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Production of Bacterial Cellulose from *Acetobacter xylinum* by using Rambutan Juice as a Carbon Source

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Rambutan juice was used as alternative carbon source in this research to produce bacterial cellulose (BC) by *Acetobacter xylinum* TISTR 107 and TISTR 975. The characteristics of BC such as concentration, yield and surface morphology were observed in this research. The experiment was started to select the bacterial type by cultivating *A. xylinum* TISTR 107 and *A. xylinum* TISTR 975 in the rambutan juice at concentration of 4 °Brix. *A. xylinum* TISTR 107 was able to produce higher BC concentration as approximately 2.66 g/l compared to other inoculum. *A. xylinum* TISTR 107 was then inoculated under different rambutan juice concentration of 4, 8 and 12 °Brix to observe optimum concentration to produce BC. The rambutan juice concentration of 8 °Brix was found as optimum concentration for *A. xylinum* TISTR 107 to produce BC base on BC concentration and production yield, as approximately 5.89 g/l and 44.45%, respectively. The surface morphology of BC at each rambutan juice concentration was observed by field emission scanning electron microscopy (FE-SEM). The results showed that pure and contaminant-free BC was produce in all conditions. The micrographs of BC provided the dispersion and the strong interfacial adhesion between the BC fibers. More interfacial adhesion was detected when the concentration of juice increased.

Keywords: bacterial cellulose, *Acetobacter xylinum*, rambutan juice

Introduction

Rambutan (*Nephelium lappaceum* Linn.), family Sapindaceae is an important fruit in Thailand, especially in the East (Chanthaburi and Trat province), that can be consumed as fresh and canned products (Marisa, 2006). During the growing season, rambutan is abundant and leads to the fruit overproduction. Moreover, rambutan always loss its moisture and has a

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darkening rind rapidly after it is harvested. Since it is mostly consumed as fresh product, low quality or non-standard rambutan (small size, injured, defect, etc.) will be massively discharged. Therefore, there is a challenge to increase its added value by using it as a carbon source for microorganism in food industry sector. The carbon source from non-standard rambutan can be applied to produce bacterial cellulose (BC). It has been reported that BC has many benefits such as enzyme immobilization, paper production, bio-sinsing, high performance speaker and biomedical application (Cannon and Anderson, 1991; Zhijun *et al*., 2014; Suwannapinunt *et al*., 2007; Kwak *et al*., 2015; Shah *et al*., 2013). BC is a purified form of extracellular polysaccharide produced by some bacteria such as *Gluconacetobacter xylinus*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Azotobacter* and *Sarcina* (Kuo *et al*., 2016; Kwak *et al*., 2015; Casarica, *et al*., 2014; Tanskul *et al*., 2013). Among these genera, *G. xylinus* or *A. xylunum* is commonly used to produce BC. Therefore, this research was aimed to produce the BC from *A. xylinum* by using non-standard rambutan juice as carbon source. The characteristics of BC produced from this research were also observed.

Materials and methods

Sample preparation

Rongrien rambutan type was used in this research. Firstly, rambutan was peeled and its seed was separated from flesh. The rambutan flesh was chopped and extracted by water at the ratio of 1:1 at room temperature for 5 min which was then filtered and kept at 4 °C for further experiment. The sugar composition of extracted juice, such as glucose, fructose, sucrose and total sugar were analysed using in house method base on AOAC 2012 (982.14).

Inoculum preparation

Acetobacter xylinum TISTR 107 and TISTR 975 were obtained from the Thailand Institute of Science and Technological Research, Ministry of Science and Technology. *A. xylinum* was grown on Hestrin Schram (HS) medium that consisted of glucose 20 g/l peptone 5 g/l yeast extract 5 g/l Na_2HPO_4 2.7 g/l and citric acid 1.15 g/l, the initial pH value 4.0. Microbial cultures were incubated at 30 °C for 72 h under static condition.

BC production

The BC production was carried out according to Kurosumi *et al*. (2009).

BC production using different inoculum

50 ml of prepared rambutan juice was added to the 500 ml Erlenmeyer flask and total soluble solid (TSS) was adjusted to 4 °Brix (equal to glucose content approximately 20 g/l) under initial pH of 4.0. 10% (v/v) of prepared inoculum was applied and incubated at 30 °C for 120 h under static condition.

BC production using different juice concentration

50 ml of prepared rambutan juice was added to the 500 ml Erlenmeyer flask and TSS was adjusted to 4, 8 and 12 °Brix under initial pH of 4.0. 10% (v/v) of optimum inoculum obtained from the first experiment was applied and incubated at 30 °C for 120 h under static condition.

BC harvesting

After the incubation period, BC membranes were collected from the culture medium and treated with 0.5 M NaOH at 90 °C for 30 min. This step was repeated 3 times in order to eliminate the attached cells. Then, the membranes were washed with distilled water to remove the components of the culture medium and other residues until showed white color and reach pH 7.0. The purified BC membrane was dried at 105 °C to constant weight and the BC concentration (BC-C) was determined in term of mass of BC (g)/volume of culture media (l).

Calculations

The efficiency of BC production was evaluated after 120 h of cultivation. The substrate conversion ratio (%), BC production rate (g/l.h) and BC production yield (%) were calculated as equation (1)-(3), respectively.

$$\text{Substrate conversion ratio; SCR (\%)} = \frac{S_i - S_f}{S_i} \times 100 \quad \ldots.(1)$$

$$\text{BC production rate; BC-PR (g/l·h)} = \frac{M_{BC}}{V \times t} \quad \ldots.(2)$$

$$\text{BC production yield; BC-PY (\%)} = \frac{M_{BC}/V}{S_i - S_f} \times 100 \quad \ldots. (3)$$

Where S_i is the initial concentration of substrate (g/l), S_f is the residual concentration (g/l), M_{BC} is the amount of BC produced (g), V is the reactional volume (l) and t is time of reaction (h).

Characterization of BC membrane

The dried BC membranes were characeterized for its surface morphology using FE-SEM (JEOL: JSM-6301F) which was operated at accelerated voltage of 5 kV and magnification of 20k. The dried BC membranes were mounted and coated two times with gold.

Statistical analysis

A completely randomized design (CRD) was used and the experiments were repeated three times. Data were analysed using one way analysis of variance (ANOVA). The statistical significance among the treatment was performed by Duncan's multiple comparison at a significant level of $p<0.05$.

Results

Sugar composition of rambutan juice

The total sugar in the rambutan juice was 9.62 g /100 g which composed of glucose (1.31 g /100 g), fructose (1.26 g /100 g) and sucrose (7.05 g /100 g).

BC production using different inoculum

To select the optimum inoculum for producing BC, *A. xylinum* TISTR 107 and *A. xylinum* TISTR 975 was inoculated to the rambutan juice. After cultivation and purification, the BC membrane from *A. xylinum* TISTR 107 and *A. xylinum* TISTR 975 were obtained with the concentration of 2.66±0.32 and 2.38±0.28 g/l, respectively (Table 1). BC membrane produced from *A. xylinum* TISTR 107 at different steps is showed in Figure 1. The efficiency of the BC production is showed in Table 1. The results showed that the BC-C, SCR and BC-PY from *A. xylinum* TISTR 975 and *A. xylinum* TISTR 107 were significantly different. However, inoculum of *A. xylinum* TISTR 107 showed higher BC concentration than other inoculum. Thus, this inoculum was selected as main inoculum for producing BC at different concentration of rambuntan juice.

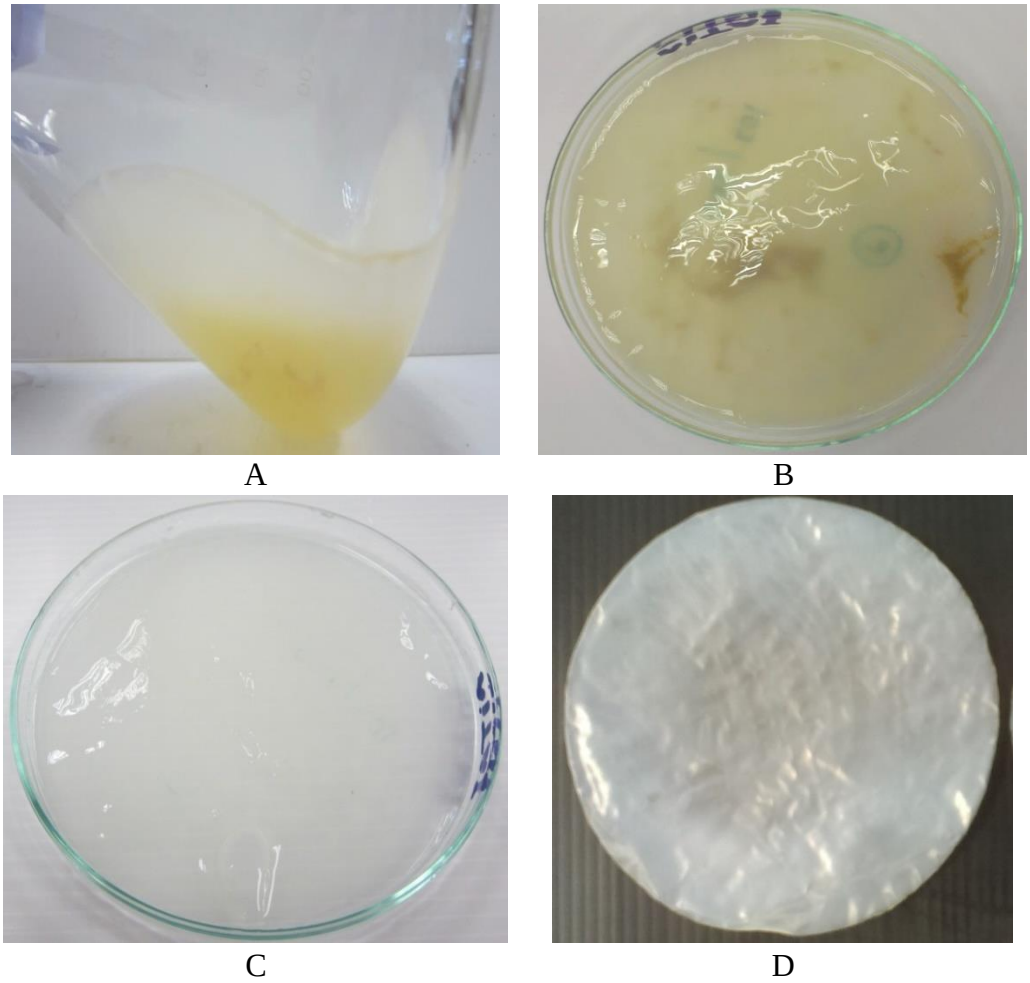


Figure 1. BC membranes produced from *A. xylinum* TISTR 107 at different steps: (A) membrane after 120 h cultivation, (B) membrane before purification, (C) membrane after purification, (D) membrane after dehydration.

Table 1. The concentration and efficiency of BC produced from *A. xylinum* TISTR 107 and *A. xylinum* TISTR 975 after 120 h cultivation

A.	M_{BC} (g)	BC-C (g/l)	SCR (%)	BC-PR (g/l·h)	BC-PY (%)
TISTR 107	0.1328±0.02[a]	2.6557±0.32[a]	20.00±4.40[a]	0.0221±0.00[a]	35.15±10.15[b]
TISTR 975	0.1188±0.01[b]	2.3769±0.28[b]	13.20±2.50[b]	0.0198±0.00[b]	46.68±11.15[a]

Significan differences in the same column are indicated by different letter.

BC production using different juice concentration

The concentration and the efficiency of BC produced by using different rambutan juice concentration are showed in Table 2. It is observed that optimum BC production was achieved under juice concentration of 8 °Brix which showed BC-C and BC-PY under this concentration higher than that under other concentrations, as approximately 5.89±0.58 g/l and 44.45±8.27%, respectively.

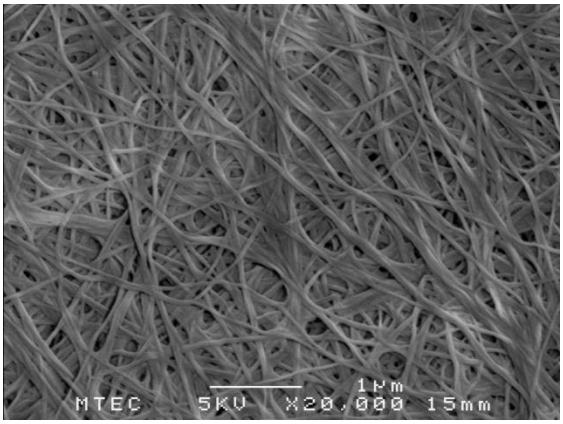
Table 2. The concentration and efficiency of BC production of *A. xylinum* TISTR 107 at different juice concentration after 120 h cultivation

TSS (°Brix)	**M_{BC} (g)**	**BC-C (g/l)**	**SCR (%)**	**BC-PR (g/l·h)**	**BC-PY (%)**
4	0.1328 ± 0.02^{c}	2.6557 ± 0.32^{c}	20.0 ± 4.3^{a}	0.0221 ± 0.00^{c}	35.15 ± 10.15^{b}
8	0.2946 ± 0.03^{a}	5.8920 ± 0.58^{a}	17.1 ± 2.8^{b}	0.0491 ± 0.00^{a}	44.45 ± 8.27^{a}
12	0.2396 ± 0.01^{b}	4.7921 ± 0.22^{b}	9.9 ± 2.0^{c}	0.0399 ± 0.00^{b}	41.82 ± 8.09^{ab}

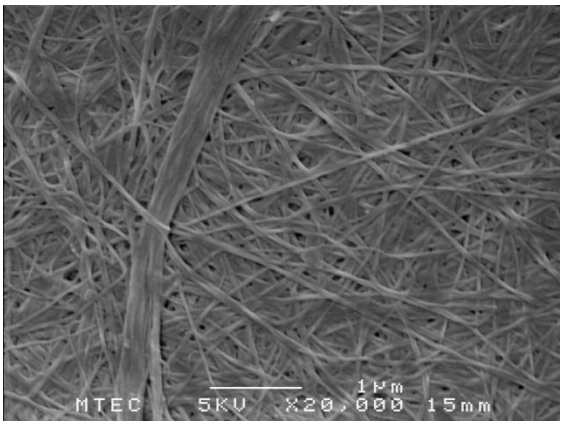
Significan differences in the same column are indicated by different letter.

Characterization of BC membrane

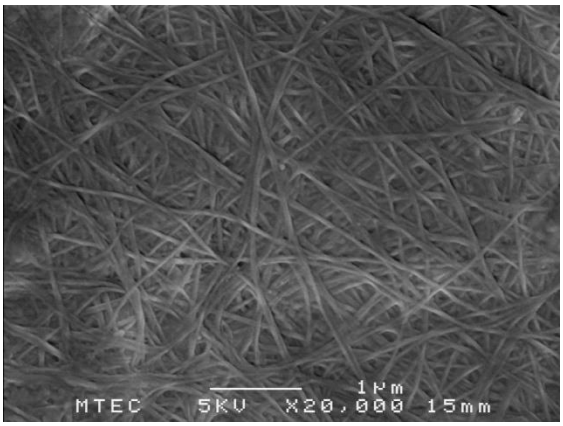
The surface morphology of BC membrane produced by *A. xylinum* TISTR 107 under different rambutan juice concentrations as carbon source are shown in Figure 2. The results showed that each concentration of rambutan juice used in this research was found to be good or suitable for BC production for the reason of clear rod shape of fiber bundles. The compact cellulose network structure was built up by random assembly of fibrils.



A



B



C

Figure 2. FE-SEM images of BC produced by *A. xylinum* TISTR 107 under different rambutan juice concentration: (A) 4 °Brix, (B) 8 °Brix, (C) 12 °Brix. All views with 20k x magnification.

Discussion

The results of total sugars showed that sucrose concentration in rambutan juice was higher, as approximately 7.05 g/100 g of juice, compared to other sugar types such as glucose and fructose. It has been reported that BC was successfully produced from fruit juice which contained high sucrose (Kurosumi *et al*., 2009). BC production from *A. xylinum* TISTR 107 and *A. xylinum* TISTR 975 with rambutan juice in this study showed similar concentration with BC produced by HS medium. The surface morphology of BC membrane under all rambutan juice concentrations showed clear and compact fiber bundles of cellulose. It indicated that rambutan juice can be considered as main carbon source to produce good quality BC. However, the rambutan juice contration of 8 °Brix showed the optimum condition in term of BC concentration and production yield.

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