Production of bacterial cellulose from tofu liquid waste and rice-washed water: morphological property and its functional groups analysis

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Abstract. Bacterial cellulose (BC) is one of the biopolymers which has a wide range of application, from medical purposes to electrical components. Previous studies show that BC could be produced from agricultural, food, and industrial waste. In this study, two kinds of liquid waste were utilized as the substrates for *Komagataeibacter xylinus* to produce BC, namely tofu liquid waste, and rice-washed water. The structure of BC was determined by Scanning Electron Microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR). The tofu liquid waste resulted higher yield after 15 days of fermentation compared to the rice-washed water. Furthermore, the FTIR spectra of both BC displayed the similar pattern as the standard cellulose. To conclude, this study revealed that both tofu liquid waste and rice-washed water were potential to be used as the production media for BC.

Keywords: Bacterial cellulose, BC production, tofu liquid waste, rice-washed water, *Komagataeibacter xylinus*

1. Introduction

Bacterial cellulose (BC) is a promising biomaterial due to its specific and excellent properties such as high cellulose purity, mechanical strength, high crystallinity, and biodegradability [1]. These properties have made BC as a potential application in electronics, cosmetics, medicine as well as food and its related products [1]. The synthetic media for BC production for research propose was introduced by Schramm and Hestrin of which eventually known as Hestrin-Schramm media [2]. However, the use of the Hestrin-Schramm (HS) medium with glucose and other supplements sources is costly and thus hinders for its wider applications [3]. On the other hand, industrialization and commercialization of BC production at commercial scale is a relatively expensive. This due to the cost of culture medium which represents approximately 30% of the production cost [4]. Meanwhile, organic wastes comprise fascinating nutrient sources for bacteria’s growth medium, but the wastes are often discarded. Therefore, cost-effective culture medium as carbon source is the challenging aspect for BC production. Previous researches have used the low-cost substrates such as industrial residues [4][5], fruit and food wastes [6][7], plantation and agricultural waste [8][9], beverages waste [7][10], by-product streams from biodiesel and confectionery industries [5].

Tofu liquid waste and rice-washed water were used for culture media. Tofu waste is waste generated in the process of tofu production or soybeans wash. Waste generated is in the form of solid
and liquid waste. In addition, rice-washed water from rice noodle industry is discarded without further utilization. Furthermore, the improper handling of food industry liquid waste can cause environmental problems. Tofu liquid waste and rice-washed water were used for BC production in the previous studies [11,12]. However, its morphological and functional group was not discussed yet whereas the knowledge of these properties are indispensable for further application.

This research aimed to characterize morphological and functional group as well as evaluate its yield of BC produced in tofu liquid waste and rice-washed water. Thus, the new media proposed in the present study provides a potentially economical and environmentally-friendly process for the production of BC.

2. Materials and Methods

2.1. Materials

The commercial bacterial starter obtained from nata de Coco Company in Cianjur was utilized as the inoculum to produce BC. Komagataibacter xylinus was screened from the starter at Research Unit for Clean Technology – Indonesian Institute of Sciences. Tofu liquid waste was obtained from a tofu small company in Cimahi, West Java. Rice-washed wastewater refers to the water used for wash the rice before cooking.

2.2. Production of BC

Pre-treatment of both tofu and rice wastewater was performed by filtering wastewater using Whatman filter paper 40 prior to use for culture medium. The commercial sucrose was added to the filtrate with the concentration of 7.5 % (w/v), then pH of the mixture was adjusted to 3 using glacial acetic acid. The mixture then boiled for 30 min. or sterilization and cooled down at room temperature and used for inoculation. The prepared seed inoculum (20 %, v/v) was transferred into a glass vessel (500 mL) containing 300 mL of each wastewater as described above after adjusting their pH to 3 using glacial acetic acid and they were cultivated statically at 30 °C for 15 days.

2.3. Harvesting of BC

The harvest was done after 5, 10, and 15 days of incubation. The BC layer which produced on the surface of medium was taken out from each flask using a clean tweezer and boiled with deionized water until it reached neutral pH. Prior to its measurement, BC was squeezed using vacuum filtration. The weight and thickness of BC were measured before and after the squeezing process. For characterization specimen, BC was hot-pressed (100 °C, 40 kgf/cm²). Dependant to the thickness of BC, the pressing process was carried out for 60 to 240 min.

2.4. Analysis of BC

2.4.1. Fourier transform infrared spectroscopy (FTIR)

The infrared spectra were recorded with a Thermo Nicolet from Thermo scientific USA type iS5, compartment model iD5 for ATR and model iD1 for. For each sample, the scan range was from 4000 cm⁻¹ to 400 cm⁻¹, using a resolution of 4 cm⁻¹.

2.4.2. Scanning electron microscopy (SEM)

The morphology of BC membrane surfaces were characterized by SEM JEOL JSM-IT300LV Japan. The pressed samples were coated with approximately 20 nm gold–palladium before observation. The samples were measured using the SEM with a sputtered time of 30 s and an accelerating voltage of 15 kV. Images with 1000×, 5,000×, 10,000× and 30,000 × magnifications were recorded.
3. Results and Discussions

3.1. BC yield

After harvesting, the produced BC was measured for its yield which refers to the dry weight of BC produced per liter of medium. We conducted the fermentation of BC for 15 days, with three sampling point at day 5, 10, and 15. As presented in Figure 1, the yield increased with the extension of the incubation time. However, the yield from the rice-washed water was similar for the fifth and tenth day. Both media reached its maximum yield after the final day of fermentation, which were 10.60 g/l for tofu liquid waste and 6.57 g/l for rice-washed water (Figure 1). The yield from this study were higher compared to yield from other studies (Table 1), as the BC yield from tofu liquid waste was around 2.5 times higher than BC from soya bean whey [11], and the yield from rice-washed water in this research was 3.8 times higher than the reference [12]. The difference of results might be caused by the difference of some conditions and parameters which affect BC production, such as initial pH, medium and inoculum size, temperature, carbon, and nitrogen source [13,14]. Accordingly, it is important for further study to focus on the optimization of BC production by waste.

The tofu liquid waste produced higher maximum yield compared to the rice-washed water. The composition and availability of carbon and nitrogen source in medium play important role to the successful bacterial cellulose production [15]. This result might be due to the tofu liquid waste contains the richer and more favourable carbon and nitrogen source than the rice-washed water. It is confirmed by the measurement of carbohydrate and protein of tofu liquid waste and rice-washed water for this study. Tofu liquid waste contains 0.17 % of carbohydrate and 0.14 % of protein, while rice-washed water consists of 0.08 % of carbohydrate and 0.08 % of protein.

Table 1. Comparison of BC from this study with references.

| Medium                | Added carbon source (%) | Added nitrogen source (%) | Inoculum (%) | Incubation time (days) | Dry weight (g/L) | References |
|-----------------------|-------------------------|---------------------------|--------------|------------------------|------------------|------------|
| Tofu liquid waste     | Sucrose 0.75            | -                         | 20           | 15                     | 10.6             | This study |
| Soya Bean Whey        | -                       | -                         | -            | 9                      | 4.14             | [11]       |
| Rice-washed water     | Sucrose 0.75            | -                         | 20           | 15                     | 6.57             | This study |
| Rice-washed water     | Sucrose 10 Urea 0.5     | 0.5                       | 20           | 14                     | 1.735            | [12]       |

Figure 1. Yield of BC after incubation of 5, 10, and 15 days
3.2. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra represent the functional groups found in the bacterial cellulose. The produced BC from both tofu liquid waste and rice-washed water exhibit a similar FTIR spectra when scanned in the wavelength ranging from 4000 to 400 cm\(^{-1}\) (Figure 2). It can be seen from Figure 2, there were some slight changes in the spectra after each sampling day. For instance, the spectra from tofu after 15 days in the range of 1000 and 1500 cm\(^{-1}\) shows relatively clearer peaks compared to the ones from 5 and 10 days of tofu fermentation. Moreover, the changes also can be found from the BC of rice water. After the initial days of fermentation, spectra between region 3000 and 2000 cm\(^{-1}\) were observed, however, the spectra were almost absent in the final day (15\(^{th}\) day). These results might indicate that the length of fermentation period can affect the structure of the produced BC.

As depicted in Figure 2, the spectra show the absorption at 3279.84 and 3341.79 cm\(^{-1}\), which refers to O-H stretching for BC produced from tofu liquid waste and rice-washed water, respectively. Narrow regions between 3000 and 2800 cm\(^{-1}\) in cellulose structure are known for the C-H moieties stretching [16]. This C-H stretching is found at a similar peak for both BC, namely at 2922.70 cm\(^{-1}\) for BC from tofu waste and 2922.09 cm\(^{-1}\) for the rice-washed water. From the scan of the standard cellulose, the next region represents -OH bending [17] in which found at 1626.28 and 1626.02 cm\(^{-1}\) for BC from tofu liquid waste and rice-washed water respectively. Adjacent to this region is the most complicated region, which can be found between 1430 and 850 cm\(^{-1}\) as the various sp\(^3\) single bond vibrations being scanned. In other words, it is called the fingerprint region [16]. From our BC samples, peak from tofu waste’s BC at 1369.08 cm\(^{-1}\) and 1314.51 cm\(^{-1}\) from the rice waste might represent -CH bending in this area. Furthermore, peak at 1024.85 cm\(^{-1}\) and 1030.43 cm\(^{-1}\) from the tofu and rice waste, respectively, indicate the presence of C-OH, C-C, C-H ring, and C-O-C stretching at \(\beta\)-(1→4) glycosidic linkage [17]. Lastly, the regions from 850 cm\(^{-1}\) to 400 cm\(^{-1}\) displays the bending and rotation of different heavy atom [16].

3.3. Scanning electron microscopy (SEM)

The observation using SEM intends to analyse the morphological structure of BC, which correlates with its mechanical properties [18]. Figure 3 shows SEM images of BC produced from tofu and rice wastewater with the magnitude of 35000×. BC has its advantage over plant cellulose as the bacteria can produce nanofibrils naturally, with its diameters generally ranging from 25 to 100 nm [19]. Moreover, ultrasonication on BC and other natural fibres could produce the finest fibres up to 6-7 nm [19]. According to Figure 3, the structure of both BC was intertwined microfibrils and nanofibrils network, alike with previous studies [20,21]. The detection on some fibres of BC produced from tofu liquid waste reveals the fibrils’ size ranging from 24.7 to 83.6 nm. On the other hand, BC from rice-washed water contains fibrils with the size of 20.5 until 55.7 nm (Figure 3). Komagataibacter xylinus is known to be able to produce the cellulose ribbon or is often called as bundle. This bundle consists of microfibrils that are made up by six parallel glucan chains. In the liquid medium, G. xylinus excretes glucose in its crystalline form as pellicles, which can float on the surface of the medium and be separated from water [22].

Based on the images, BC from rice-washed water looks more condensed than BC from tofu liquid waste as there are fewer spaces between the fibrils. A similar finding was found from the study on BC production from soybean oil refinery waste [21]. The structure of BC from the mentioned soybean waste had more loose structure with larger pores compared to BC, which was produced from rich medium containing 0.5 % glucose, 0.5 % mannitol, 0.5 % polypeptone, 0.5 % yeast extract, and 0.1 % magnesium sulphate [21]. This loose structure might be produced from the slower usage of fatty acid by K. xylinus compared to the utilization of carbohydrates [21]. As tofu liquid waste also contains around 25 % of fat [23], the same reasoning might also be the cause of the more loose structure of BC from tofu waste (Figure 3).
Figure 2. FTIR spectra of BC; from below to above: Tofu 5, Tofu 10, Tofu 15 represent BC from tofu liquid waste after 5, 10, and 15 days of incubation, respectively. Rice 5, Rice 10, Rice 15 refer to BC from rice-washed water after 5, 10, and 15 days, accordingly.

Figure 3. SEM observation of BC produced from tofu liquid waste (a) and rice-washed water (b) with the magnitude of 35000×
4. Conclusion
According to this study, both tofu liquid waste and rice-washed water were feasible for BC production. After 15 days of incubation, tofu liquid waste yielded a higher mass per volume of media. However, SEM images showed that BC from rice-washed water was relatively more condensed. The FTIR spectra indicated that the produced BC consists of the functional group which is found in standard cellulose. More extensive research is required for a better understanding of the bacterial metabolism, the improvement of the structure and mechanical properties, and the application of each BC.

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