Characterization of bacterial cellulose produced via fermentation of *acetobacter xylinum 0416*

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A R T I C L E   I N F O

A B S T R A C T

The production of plant-based cellulose products had contributed to the increasing rate of deforestation activities. Bacterial cellulose (BC) produced via fermentation process can be considered as an alternative. In this study, BC was produced by fermentation of *Acetobacter xylinum 0416* and further analyzed to determine its physiochemical properties. The characterization of the BC was carried out through fourier transform infrared spectroscopy (FTIR), field emission scanning electron microscope (FESEM), thermogravimetric analysis (TGA), pH, moisture content, compressibility index and swelling properties. Then, it was compared with plant-based cellulose products which are carboxymethyl cellulose (CMC) and cellulose powder (CP). FTIR analysis demonstrated the similar properties between BC, CMC and CP while FESEM depicted a smoother surface and nanostructure of the BC. The TGA analysis indicated that BC has the highest thermal stability compared to CMC and CP. The other characterization results showed that BC displayed promising properties compared to CMC and CP. These findings further support the potential of substituting the use of plant-based cellulose products in the market with BC produced by A.xylinum 0416.

1. Introduction

In recent years, plant-based cellulose was widely used in the industries for the production of various products. However, the use of cellulose crops from wood and cotton give direct impact on the earth's carbon cycle as it used up forest resources. Additionally, the manipulation of trees for these purposes has continuously depleted forest resources lead to highly needed for other alternative. The use of alternative non-wood sources of fiber in preparation of pulp for industrial applications has received substantial attention. The main reason for the continuous attention increase in this area is a decrease in wood availability with the increasing demand for market pulp in some rapidly developing countries in Asia, Africa and Latin America (Ohwoavworhua et al., 2009). Therefore, bacterial cellulose (BC) can be used as an alternative for plant-based cellulose products.

BC is an exopolysaccharide produced by various species of bacteria (Jonas and Farah, 1998). BC possesses special and unique properties that differ from plant-based cellulose. It is chemically pure and free of lignin and hemicelluloses. When compared to plant-based cellulose, BC has a higher crystallinity, degree of polymerization, tensile strength, moldability, biocompatibility and transparency. Furthermore, the ultra-high strength and toughness of BC make it an ideal raw material for high performance applications such as composites, pharmaceuticals, textiles, mining, and refinery. In food applications, BC can be used in diverse ways such as low-calorie additive, thickener, stabilizer and texture modifier (Chawla et al., 2009).

Other researchers also reported the use of BC as carrier support for probiotic (Jagannath et al., 2010) and for enzyme immobilization (Wu and Lia, 2008). In practice, the industrialist might take advantage towards the use of BC in the cases where the chemical purity, mechanical strength, degree of crystallinity or adsorptive capacity in particularly important for their end products. Therefore, it is necessary to determine the physiochemical

properties of BC to ensure the feasibility of BC to be use in the industries.

Hence, in this study, the physiochemical properties of BC produced by *Acetobacter xylinum 0416* were investigated. The physiochemical analysis is a method used to determine the characteristic, physical and chemical properties of a material. This includes Fourier transform infrared (FTIR) analysis, field emission scanning electron microscopy (FESEM), thermal stability using thermogravimetric analysis (TGA), pH, moisture content, compressibility index and swelling index. Additionally, the properties of BC also had been compared with the plant-based cellulose products; carboxymethyl cellulose (CMC) and cellulose powder (CP).

2. Material and methods

2.1. Fermentation process

The fermentation medium was prepared in 3 L distilled water containing 60 g glucose, 8 g sodium hydrogen phosphate (Na_2HPO_4), 15 g bactopeptone, 15 g yeast extract and 3.45 g citric acid. The fermentation medium was sterilized using an autoclave at 121°C for 15 min. The fermentation medium was poured into ten separated containers which each container was filled with 300 ml of fermentation medium. Then 10% (v/v) of the strain *A.xylinum 0416* was transferred into each of the containers. The fermentation medium was incubated for 14 days at 28±1°C as the medium went through a static fermentation to obtain the BC pellicles (Zahan et al., 2015).

2.2. Preparation of BC powder

The BC pellicle obtained was washed thoroughly with water. Then it was soaked and treated with boiling water at 100°C for 1 h using a water bath. Then the BC pellicle was ground using a heavy duty blender and poured into a tray. It was dried in the oven at 50°C for 24 h. The dried BC was then ground again until a fine BC powder was obtained (Halib et al., 2012).

2.3. Fourier transforms infrared (FTIR) analysis

The BC, CMC and CP samples were mixed with potassium bromide (1:100), pulverized with a mortar and pestle and compressed into tablets separately. The FTIR analysis was carried out using FTIR spectrometer with the resolution of 4 cm^{-1} in the range of 4000 to 400 cm^{-1} (Shaharuddin and Muhamad, 2015).

2.4. Field emission scanning electron microscope (FESEM)

The BC, CMC and CP samples were analyzed using the FESEM at 28±1°C with an accelerating voltage at 10 kV. All samples were mounted and observed on the aluminum stub with carbon tape. An imaging magnification of approximately 15000, 10000, 5000 and 2500 times were used. The micrograph of all samples was collected and recorded (Alyamani and Lemine, 2012).

2.5. Thermogravimetric analysis (TGA)

Ten milligrams (10 mg) of BC, CMC and CP samples were prepared and placed in a sample pan separately. The analysis was performed at 10°C/min from 30 to 950°C for 65 min. The differential thermogravimetric (DTG) curve of all samples was derived using Pyris 1 software provided by Perkin Elmer (Selvakumaran and Muhamad, 2015).

2.6. Swelling properties

Two grams (2 g) of BC, CMC and CP samples were mixed with 40 g of different solution separately (distilled water, acetone, dimethyl sulfoxide (DMSO) and acetic acid). The suspension was allowed to stand for 2 h. The suspensions were centrifuged at 3500 rpm for 30 min. The supernatant was removed and the wet precipitate was weighed. The steps were repeated three times to get the average reading (Goh et al., 2012). The result was expressed as a gram of water per gram of sample (w/w) by using Eq. 1.

$$\text{liquid retention level} = \frac{M_1}{M_2} - 1 \times 100 \tag{1}$$

where, M_1 = weight of wet sample and M_2 = weight of dry sample.

2.7. Compressibility index

Five grams (5 g) of BC, CMC and CP samples were accurately weighed and poured into a 100 ml graduated cylinder separately. Then, the bulk volume was taken (the initial reading). After that, the cylinder was tapped at height of 2.5 cm on the hard surface until a constant volume was obtained. The tap volume was taken as a final reading. The procedure was repeated three times to get the average value (Azubuike and Okhamafe, 2012). The bulk density, D_{bulk} and tapped density, D_{tap} were calculated using Eqs. 2 and 3.

$$D_{bulk} = \frac{W}{V_0} \tag{2}$$

$$D_{tap} = \frac{W}{V_1} \tag{3}$$

where, V_0 = volume of the bulk, V_1 = the volume tap density and W = weight of the celluloses (5g).

The Carr's index was calculated using Eq. 4 and the Hausner ratio was calculated using Eq. 5.

$$\text{Carr's index} = \frac{D_{tap} - D_{bulk}}{D_{tap}} \times 100 \tag{4}$$

$$\text{Hausner ratio} = \frac{D_{tap}}{D_{bulk}} \tag{5}$$

2.8. Determination of pH

One gram (1 g) of BC, CMC and CP samples were mixed with 40 ml of distilled water at pH 6.8 separately. The solution was stirred for 15 min using a hot plate and magnetic stirrer. The pH was checked using the pH meter and the reading was recorded. The steps were repeated three times and the average reading was calculated (Halib et al., 2012).

2.9. Determination of moisture content

Five grams (5 g) of BC, CMC and CP samples were accurately weighted separately. Then, the samples were placed in the moisture analyzer and the reading was recorded. The steps were repeated

three times and the average reading was calculated (Azubuike and Okhamafe, 2012).

3. Results and discussion

3.1. Fourier transforms infrared (FTIR) analysis

Fig. 1 shows the functional group and chemical bonding of BC, CMC and CP samples. The main peaks depicted for all samples are –H bond and –OH group (3200–3500 cm^{-1}), -C-H stretching (2800–2900 cm^{-1}), -C=O stretching (1600–1700 cm^{-1}), the carbonyl groups of the asymmetric –CH$_2$ bending and wagging (1300–1500 cm^{-1}) and–C–O–C– stretch of the β-1,4-glycosidic linkage (1000–1200 cm^{-1}) which similar to non-microbial source of natural cellulose (Pachuau, 2015; Barbara et al., 2008).
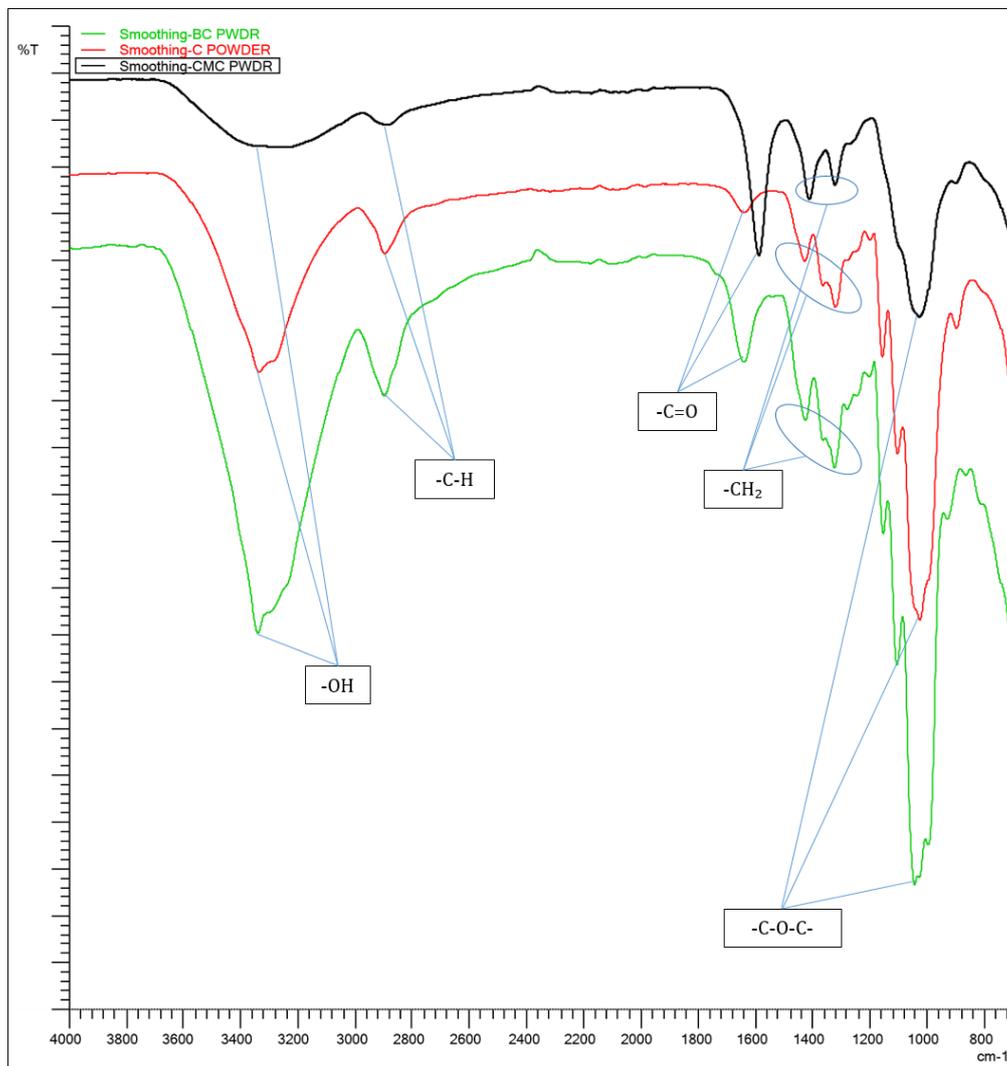

Fig. 1: Fourier Transform Infrared spectroscopy (FTIR) for BC, CMC and CP

As overall, this result particularly proved that BC, CMC and CP have a similar functional groups and chemical bonding. Thus, the chemical nature of BC was confirmed by the infrared spectra of CMC and CP. These results also had depicted that the BC produced by *A.xylinum 0416* was confirmed as pure cellulose

3.2. Field emission scanning electron microscope (FESEM)

The morphological structures of BC, CMC and CP sample was done using FESEM analysis at the magnification of 2500 times as shown in Fig. 2.

The image obtained shows that BC poses a very similar structure with the CMC and CP, plant based cellulose powders. Additionally, the fibrils were organized and tightly packed which conferred a morphological feature similar to the plant based cellulose powder. Besides, the surface of the BC was smoother compared to the plant based cellulose powder.

Further analysis of the BC under the magnification of 5000, 10000 and 15000 times had revealed that the BC poses a nanostructure with an irregular size and shape of fibrous network as shown in Fig. 3. The nanoparticle provide numerous advantages and applications in the industries since more surface area can be utilize thus enhance their overall capability. This include, nanoparticles can be easily manipulated for both passive and active drug targeting, availability of appropriate matrix helps in increasing the efficacy and reducing side effects of the drug, and finally important to control and sustain release of the drug during the transportation as well as the location of the release.

3.3. Thermogravimetric analysis (TGA)

Fig. 4 shows the maximum rates of weight loss for BC, CMC and CP at different temperature. The result obtained was in agreement with Halib et al. (2012) and Barbara et al. (2008) who stated that the maximum rate of weight loss was at 330 to 370°C.
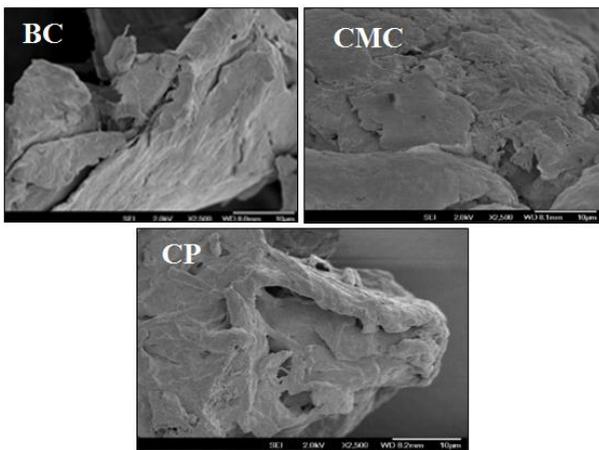

Fig. 2: Micrograph of BC, CMC and CP under the magnification of 2500 times

3.4. Swelling properties

Fig. 5 shows the liquid retention level of BC and CMC in different type of solvent. As overall, BC had shown higher liquid retention level in all solvent compared to CMC which reflect higher swelling properties of BC. The swelling power of the cellulose in the solvent was influenced by the degree of molar volume of the solvent which contribute to the hydrogen bonding capability and the cohesive energy density of the solvent (Goh et al., 2012; Cheng et al., 2010).

The result also depicted that BC show the highest temperature for the maximum rate weight loss compared to CMC and CP. On the other hand, the differential thermogravimetric (DTG) curve obtained depicted that the temperature of the maximum weight loss for BC was shifted almost 63°C higher than CMC and almost 11°C higher than CP. This reflects that BC possessed the highest thermal stability compared to plant cellulose (Cheng et al., 2009). Additionally, the TGA curve of BC showed a typical decomposition curve of pure compound where only one step of decomposition was observed (Bottom, 2008). Thus this result had indicated the purity of BC produced by *A.xylinum 0416*.
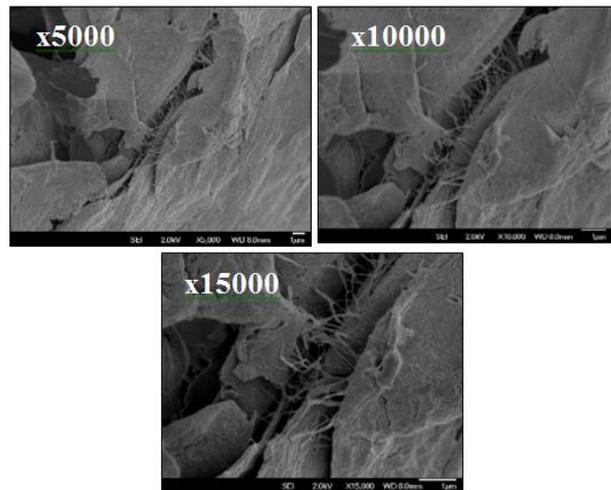

Fig. 3: Micrograph of BC under the magnification of 5000, 10000 and 15000 times

Swelling power also indicates their water holding capacity. The water binding capacity in commercial cellulose-based products is important especially when it is used as a thickener, adhesive, binder, encapsulating agent, film former, gelling agent, texturizer and fat-sparing agent (Kaur et al., 2011).

3.5. Compressibility index, moisture content and pH

Table 1 summarized the compressibility index, moisture content and pH value for BC and CMC. The compressibility index of BC and CMC was determined by Carr's index and Hausner ratio. The Carr's index of BC and CMC powder are 4.90% and 6.10% respectively. While the Hausner ratio for BC and CMC are 1.05 and 1.06 respectively. According to Goh et al. (2012), the excellent Carr's index range was at the range of 5 to 10%. These results indicate that BC shows a better flow property compared to CMC. While Azubuike and Okhamafe (2012) stated that the smaller Carr's index exhibits a smaller compressibility index and angle of repose, indicating and confirming its better flow property.

Table 1 also depicted that the moisture content of CMC was slightly higher than BC. According to Goh et al. (2012), the acceptable range of the moisture content for cellulose powder product was in the range of 5 to 7%. Finally, the pH recorded for both BC and CMC was almost the same and nearly to the neutral values.

4. Conclusion

The study was set out to determine the properties of BC produced via fermentation using the *A.xylinum 0416*. The data collected had proven that BC shows a better and promising characteristic compared to plant based cellulose products (CMC and CP). In conclusion, BC can be used as one of the alternative to replace the plant based cellulose product in the industries which finally help us to preserve our forest for the benefit of the future generations.
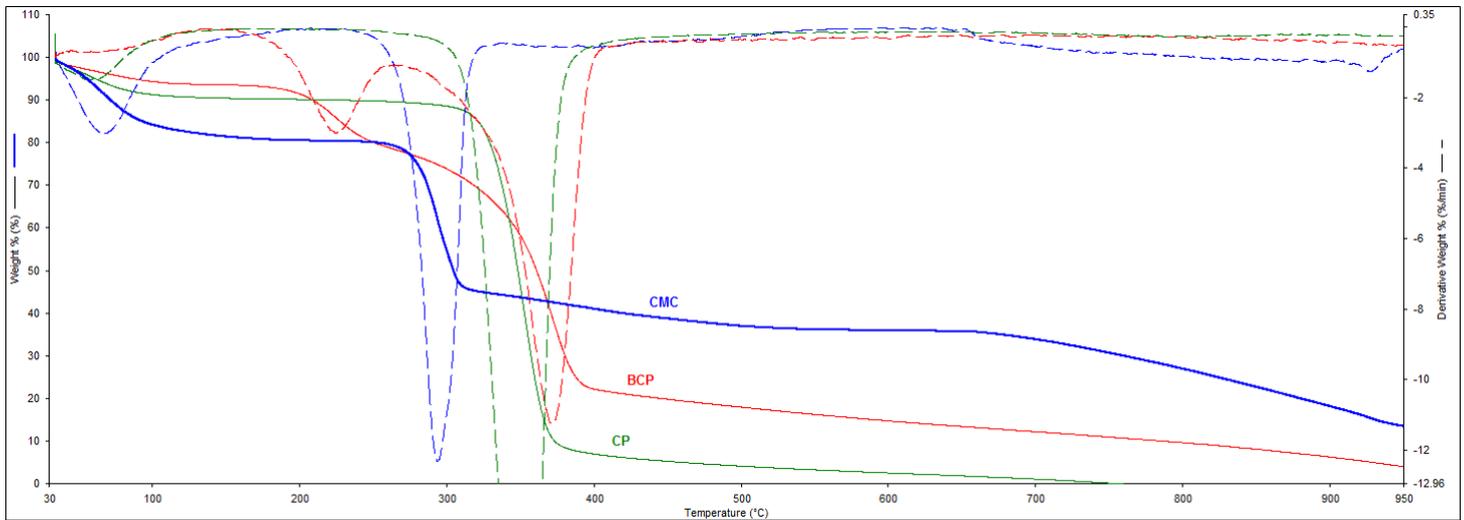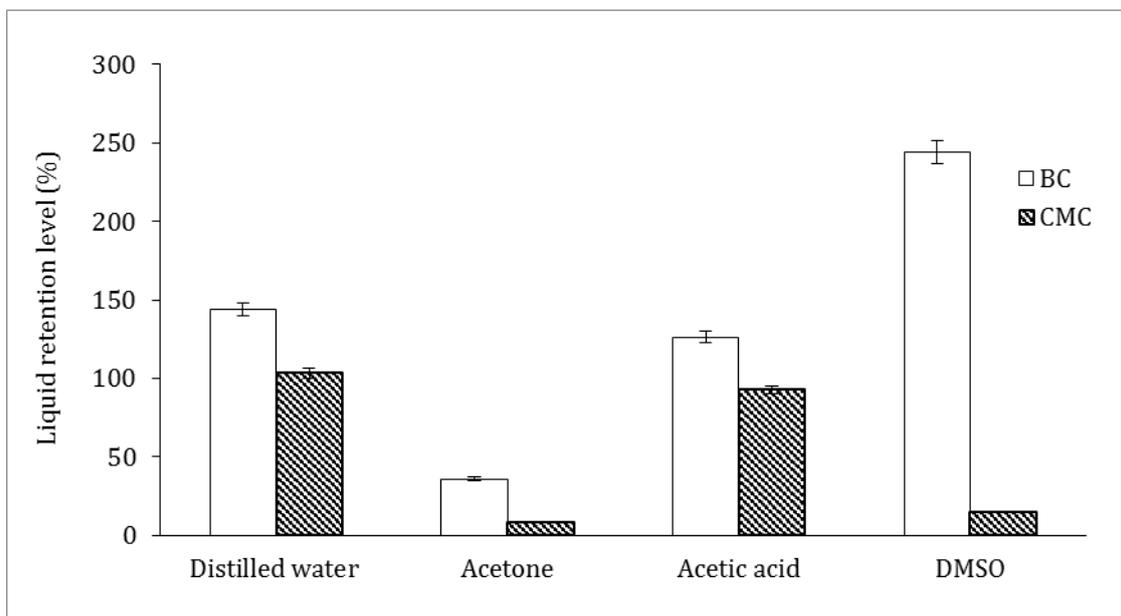

Fig. 4: The TGA and DTG curve of BC, CMC and CP

Fig. 5: The liquid retention level of BC and CMC in four different solvent

Table 1: The compressibility index, moisture content and pH of BC and CMC

	Compressibility Index (%)	Moisture Content (%)	pH
BC	5.00	8.75	6.60
CMC	6.00	11.84	7.10

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