high biodegradability, strong mechanical strength and high degree of polymerization and crystallinity [7]. Furthermore, BC can be produced in a variety of substrates, and its structure can be modified during the fermentation process to achieve any specific target or requirement.

## 2. Experimental procedures

### 2.1. Production of bacterial cellulose (BC)

BC was produced in a static fermentation. The fermentation medium (Hestrin-Schramm medium) was prepared by mixing 20 g of glucose, 2.7 g of disodium hydrogen phosphate, 5 g of peptone, 5 g of yeast extract and 1.5 g of citric acid in a 1 L distilled water. After homogenous, the fermentation medium was autoclaved at 121 °C for 15 min. Then the fermentation medium was cooled to 27 ± 1 °C. 200 ml of fermentation medium was poured into five sterilized containers aseptically. Then, 10% v/v of inoculum *Acetobacter xylium* 0416 was transferred into each container in an aseptic condition and incubated at 27 ± 1 °C for 7 days [8].

### 2.2. Preparation of lauric acid (LA) solution

In this study, LA had been used as an antimicrobial (AM) agent. The LA (in a solid form with 99% concentration) was weighted and dissolved in 100 ml distilled water to obtained different concentration at 2, 4 and 6% w/v. The mixture was heated and stirred using a hot plate at 85 ± 1 °C until a homogenous solution was obtained.

### 2.3. Preparation of BC-LA films

After harvesting the BC from the fermentation container, the BC was washed with plenty of tap water. Then, the BC was boiled in 200 ml of distilled water for 1 hr to remove all the cell entrapped within the pellicle. After that, the BC was drained and rewashed with distilled water for several times before soaked in a distilled

water until the pH of the water become 7.0 [8]. Then, the BC was soaked for 14 hr in the LA solutions at different concentration (2, 4 and 6% w/v) prepared in section 2.2. As for control, the BC was also soaked in the distilled water (0% of LA). After that, the BC was taken out from the solutions and dried at 27 ± 1 °C (natural drying process) for 24 hr. The dried BC-LA film was stored in a sealed plastic bag for further analysis. The experiment was repeated using 2% w/v LA solutions for different soaking times at 4 and 24 hr, respectively [9].

### 2.4. Zone inhibition assay for antimicrobial (AM) activity test

The AM activity test was carried out using the disc diffusion method. The cultures of *Bacillus subtilis* and *Escherichia coli* were aseptically overlaid onto hard nutrient agar plates using a cotton swab. Each BC-LA and control film (pure BC) prepared in section 2.3 was cut into a circular shape with a diameter of 5–6 mm using a sterilized puncher and placed on the bacterial lawns using a sterilized caliper. The plates were incubated at 37 °C for 48 hr in the incubator. The plates were visually examined for zones of inhibition around the films. The size (diameter) of the inhibition zone was measured at two cross sectional-points, and the average was taken as the inhibition zone [2,3,10].

### 2.5. Biodegradation test

The biodegradation test was performed using pure BC film. The BC film was cut into 100 × 150 mm. The initial weight and tensile strength of the film were also recorded. Then, a 13 × 20 inch polybag was filled with compost soil. Then, the BC film was buried at 5 cm from the bottom of the polybag and left for 1 day at the natural environment. The procedures were repeated for 3, 5, 14 and 21 days [11,12]. After that, the BC film was harvested from the soil and analyzed for its final weight, length, and tensile strength. The final weight was measured using an analytical balance, while the length was measured using a ruler. The tensile strength was measured using a Universal Testing machine at a crosshead speed of 10 mm/min.

### 2.6. Determination of BC degrading microbes

Firstly, Potato Dextrose Agar (PDA) and Nutrient Agar (NA) were prepared in the petri dish. Then, the remaining degraded BC films obtained in section 2.5 were placed on the surface of the agar and incubated at 37 °C for 3 days in the incubator. The growth of microbes on both agar was observed every 24 hr. The grown microbes were characterized by the determination of its morphology using naked eyes and under the microscope using a wet mount method. Here, a drop of water was placed on a clean piece of a slide. The microbes were then isolated by using an inoculation loop and mixed in the drop of water until it becomes suspended. Then, the coverslip was placed on the suspension without introducing any bubbles. The slide with the specimen was then placed on the microscope at magnifications of 40× and 100× to identify the types of grown microbes [13].

## 3. Results and discussion

### 3.1. Antimicrobial (AM) activity analysis

The AM activity of bacterial cellulose-lauric acid (BC-LA) films against *Bacillus subtilis* and *Escherichia coli* were expressed in terms of inhibition zone diameter. The disc diffusion agar had been used as a prototype of wrapping foods which could be used to estimate

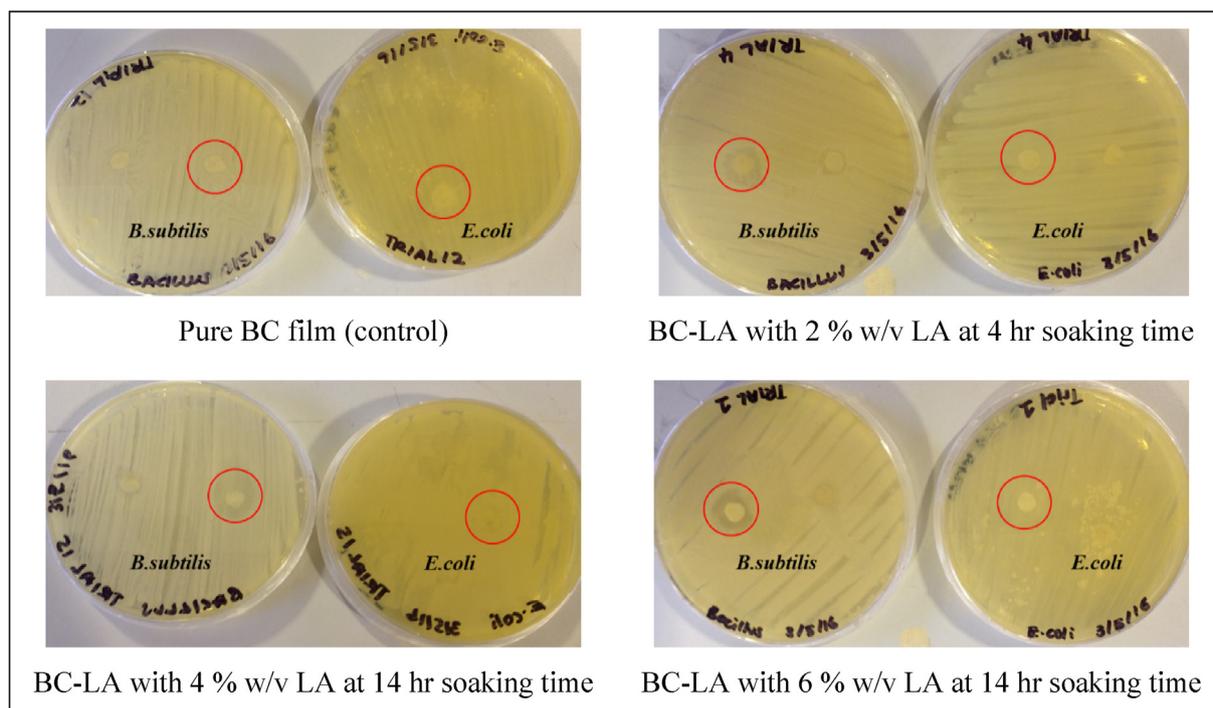


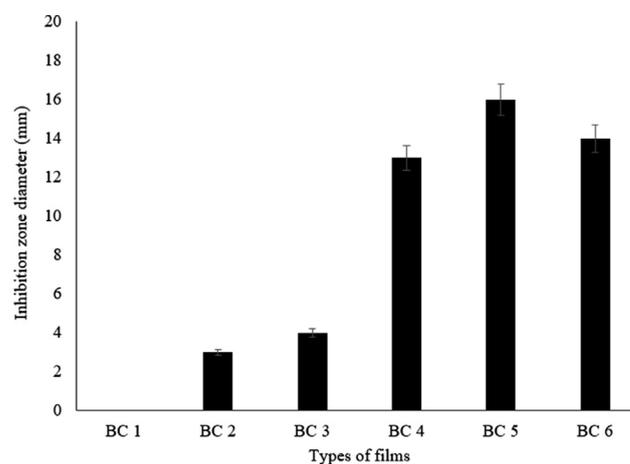
Fig. 1. The inhibition zone of pure BC film and different type of BC-LA films on *B. subtilis* and *E. coli*.

the amount of the AM agent migrates from the film when in contacts with the contaminated surfaces [2,14].

Fig. 1 shows the inhibition zone obtained during the disc diffusion agar test. The *B. subtilis* and *E. coli* were selected because both strains are commonly known as foodborne microbes. Additionally, *B. subtilis* represents a gram-positive bacteria, while *E. coli* represents a gram-negative bacteria, which could help in explaining the inhibition effect of LA well. From Fig. 1, the inhibition zone was absent in the case of pure BC film and *E. coli*, while the inhibition zone of *B. subtilis* could be observed by a different type of BC-LA films. Here, the larger the diameter of inhibition zone, the larger the AM effect of BC-LA films towards the foodborne microbes. In short, the incorporation of LA into the BC films had developed an inhibitory activity by the BC-LA film towards *B. subtilis* only which in agreement with previous research who determined that the AM effect of LA only effective towards the gram-positive bacteria [3,5,15].

The effect of LA concentration and soaking time on the inhibitory activities toward *B. subtilis* can be observed in Fig. 2. Fig. 2 depicted that, the inhibition zone of BC-LA films was recorded between 13 and 15 mm when the concentration of LA varied between 2 and 6% w/v. Although the differences recorded was insignificant, the highest inhibitory effect was shown by the BC-LA film prepared with 4% w/v of LA. In term of soaking time, the longer the BC film was soaked in the LA solution, the better the inhibitory effect. Here, the effect of soaking time shows more significant impact towards the inhibition zone, where in the case of BC-LA film with 2% w/v of LA, the inhibition zone increased by 77% when the soaking time was extended from 4 to 24 hr. This is due to the ultra-fine fiber network structure and hydrophilic nature of BC, which enhanced the entrapment of a high amount of LA in the film structure, thus improved the inhibitory activities.

The efficacy of LA as the most potent AM agent have been reported since a long time ago [16]. Lauric acid (C12:0) is a medium-chain free fatty acids (FFAs) and a minor component in the sebum, which secreted from sebaceous glands as sebum triacylglycerides [17]. The FFAs was recognized to have a broad spectrum of AM activity towards various types of microbes, especially



Legends:

BC 1: Pure BC

BC 2: BC-LA with 2 % w/v LA at 4 hr soaking time

BC 3: BC-LA with 2 % w/v LA at 14 hr soaking time

BC 4: BC-LA with 2 % w/v LA at 24 hr soaking time

BC 5: BC-LA with 4 % w/v LA at 14 hr soaking time

BC 6: BC-LA with 6 % w/v LA at 14 hr soaking time

Fig. 2. The diameter of inhibition zone for *B. subtilis* by pure BC film and different type of BC-LA films.

the FFAs with chains length of C8–C18. Although the mechanism of FFAs in killing the microbes was not well known, Anzaku et al. [15] reported that the FFAs disrupt the cell membranes of the microbes.

### 3.2. Biodegradation analysis

Fig. 3 shows the physical appearance of pure bacterial cellulose (BC) film during the biodegradation study. The changes could be observed since day one of the study, which indicates that the

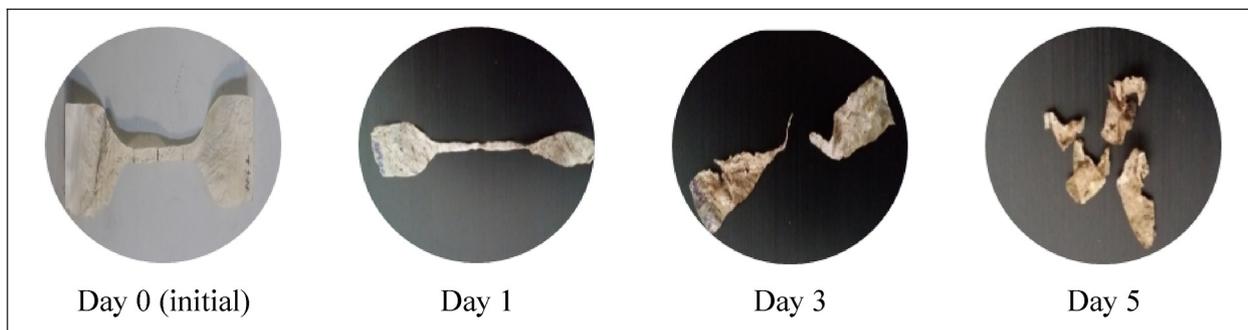


Fig. 3. The physical appearance of BC films during biodegradation test.

biodegradation of BC film was started. This will cause the physical changes of BC film, especially in terms of size and shape. The size of BC film became smaller as the compactness of the BC film has been decreasing throughout the period, which finally causes the BC film to lose its initial shape and thickness [12,18].

Fig. 4 depicts the detail changes in physical and mechanical properties of BC film in three different aspects. Fig. 4(A) shows that the weight of BC film decreased from 0.21 g on day 1–0.15 g on day 2. The weight was further decreased to 0.05 and 0.04 g on day 3 and 5 respectively. On the other hand, Fig. 4(B) shows that the length of BC film decreased from 100 mm on day 1–90 mm on day 2. Then the length was decreased to 33 and 9.3 mm on day 3 and 5 respectively. Meanwhile, Fig. 4(C) shows that the tensile strength of BC film was dramatically decreased from 30.35 MPa on day 1–8.66 MPa on day 2. The tensile strength of BC film could not be measured on the third day onwards because the BC film had

lost its initial shape. Interestingly, the BC film was fully degraded on the seventh day of the experiment. This result is way better than the result obtained by Abdul Aziz [12] who reported that the 100% degradation of BC incorporated with chitosan and starch (size  $200 \times 200$  mm) was achieved within 18 days of composting. While Alshabanat [19] reported that, the biodegradation of date palm fiber incorporated with linear low-density polyethylene (LLDPE) (size  $20 \times 40$  mm) reach around 32% of weight loss after 40 days of burial.

The changes in physical and mechanical properties of BC film has resulted from the biodegradation process occur in the soil [20]. When BC film was directly composed, the microbial consortium inside the soil transforms the available biodegradable components into carbon dioxide, methane, water, and biomass [18].

In order to determine the responsible microbes that degrade the BC film, the remaining BC film after the experiment was placed on

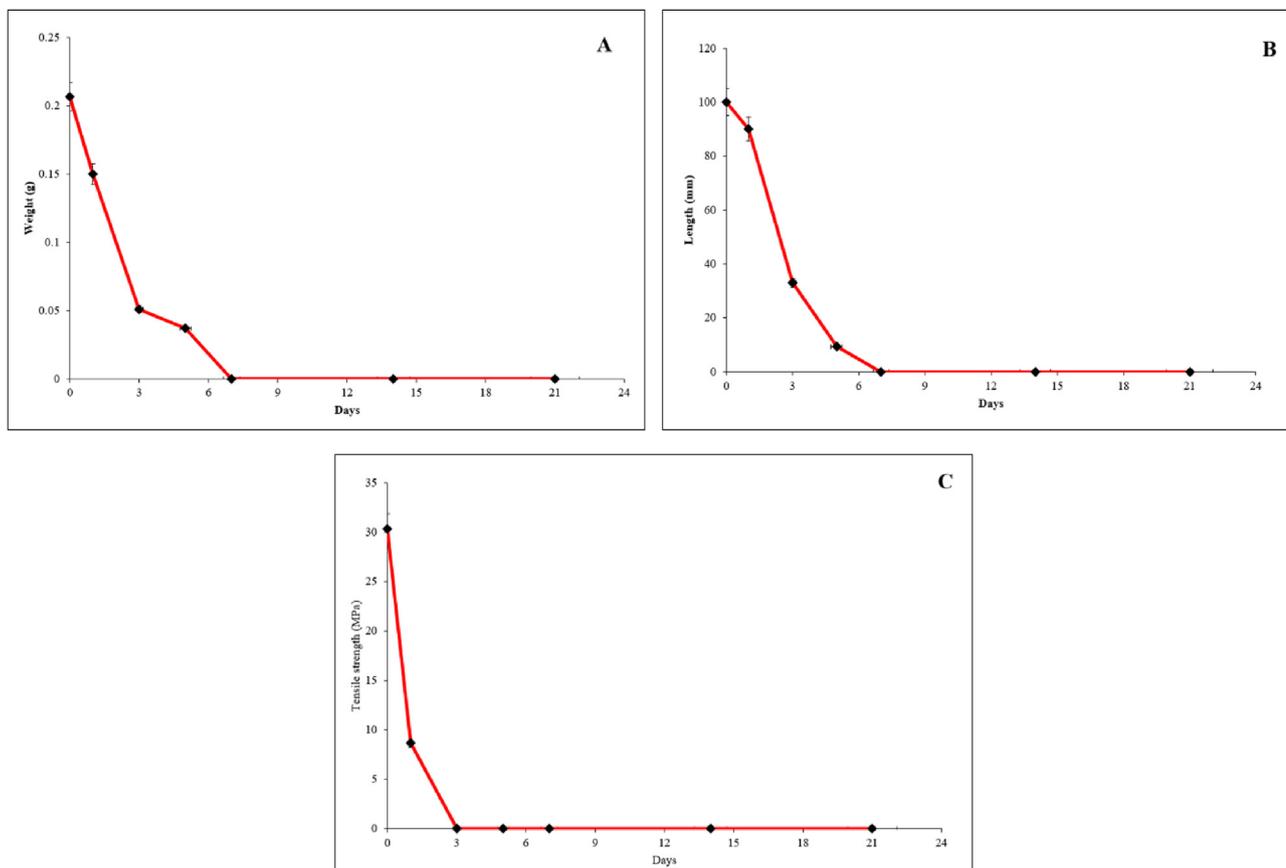
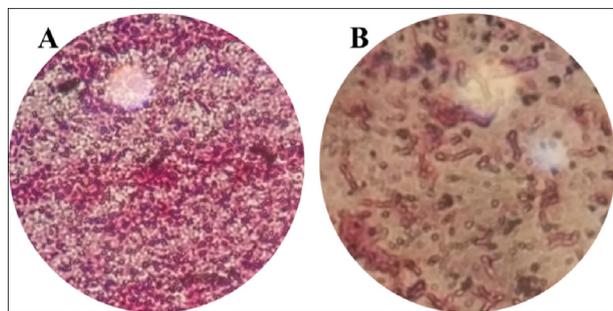


Fig. 4. The (A) weight, (B) length and (C) tensile strength of BC films during biodegradation test.



**Fig. 5.** The grown microbe on nutrient agar under the microscope with magnification (A) 40 $\times$ ; (B) 100 $\times$ .

**Table 1**

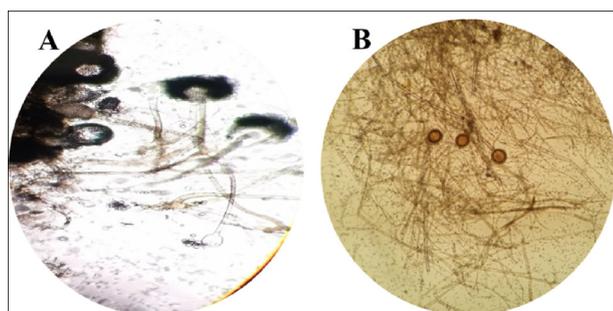
The morphology of microbe grown on nutrient agar.

Colony characteristics	Observations
Size	Medium
Form	Irregular
Elevation	Flat
Margin	Entire
Colour	Pale yellow
Possible microbe	<i>Bacillus</i> sp.

the nutrient agar (NA) and potato dextrose agar (PDA). Fig. 5 shows the grown microbe isolated from the NA plate and observed under the microscope. Here, the grown microbe on the NA plate had been identified as *Bacillus* sp. based on the characteristics of the colonies as tabulated in Table 1. The *Bacillus* sp. is a gram-positive and mesophilic bacterium with aerobic heterotrophs that produce heat-resistance endospores. The *Bacillus* sp. colonies are typically white and pasty looking, while the cells usually rod in shape. *Bacillus* sp. is one of the common microbes present in the soil and air.

The *Bacillus* sp. had been well known to have the highest cellulase activity, whereby cellulase is the enzyme responsible for the cellulose breaks down [11,21,22]. Cellulase breaks down the cellulose in 3 synergies actions: (i) the 1,4- $\beta$ -endoglucanases randomly cleave the internal chains of cellulose and creating new ends of shorter chains, (ii) the 1,4- $\beta$ -exoglucanases attack non-reducing or reducing ends of cellulose and split the fibrils from crystalline cellulose, (iii)  $\beta$ -1,4- $\beta$ -glucosidase hydrolyses cellobiose and water-soluble cellodextrin into glucose [23,24].

On the other hand, the grown microbe on the PDA plate was pictured in Fig. 6 and had been identified as *Rhizopus* sp. based on the observation of the colonies as tabulated in Table 2. According to Sharma and Singh [25] and Ribes et al. [26], *Rhizopus* sp. proliferates in colonies and resemble like cotton candies. The colonies will darken with age and turn into grey or yellow-brown. The



**Fig. 6.** The grown microbe on potato dextrose agar under the microscope with magnification (A) 40 $\times$ ; (B) 100 $\times$ .

**Table 2**

The morphology of microbe grown on potato dextrose agar.

Colony characteristics	Observations
Size	Very large
Form	Filamentous
Elevation	Raised
Margin	Filamentous
Colour	Grey
Possible microbe	<i>Rhizopus</i> sp.

*Rhizopus* sp. usually has long, terminate in a columella and dark round sporangium containing oval colourless or brown spores [27]. *Rhizopus* sp. which also commonly found in the soil had been identified to have an ability to produce cellulase which responsible for cellulose break down [25,28,29].

#### 4. Conclusion

A new biodegradable and antimicrobial (AM) packaging material was successfully developed by incorporating lauric acid (LA) into the bacterial cellulose (BC) film. The biodegradable characteristic and AM activity of BC-LA films were also evaluated and showed a good result. The BC was fully degraded in the compost soil at the seventh day of burial, while the responsible microbes for the biodegradation were identified as *Bacillus* sp. and *Rhizopus* sp. Moreover, in this study, the AM activity of BC-LA films was effective towards the gram-positive bacteria known as *Bacillus subtilis*. Finally, BC film had been proven to have great potential to be used as an alternative for synthetic plastic in order to reduce environmental pollution. By the addition of active substance into the BC film like AM agent, the functionality of the BC could be enhanced, thus opening new doors for other applications in the industries.

#### CRedit authorship contribution statement

**Khairul Azly Zahan:** Conceptualization, Funding acquisition, Resources, Software, Supervision, Writing - review & editing, Project administration, Writing - original draft. **Nur Maisarah Azizul:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization. **Mahfuzah Mustapha:** Conceptualization, Funding acquisition, Resources, Software, Supervision, Writing - review & editing. **Woei Yenn Tong:** Conceptualization, Funding acquisition, Resources, Software, Supervision, Writing - review & editing. **Muhammad Shahrir Abdul Rahman:** Conceptualization, Funding acquisition, Resources, Software, Supervision, Writing - review & editing. **Iylia Syahmi Sahuri:** Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Project administration, Writing - original draft.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

The authors would like to thank Universiti Kuala Lumpur (UniKL) and Majlis Amanah Rakyat (MARA), Malaysia.

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