Oil palm frond juice and coconut water as alternative fermentation substrate for bacterial cellulose production

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Abstract. Bacterial cellulose (BC) has emerged as an alternative and sustainable biomaterial due to its remarkable structural and mechanical properties, which can be used in various applications. BC is a form of biopolymer which far superior to its plants equivalent because of its high purity, high strength construction material, good mechanical strength, elasticity and high crystallinity. The research aims to produce BC using biological process synthesis of A. xylinum in oil palm frond juice (OPFJ) based medium. Oil palm frond (OPF) is Malaysia’s largest biomass produced from the oil palm plantation industry in Malaysia and contain fermentable sugar required to grow BC, glucose, sucrose and fructose. The experiment was conducted under optimum temperature 30 °C in static culture condition which were applied with different ratio of medium (Coconut water, OPFJ and distilled water). Our findings revealed that highest BC yield (4.50 g/L) was obtained in the mixture medium of OPFJ: Coconut water (60:40). FTIR analysis confirmed the pellicles as cellulosic material. FE-SEM analysis showed the ribbon network consisting of nanosize fibrils with diameter ranging from 50 to 60 nm. Overall, the work demonstrated the potential of producing high value-added polymer from OPFJ-based medium.

1. Introduction

Bacterial cellulose (BC) is an exopolysaccharide which can be synthesized by several aerobic bacteria, such as Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alcaligenes, Azotobacter, Pseudomonas, Rhizobium and Sarcina [1]. However, only Acetobacter species could efficiently produce high-quality cellulose to justify commercial interest [2]. The chemical composition of BC is similar to plant cellulose; however, the physicochemical and mechanical properties of BC are distinct owing to the uniform, continuous and nanoscale cellulosic fibers network. BC is known for its unique properties, including high purity, high degree of polymerization, high crystallinity, high water holding capacity, high tensile strength and good biocompatibility, relative to plant cellulose [3–5]. These properties make BC ideal for numerous possible applications in various fields, such as biomedicine, cosmetics, high-end acoustic diaphragms, papermaking, food industry and other areas [6,7].

Despite the remarkable properties of BC, its industrial production and commercial applications are often limited by the expensive BC standard growth medium and low production yield. It has been reported that the cost of fermentation medium accounts for 50 to 65% of the overall process cost [8]. Therefore, to minimize production costs, the use of alternative low-cost carbon sources as fermentation substrates should be explored [9]. In recent years, several studies utilizing industrial by-products and agricultural wastes for BC production has been reported: mango waste [10], crude distillery effluent...
[11], food wastes [12], whey [13], etc. Among these wastes, the use of agricultural wastes improves the cost effectiveness of BC production due to the large-scale availability and higher BC productivity [14].

In Malaysia, oil palm frond (OPF) contributes the largest portion (70 %) of the palm oil biomass, amounted to approximately 83 MMT per annum [15]. OPF can be pressed to obtain oil palm frond juice (OPFJ) comprising high quantities of sugars (up to 40 g/L total sugars consisted of glucose, sucrose, and fructose) and minerals and nutrients [16]. Therefore, the use of OPFJ as fermentation substrates to produce other valuable chemicals such as PHA and PHB have been widely reported [17-18]. However, to the best of our knowledge, there have been limited studies on the valorisation of the OPFJ as the raw material for BC production. On the other hand, coconut water (CW), by-product from copra and coconut milk industries has been demonstrated as a potential substrate for BC production [19]. Nevertheless, the combination of both OPFJ and CW has never been reported in the literature. Therefore, with the aim to manipulate the agricultural waste into a valuable material, utilization of both OPFJ and CW as culture medium to produce BC not only can make the BC production more cost-effective but also could decrease the environmental pollution associated with the disposal of OPF.

In this study, we explored the use of OPFJ and CW as the substrate for the static cultivation of BC by *Acetobacter xylinum*. To evaluate the feasibility of using OPFJ as substrate for bacterial cellulose production by *A. xylinum*, medium formulations comprised of different volume ratio of OPFJ: DW, CW: DW and OPFJ: CW (20:80, 40:60, 60:40, 80:20 and 100:100) were chosen in this study. The five different volume ratio of culture medium formulations were prepared by adding either CW or DW with OPFJ in the shake flask with respective volume to obtain desired concentration. The mediums were autoclaved at 121 °C for 15 min prior to inoculation. The initial pH of the OPFJ medium was adjusted to pH 4.5 by 50 %v/v acetic acid. The medium was then inoculated with 10 %v/v inoculum, followed by static cultivation at 30 °C for 7 days.

2. Materials and Methods

2.1. Preparation of oil palm frond juice (OPFJ)
Fresh oil palm fronds (petiole or basal part without leaves) were obtained from a local palm oil mill plantation located at Lepar, Kuantan, Pahang. OPFJ were extracted by pressing the petioles using a pilot scale hydraulic pressing machine (MATSUA Inc., Japan) at Forest Research Institute of Malaysia (FRIM). The extracted OPFJ were filtered using 0.5 mm steel sieve and filter cloth to discard the undesired substances such as fibre debris and solid particles. The OPFJ were further clarified by centrifuging at 10 000 rpm for 20 min at 4 °C using an Eppendorf centrifuge. The resultant supernatant of OPFJ were stored at -20 °C before used.

2.2. Production of bacterial cellulose by *A. xylinum*
To evaluate the feasibility of using OPFJ as substrate for bacterial cellulose production by *A. xylinum*, medium formulations comprised of different volume ratio of OPFJ: DW, CW: DW and OPFJ: CW (20:80, 40:60, 60:40, 80:20 and 100:100) were chosen in this study. The five different volume ratio of culture medium formulations were prepared by adding either CW or DW with OPFJ in the shake flask with respective volume to obtain desired concentration. The mediums were autoclaved at 121 °C for 15 min prior to inoculation. The initial pH of the OPFJ medium was adjusted to pH 4.5 by 50 %v/v acetic acid. The medium was then inoculated with 10 %v/v inoculum, followed by static cultivation at 30 °C for 7 days.

2.3. Purification and quantification of bacterial cellulose
At the end of 7 days incubation period, the BC pellicles covering the surface of the liquid medium were separated and removed from the flasks. The BC samples were then subjected to the twice washing with the distilled water. After that, the BC samples were transferred into 0.5 M NaOH solution for 20 min to remove any attachment of bacterial cells from the BC surface, followed by repeated soaking and washing with distilled water. The BC was then soaked in distilled water overnight to remove the alkaline solution.
in the BC. Finally, the wet BC was weighed before freeze-dried for 3 days. The dry weight of BC was also recorded, and the BC yield was calculated using the following formula:

\[
\text{BC yield (g/L)} = \frac{\text{Dry weight of BC (g)}}{\text{Volume of culture medium (L)}}
\]

(1)

2.4. Structure and Composition Analysis of BC
All BC pellicles obtained in the experiments were characterized in terms of chemical structure and surface morphology using FTIR and FE-SEM.

2.4.1. Fourier Transform Infrared Spectroscopy (FTIR). The presence of functional groups of the freeze-dried BC samples was observed by FTIR spectroscopy on a Thermo Scientific Nicolet iS5 FTIR Spectrometer. The FTIR spectra was recorded for 64 scans, at a resolution of 4 cm\(^{-1}\) in the range of 4000–400 cm\(^{-1}\).

2.4.2. Field Scanning Electron Microscopy (FE-SEM). The microstructure of the freeze-dried BC sample was observed by a DSM 940A high resolution Field Emission Scanning Electron Microscope (FE-SEM; Zeiss, Germany) operated at 15 kV.

3. Results and Discussion

3.1. Effect of volume ratio of OPFJ: DW, CW: DW and OPFJ: CW on BC dry weight

OPF is an abundantly available type of agro-industrial wastes that is usually left out at the plantation sites for uses as fertilizer. However, previous studies demonstrated the promising potential for OPF to be converted into fermentable sugars. As pointed out by Zahari et al. (14), the OPFJ was mainly consisted of glucose, sucrose and fructose, which is suitable to be used as an alternative substrate for BC production. Five different volume ratios of OPFJ: DW, CW: DW and OPFJ: CW (20:80, 40:60, 60:80, 80:20 and 100:100). Figure 1 reveals the production of BC in different type of medium with respect to yield of BC (g/L). Of all the different formulation medium tested in this work, dilutions of OPFJ in DW consistently produced lowest BC yield compared to the other two types of medium, regardless of the volume ratio of OPFJ: DW used. As shown in Figure 1(a), the lowest yield of BC (0.90 g/L) was recorded at the volume ratio of OPFJ: DW of 20:80. Increasing the volume ratio of OPFJ: DW also increased the yield of BC produced, as indicated by the highest yield of BC (1.52 g/L) recorded at the volume ratio of OPFJ: DW of 20:80. The high yield of BC proved that the sugars content in OPFJ was successfully converted into cellulose. The effect of CW medium and DW on the production of BC were demonstrated in Figure 1(b). When the volume ratio of CW: DW used was 20:80, maximum yield of BC was obtained (2.44 g/L). However, further increases of the volume ratio caused the yield of BC to also decreased, until the minimum BC yield was found at the volume ratio of 100:100 of CW: DW (1.55 g/L). Increasing volume ratio of CW: DW also means that there were more sugars content in the medium. According to A.W Indrianingsih et al. [20], the accumulation of sugars can cause the plasmolysis in these bacterial cells, resulting in the decline in the formation of BC. On a contrary, higher dry weights of BC (4.50 g/L) was achieved when the volume ratio of the OPFJ: CW was fixed at 40:60 and higher, indicating that they should be used when attempting to achieve high amount of BC (Figure 1(c)).
Figure 1. BC yield for different concentrations of (a) OPFJ in distilled water, (b) coconut water in distilled water and (c) OPFJ in coconut water.

3.2. FTIR Analysis of bacterial cellulose

Figure 2 shows the FTIR spectra of the BC samples evaluated in the wave number of 4000-400 cm\(^{-1}\). As indicated in Figure 2(a), (b) and (c), the characteristic bands of cellulose for the BC samples synthesized in OPFJ, 60:40 of OPFJ: CW and CW appeared at 3340 - 3343 cm\(^{-1}\) for the stretching vibration of hydroxyl groups (-OH), at 2919 - 2920 cm\(^{-1}\) for the asymmetric stretching vibration of methylene (-CH\(_2\)-) and at 1056 – 1057 cm\(^{-1}\) for C-O-C and C-O-H stretching vibration of sugar ring and at 897 cm\(^{-1}\) for γ (COC) in plane, symmetric stretching [24]. Overall, no obvious difference was observed on the BC samples obtained from the three different BC samples produced from three different types of mediums indicating that no influence of OPFJ as substrate on the functional groups of BCs. Obviously, the BC obtained has all the characteristic bands of cellulose, indicating its high purity of cellulose when compared with other plant cellulose.
3.3. Morphological analysis of bacterial cellulose using FE-SEM

The morphologies of BC samples were shown in Figure 3. As it depicted, the BC samples showed a reticulated structure consisting of ultrafine fibrils with dimensions ranged from 50 to 60 nm. Detailed examination of the micrographs revealed profound morphological features of the BC samples. The fibrils of BC produced from the OPFJ appeared as crossed, superimposed layers of cellulose ribbons that were randomly oriented and almost uniform in size. The SEM figures showed that the nanostructure of BC obtained in this work is much clearer than that got from elephant grass acid hydrolysate [21].
4. Conclusion
This study demonstrates that combination of OPFJ and CW were feasible to be utilized as alternative substrate for BC production by *A. xylinum*. After 7 days of fermentation, culture medium formulated by the combination of OPFJ and CW yielded higher BC dry weight (4.50 g/L) than just OPFJ and CW alone. FTIR spectra of the BC produced in OPFJ, CW and OPFJ/CW show identical chemical profile assigned to cellulose. FE-SEM analysis found that the BC samples were fibrous with irregular size and form, with the ribbon’s diameter ranging between 50 and 60 nm. More comprehensive is required in the future to further understand the metabolism of bacteria and the structure and mechanical properties of BC.

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