The utilization of wastewater of Thai fermented rice noodle (Kanom-jeen) manufacturing process for the production of bacterial cellulose by *Acetobacter xylinum* TISTR 975

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Abstract. Bacterial cellulose (BC) is a naturally produced as an exopolysaccharide from some bacteria. It has excellent properties over the plant cellulose and has numerous applications in many fields including food, pharmaceutical, textile, paper manufacturing and other industries. However, a major limitation of bacterial cellulose production is the high cost of carbon substrate. The study aims to reduce the cost of bacterial cellulose using a cheap carbon source. This study presents feasibility in the production of bacterial cellulose using the starchy effluent waste from the Thai fermented rice noodle manufacturing process as a low-cost substrate by *Acetobacter xylinum* TISTR 975. The optimizations of culture conditions for bacterial cellulose production were also investigated under static culture. The results indicated that starchy effluent waste from the Thai fermented rice noodle manufacturing process performs well for the production of bacterial cellulose by supplementing with 50 g/L sucrose and 2% olive oil under the static condition. The structure and physical properties of bacterial cellulose were characterized using SEM, FTIR and XRD. In summary, the starchy effluent waste from the Thai fermented rice noodle manufacturing process can be used to produce bacterial cellulose which is a high value-added, sustainable and green product.

1. Introduction

Bacterial cellulose (BC), an exopolysaccharide, is produced by various species of bacteria, such as those of the genera *Acetobacter strains*, *Gluconacetobacter*, *Agrobacter*, *Sarcina*, and among others. They are hence called bacterial cellulose. BC demonstrates unique properties superior to cellulose from plants. These include high purity, good shape retention, high water binding capacity, high degree of crystallinity, good water retention, excellent tensile strength and moldability [1, 2, 3]. However, a limitation of the commercial uses of BC is its high cost of substrate (mainly carbon source). The carbon source may determine the cost of a production process, and in the case of cellulose production, it is up to 65% of the entire cost of biotechnological process [4]. More recently, some cheap and renewable substrates based on agro-industrial wastes and by-products can be used as the culture medium for BC production [5,6,7]. The wastewater derived from Khun Boonruen’s Thai fermented rice noodles (TFRN) factory in Prong Madua Community, Nakhon Pathom, Thailand, was discharged at approximately 80-100 L per day. The study aimed to investigate the feasibility of the production of BC using wastewater from Thai fermented rice noodles (TFRN) without any pre-treatment in order to decrease cost of BC production. This would be a potential renewable carbon source differing from the traditional one. In
addition, this study determined the structure and properties of these BC. So this study would benefit the provision of a potential low-cost and environmentally-friendly.

2. Experimental

Bacterial strain: *Acetobacter xylinum* TISTR 975 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR) Culture Collection, Thailand.

The wastewater of Thai fermented rice noodles was obtained from Khun Boonruen Knom-jeen’s factory in Prong Madua Community, Nakhon Pathom, Thailand. The pH, BOD and COD of the wastewater were 6.30, 0.30-1.26 mg/L and 17,000 - 17,600 mg/L, respectively [8]. For the preparation of wastewater from Thai fermented rice noodles (TFRN) medium, the wastewater was centrifuged at 12,000 rpm for 10 min to remove particles. The supernatant was then filtered and kept as a stock solution and sterilized in an autoclave at 121 °C for 15 min. The TFRN medium containing (g/L): glucose 50, ammonium sulfate 5 and 1% (v/v) acetic acid. The seed medium containing (g/L): glucose 4, peptone 5, yeast extract 5, disodium hydrogen phosphate 2.7, citric acid 1.15, and pH 6.0 [9].

*Acetobacter xylinum* TISTR 975 was cultivated in 500 mL flasks with 100 mL of seed medium and then incubated at 30 °C under static culture conditions for 3 days. The seed inoculum (10% v/v) was then transferred into the TFRN medium and then incubated at 30 °C under static culture conditions for 15 days. The thickness of BC was determined using a Vernier caliper. The experiments were done in triplicate.

To study the effects of olive oil for BC production, the olive oil (Bertolli®, Sino-Pacific Trading Thailand) concentration of 0.5%, 1% and 2% (v/v) was added to the TFRN medium and then incubated at 30 °C under static culture conditions for 15 days. The thickness of BC was determined using a Vernier caliper. The experiments were done in triplicate.

After cultivation, the BC was picked from the surface and treated with 0.1 N NaOH at 90 °C for 10 min to remove the microorganisms. The cellulose was immersed overnight in distilled water or until the pH of the water became neutral. Purified BC was washed twice with distilled water. After that, the purified BC was freeze-dried for the characterization of the structure and physical properties.

Scanning Electron Microscopy (SEM). The morphology and microstructure of BC were characterized by SEM. After freeze drying, the BC was mounted on SEM grids, and gold particles were sputter coated with Rotary-pumped sputter (Q150R S model, Quorum, England) to the BC surfaces. The specimens were observed with a scanning electron microscopy at an accelerating voltage of 3 kV (MIRA3 model, TESCAN, Czech Republic).

Fourier Transform Infrared Spectroscopy (FTIR). The surface properties of the BC were analysed by a Nicolet iS5 FTIR Spectrometer (Thermo Fisher Scientific, USA). For each analysis, the scan range was 4,000 - 400 cm⁻¹ with a resolution of 4 cm⁻¹ [10].

X-ray diffraction (XRD). Dried BC was investigated using a MiniFlex II X-ray diffractometer. The samples were scanned from 20 = 3° to 20 = 70° at a scanning rate of 2° 20 min⁻¹. The operating voltage and current were 40 kV and 30 mA, respectively [10].

3. Results and discussion

This result shows feasibility in the production of bacterial cellulose by *Acetobacter xylinum* TISTR 975 using the starchy effluent waste from the Thai fermented rice noodle manufacturing process without any pre-treatment in order to save the costs of energy, chemicals and time for BC production. The thickness of the BC film obtained from TFRN and TFRN medium with sucrose concentration of 50 g/l that were significantly different in the static cultures. After 15 days of incubation under static culture, the thickness of the BC film obtained from TFRN and TFRN medium with sucrose concentration of 50 g/l were 5.33 and 13.67 mm, respectively. These results show that the TFRN medium could be used for BC production. It indicated that starchy effluent waste from the Thai fermented rice noodle manufacturing process performs well for the production of bacterial cellulose by supplementing with 5 g/L sucrose and
2% olive oil under the static condition as shown in Figure 1. The thickness of the BC film significantly increased by adding 2.0% (v/v) olive oil. These conditions gave the maximum thickness of 18.3 mm compared with 14.0 mm without olive oil (Table 1). Similarly, Żywicka et al. (2018) report that the increased production of BC was achieved by supplementing culture medium with vegetable oil [11]. SEM displayed the morphology of the surface structure of the BC at a magnification of 25,000 times as shown in Figure 2. The width of BC fibrils was less than approximately 100 nm in size. The network of thin layers of BC structures composed of very neat fibrils. The two conditions of BC had similar microstructures.

![Figure 1](image1)

**Figure 1.** The production of bacterial cellulose using starchy effluent waste from the Thai fermented rice noodle manufacturing process (a) without and (b) with 2% olive oil.

| olive oil concentration (v/v) | Thickness (mm)          |
|------------------------------|-------------------------|
| 0.0                          | 14.0 ± 1.73             |
| 0.5                          | 11.7 ± 1.15             |
| 1.0                          | 17.0 ± 1.00             |
| 2.0                          | 18.3 ± 1.52             |

**Table 1.** Effects of olive oil on BC production by *Acetobacter xylinum* TISTR 975 in TFRN medium with sucrose concentration of 50 g/l under static condition for 15 days.

![Figure 2](image2)

**Figure 2.** SEM images of the surface morphological structure of freeze-dried BC in TFRN medium with sucrose concentration of 50 g/l (a), with sucrose and olive oil concentration of 50 g/l and 2% (v/v) (b).

FTIR was used to obtain the specific functional groups or chemical bonds of the BC samples. The FTIR spectra of the BC from TFRN medium with sucrose concentration of 50 g/l (blue curve) and TFRN medium with sucrose and olive oil concentration of 50 g/l and 2% (v/v) (red curve) are shown in Figure 3. The two conditions of BC showed several important characteristic peaks at 3344 cm\(^{-1}\) (stretching vibration of hydroxyl groups (OH)), at 2895 cm\(^{-1}\) (asymmetric stretching vibration of -CH\(_2\)CH), at 1427
cm⁻¹ (asymmetric deformation vibration of methyl and methylene), at 1161 cm⁻¹ (asymmetric stretching of C-O at ring) and at 1056 cm⁻¹ (stretching of C-O) [10,12]. The results confirmed that no obvious difference is observed for functional groups of BC samples obtained from both media. The X-Ray Diffraction patterns of BC from TFRN medium with sucrose concentration of 50 g/l (blue curve) and TFRN medium with sucrose and olive oil concentration of 50 g/l and 2% (v/v) (red curve) are shown in Figure 4. The blue curve, it had two main characteristic peaks at 14.64° and 22.95°. The red curve, it showed two main characteristic peaks at 14.89° and 23.23°.

Figure 3. FTIR spectra of BC produced by TFRN medium with sucrose concentration of 50 g/l (blue curve), with sucrose and olive oil concentration of 50 g/l and 2% (v/v) (red curve).

Figure 4. X-ray diffraction diagrams of BC produced by TFRN medium with sucrose concentration of 50 g/l (blue curve), with sucrose and olive oil concentration of 50 g/l and 2% (v/v) (red curve).

4. Conclusions
In this study, the TFRN without any pretreatment could be used directly as substrate for BC production by Acetobacter xylinum TISTR 975. The thickness of the BC film was significantly increased by adding sucrose and olive oil concentration of 50 g/l and 2.0 % (v/v) respectively. Overall, the fermentation of TFRN showed a small influence on BC structure as indicated by DSC, FTIR, and XRD technique. The BC from both media had similar properties. Using inexpensive waste materials as substrates in
fermentation media can significantly reduce the production cost of BC. It also has an environmentally friendly effect by the removal of such waste from the environment.

5. References
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