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Bacterial Cellulose as a Potential Hard Gelatin Capsule

(Potensi Selulosa Bakteria Sebagai Kapsul Gelatin Keras)

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ABSTRACT

Matured coconut water is considered as an industrial waste where about 5.3m³ of this solution was discarded everyday especially in the coconut processing industry. An improper disposal process can cause environmental pollution especially to nearest river of processing industry. Despite all the advantages of bacterial cellulose (BC) over plant cellulose, its production is relatively expensive process primarily due to the low productivity and expensive culture medium. Thus, coconut water can be used as a medium for fermentation bacterial cellulose and the production cost can be reduced up to 50 to 60%. Acetobacter xylinum 0416 is often used in industry because they can be produced large quantity of BC. There are many applications of BC in industries such as in the food industry, pharmaceutical industry, textiles and etc. However, this study only focuses on the characteristics of hard gelatin capsule (pharmaceutical industry) and BC. This is because, in market there are about 80-90% hard gelatin capsule are non-halal and this situation cause very high demand of halal hard gelatin capsule especially in Islamic countries. This study focus on determination of optimum condition for BC production by manipulation of variable with a certain pH range which is (4-6), the incubator temperature (28-32°C), and time of fermentation (3-7 days). Comparison of BC and hard gelatin capsule properties were made through FESEM, XRD and FTIR analysis. Study found that optimum BC was obtained about 2.28 g/L at 32°C, in pH4 for 7 days fermentation period.

Keywords: Matured coconut water; Fermentation; Bacterial cellulose; Hard gelatin capsule

ABSTRAK

Air kelapa tua dianggap sebagai sisa industri di mana kira-kira 5.3m³ larutan ini dibuang setiap hari terutamanya di dalam industri pemprosesan kelapa. Proses pelupusan yang salah boleh mengakibatkan pencemaran alam sekitar terutamanya sungai yang berdekatan kilang pemprosesan. Walaupun kelebihan selulosa bakteria (BC) mengatasi selulosa tumbuhan, kos pengeluarannya yang agak tinggi terutamanya disebabkan oleh produktiviti rendah dan media fementasi yang mahal. Oleh itu, air kelapa boleh digunakan sebagai medium fermentasi selulosa bakteria dan kos pengeluaran dapat dikurangkan sehingga 50 hingga 60%. Acetobacter xylinum 0416 sering digunakan dalam industri kerana mampu menghasilkan BC dalam kuantiti yang banyak. Terdapat banyak aplikasi BC di dalam industri seperti industri makanan, industri farmaseutikal, tekstil dan sebagainya. Walau bagaimanapun, kajian ini hanya memberi tumpuan kepada ciri-ciri kapsul keras (industri farmaseutikal) dan BC. Hal ini kerana, di pasaran terdapat kira-kira 80-90% kapsul keras bukan halal dan keadaan ini menyebabkan permintaan tinggi terhadap kapsul keras terutama di negara-negara Islam. Kajian ini menumpukan pada penentuan keadaan optimum untuk pengeluaran BC dengan manipulasi pemboleh ubah dengan julat pH tertentu iaitu 4-6, suhu inkubator (28-32°C), dan masa penapaian (3-7 hari). Perbandingan sifat-sifat kapsul BC dan kapsul keras dibuat melalui analisis FESEM, XRD dan FTIR. Kajian mendapati bahawa keadaan optimum BC adalah sebanyak 2.28 g/L pada 32°C, dalam pH4 selama tempoh 7 hari fermentasi.

Kata kunci:Air kelapa tua; Fermentasi; Selulosa bakteria; Kapsul gelatin keras

INTRODUCTION

Cellulose is a non-toxic biopolymer mainly extracted from plants and has been use for centuries as raw material in a variety of usage (Chiciudean 2011). Cellulose that produced by bacteria is known as bacterial cellulose (BC) with better physiochemical properties than plants in nature. *Agrobacterium, Rhizobium and Sarcina* species manage to synthesize BC but *Acetobacte*r was found capable to generate BC in high quantity that sufficient for industrial

purpose (Chiciudean 2011; Afreen, 2014). The cellulose producing bacteria consume either glucose, other type of sugars or glycerol to produce pure cellulose. A cell can convert about 108 molecules of glucose to cellulose within an hour (Nugroho & Aji 2015; Zainudin et al. 2016; Lina Fu et al. 2011).

Both BC and plant cellulose has an identical molecular formula, $(C_6H_{10}O_5)n$ but exist contrarily in nature. BC was excreted extracellularly with high purity structure while plant cellulose as a dominant structure in cell wall is branches with hemicellulose, lignin and pectin. The purity of BC enable it to hold high water capacity (Chawla et al. 2009). Matured coconut water is a nutritional agro waste that contains sucrose, glucose and fructose as the main sugar including inorganic ions, amino acids and vitamins. The sugars can act as carbon supplier while other nutrition prepare a conducive condition that meet the demand for bacteria growth and BC synthesis (Yong et al. 2009). Instead of having rich beneficial composition, the usage of matured coconut water as fermentation medium can reduce the cost of total production process and easy to handle.

For capsule manufacturing industry in Malaysia, about 10% of gelatine imported from Taiwan are believed to be halal while the other 90 % are from bovine gelatin of unknown halal status (Gama et al. 2017). Hard gelatin capsule is easy to produce, to storage and has no taste and smell. In addition, the drug absorption of hard gelatin capsule was faster than a pill or tablet. However, the usage of gelatine capsules is limited due to difficulties in having halal source of gelatin or it replacement. Hence, this study was run to look at the potential of BC as substrate in hard gelatin capsule (HGC) production.

MATERIAL AND METHODS

ACETOBACTER XYLINUM 0416 INOCULUM

Inoculum medium was prepared by dissolving 8.0 g of glucose ($C_6H_{12}O_6$) and 0.5 g of ammonium sulphate $(NH_4)_2SO_4$ into 100 ml of matured coconut water. The pH of medium was adjusted with 0.5M acetic acid (CH_3COOH) or 0.5 M sodium hydroxide (NaOH) to reach pH4.5. The mixture was sterilized at 121°C for 15 minutes. About 10 ml of *Acetobacter xylinum 0416* stocks culture received from Malaysian Agricultural Research and Development Institute (MARDI) was added into the culture medium and incubated in static condition for 3 days at 30°C. The whole preparation process was carried out in laminar flow chambers aseptically to avoid contamination.

OPTIMIZATION ON BC PRODUCTION

A response Surface Methodology (RSM) design has been used to determine optimum condition of BC production and was analyzed with Design Expert 6.0.10®. For BC fermentation process, a total of 10 mL inoculum was mixed with 100 mL matured coconut water that has been set at a certain pH ranged from pH4 to pH6. The pH value was adjusted by adding 0.5 M NaOH or 0.5 M CH_3COOH into the solution. Then, the mixture was incubated at certain temperature (30°C - 32°C) and within a certain fermentation period (3 – 7 days) as proposed by RSM. The fermentation processes were run in static state throughout the process.

DETERMINATION OF BC DRY WEIGHT

The BC pellicle formed on the surface of medium was washed with distilled water and boiled in 0.5 M NaOH for 20 minutes. It was then washed several times with distilled water, soaked in 10% H_2O_2 for 30 minutes and finally rinsed with distilled water overnight. The BC was dried in incubators at 60°C for several days until a constant weight achieved.

FOURIER TRANSFORM INFRARED (FTIR) SPECTRAL STUDIES

To prove the structure of bacterial cellulose and the existence of gelatine in hard gelatin capsule, FTIR spectra data were used. The FTIR spectrophotometer used in this study was a Nicolet 6700 from Thermo Scientific, United States with the wavelength range used was from 4000 to 400 cm^{-1}.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY (FESEM) STUDIES

The nanoscale topography of bacterial cellulose and hard capsule gelatine were visualised and compared using a high resolution Field Emission Scanning in Electron Microscopy (FESEM). The FESEM used this study was MERLIN (ZEISS) with resolution 0.8 nm/15 kV and magnification 5,000 to 10,000 times.

X-RAY DIFFRACTION (XRD) STUDIES

The crystallinity of BC and HGC were compared using X-ray diffractometer (XRD) spectra. A D8 Advance Bruker AXS model from Germany was used for analysis with 40 kV and 40 mA of operating voltage and current. Both samples were scanned in a 10-30° range of 2° with 1.5406 nm wavelength. Equation 1 shows the crystallinity (%) calculation of samples (Kim and Holtzapple 2006):

$$\text{Crystallinity, \%: } \frac{I_{002} - I_{am}}{I_{am}} \times 100\% \qquad (1)$$

where, I_{002} and I_{am} are the maximum scattering intensities of the diffraction from the (002) plane at 2θ = 22.6 and the background scatter diffraction intensity measured at 2θ = 18°. The crystallization size calculation was based on Scherrer formula as shown in Equation 2 below:

$$\text{Crystallization size, L: } \frac{K\lambda}{\beta \cos \theta} \qquad (2)$$

where, K is the dimensionless crystallite shape factor (0.9), λ is the X-ray wavelength (nm), β is the line broadening at half maximum intensity (radians), and θ is the Bragg angle (degree).

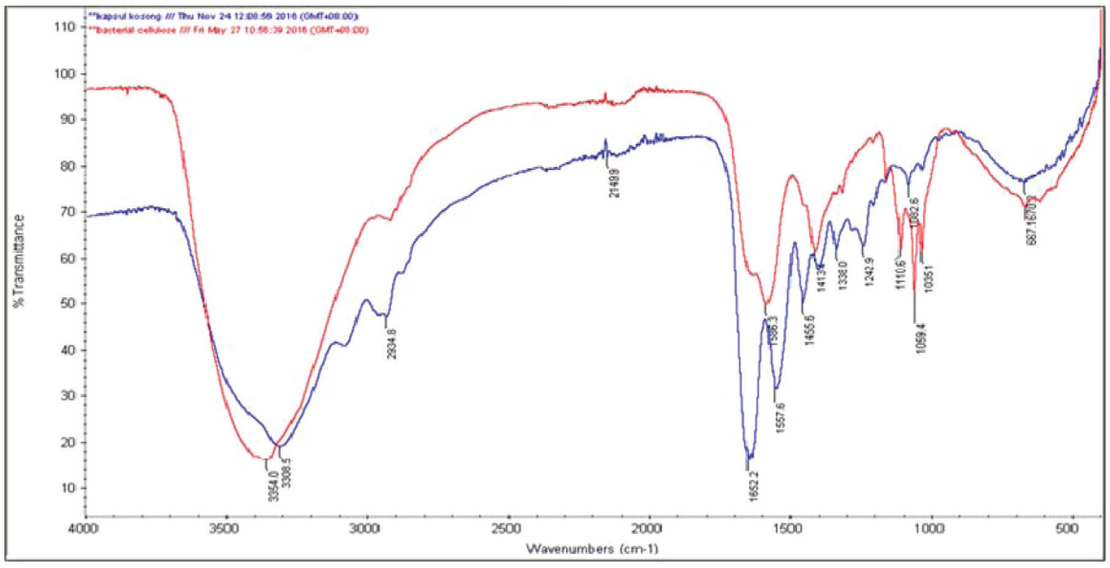

FIGURE 1. FTIR spectra of hard gelatin capsule (HGC) and bacterial cellulose (BC)

RESULTS AND DISCUSSIONS

In this study, bacterial inoculum of 3 days old with growth rate, μ_{max} = 0.59 h^{-1} in matured coconut water medium was used for fermentation (unpublished result). At this rate, the *Acetobacter xylinum 0416* is experiencing exponential phase where the bacteria cell was actively dividing. In static fermentation state, the *A.xylinum* culture consumed sugars for growth and produced a layer of cellulose pellicle at the surface of coconut water medium. The sugar level in fermentation medium however influence the rate of production as too much sugars may give negative impact on cell growth and BC production where the excessive unutilized glucose will be converted to keto-gluconic acid, lowered the medium pH and disturb the production (Halib et al. 2012; Chawla et al. 2009).

Selection of temperature, types of medium and medium conditions are essential to be considered in BC production (Sulaiman et al. 2018; Kasim & Rahman, 2016; Masaoko et al. 1993; Pa'E et al. 2014). In the response surface statistical analysis through regulating the temperature, initial pH and duration of fermentation, the optimum conditions of BC production were suggested at 32°C, in pH 4 of culture medium, and 7 days fermentation period which capable to synthesize about 2.28 g/L cellulose. Based on this finding, these parameters condition were then being used throughout the upcoming experiment.

To validate the structure and purity of cellulose produced by bacterium in this study as well the existence of gelatin in HGC, analysis by FTIR was performed. The FTIR identifies chemical functional groups and bonding in a molecule by generating a profile of broad specific infrared absorption spectrum of samples. By comparing with a distinctive molecular fingerprint, samples with variety of components can be screen and recognized.

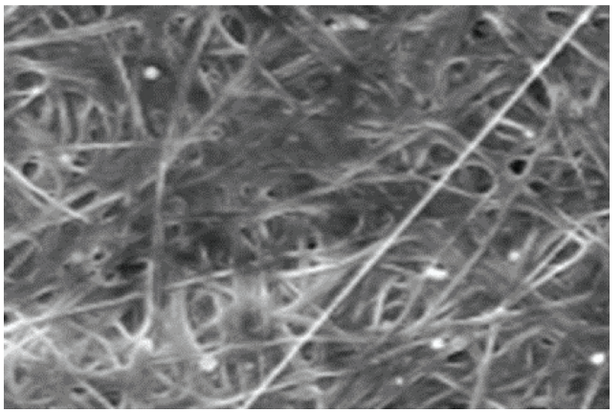

FIGURE 2. The FESEM of the surface of bacterial cellulose at 10,000x magnification

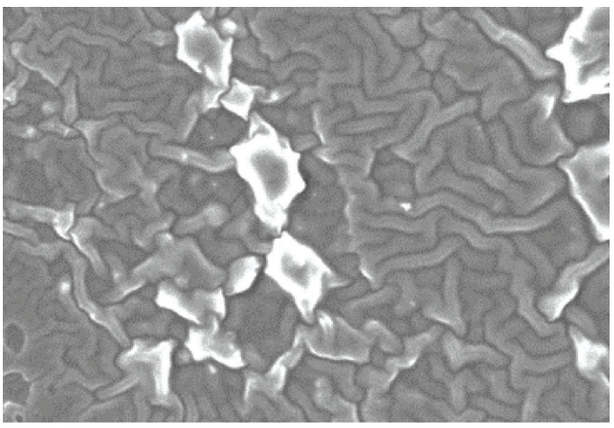

FIGURE 3. The FESEM of the surface of hard gelatin capsule at 10,000x magnification

For the bacterial cellulose produced in this study (Figure 1), a broad peak appeared at 3354 cm^{-1} represents O-H stretching vibration of hydroxyl group. A peak of absorption band round 1600 cm^{-1} (1586.3 cm^{-1}) is the stretching vibration of carboxyl group (COO^-), 1413.4 cm^{-1} is the carboxyl groups as it salts and 1035 cm^{-1} to 1060 cm^{-1} indicate C-O stretching vibration (Ismail et al. 2010; Oliveira et al. 2015; Jiang et al. 2017; Gayathry & Gopalaswamy 2014). The spectra of this sample presents a typical characteristic of cellulose and consistent with the fingerprint region of pure cellulose. Thus its confirming that bacterial cellulose produced by *A.xylinum* is a pure cellulose (Halib et al. 2012).

For HGC, the capsule is usually made of gelatin derived from collagen of animals. The source of gelatin in food capsule is always a concern due to religious restriction especially consumption of pork product in Muslim and Jewish also cattle product in Hindus community. Moreover, this issue did raise a concern in group of vegetarian people. For this HGC, the gelatin spectrum showed characteristic bands at 3308.5 cm^{-1} due to N-H stretching of amide –A, amide-B stretching at 2934.8 cm^{-1}, C = O stretching of amide-I band at 1652.2 cm^{-1}, N-H bending vibrations of amide-II at 1557.6 cm^{-1}, C-N stretching vibrations coupled with in-plane N-H bending of amide-III at 1242.9 cm^{-1} also amide-V at 667.1 cm^{-1} (Muthivhi et al. 2018; Al-Saidi et al. 2012). This spectrum prove the existence of animal product in capsule which commonly not being declared of their source.

XRD was used to determine the crystallinity index and crystal size for both samples. XRD reading for BC was 62.4% whereas the crystallinity for HGC is only 9.7%. There is a huge different between these two samples. High crystalline phase will make polymer more brittle, increasing melting temperature and hard for any substance to diffuse in. According to Bhat et al. 2017 and Kamarudin et al. 2016, HGC is flexible due to the presence of water that acts as a plasticizer. Plasticizer is the substance that acts as reducing polymer stiffness because it can decrease the cohesive intermolecular forces along polymer chain. In contrast, once BC microfibrils converted to cellulose nanofiber (CNFs), the material will become highly stiff, low thermal expansion and with high strength (Daicho et al. 2018). It also has great hydrophilicity and can evenly disperse in water which make it experiencing same performance as gelatin. Besides that, cellulose has been used as thickening agent, capsule diluent and adsorbent (Marques-Marinho & Duarte 2013).

Figure 2 and 3 show the surface structure of BC and HGC respectively through FESEM analysis at 10,000X magnification. Figure 2 shows the arrangement of BC fibril that result from the outer crossing among fibrils. The BC was extracellularly export through pores of *A. xylinum 0416* and arranged in line along the longitudinal axis. The entangled structure of BC with some spaces distributed through it, allows impregnation of other compound into BC matrix and hence improve the BC properties such as their flexibility and stiffness. The FESEM image of BC obtained is also supported by Chawla et al. 2009. Meanwhile, Figure 3 depicts the HGC surface area. The polymers was in an organized settings thus make it more flexible.

CONCLUSION

Bacterial cellulose has been widely used in different fields, such as in paper industry, electronics and tissue engineering due to its remarkable mechanical properties and porosity. In order to have an economic BC production, fermentation at optimize condition with the usage of matured coconut water as fermentation medium is greatly beneficial. Comparison made between BC and HGC shown that BC has almost similar properties with HGC. Thus, BC has potential to replace HGC as biodegradable capsule. Plus, with addition of some compound, better properties of BC composite could be made.

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