International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Physical Properties of Bacterial Cellulose Produced by *Gluconacetobacter xylinus* BTCC B796 with Different Temperature and Agitation

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Abstract: ***The objective of this work was to look at the effects agitation and growth times on the properties of bacterial cellulose produced by Gluconacetobacter xylinus. Gluconacetobacter xylinus was grown in HS medium at 50 rpm at room temperature for effect agitation and 23ºC, 25ºC, 27ºC and 30 ºC at static conditions for seven days. The cellulose produced was analyzed for crystallinity index, FTIR, SEM. The results showed The use of agitation and decreasing temperature can reduce the index crystallinity of bacterial cellulose, and shown by evidence of changes in hydrogen bonding. Decreasing crystallinity index of bacterial cellulose furthermore resulted in more void and loose cellulose arrangement.***

Keywords: Bacterial cellulose, *Gluconacetobacter xylinus*, agitation, crystallinity index, physical properties

1. Introduction

Bacterial cellulose is a fermentation product with interesting characteristics, ie small fibril size (1.5 nm) [1], semicrystalline and able of forming 3-dimensional network, so it can be used in the food field as dessert for example *nata de coco*[2]. Bacterial cellulose in the food field also used as a packaging for sausages and potentially as a stabilizer and thickener agent [2], but bacterial cellulose can have high crystallinity and sometimes influent to texture [3], decrease in WHC and low rehydration ratio [4], so it become not suitable for the food field.

Previous research suggests that the crystallinity of bacterial cellulose may be altered by fermentation conditions. The use of agitation in the production of bacterial cellulose may result in changes in the crystallinity of bacterial cellulose. The production of bacterial cellulose using agitation at a rate of 150 rpm can decrease the crystallinity index when compared to static from 81.6% to 67.1% [5], 84.1% to 51.2% [6] and 89% to 84% [7]. The crystallinity of bacterial cellulose also able change due to differences in the use of fermentation temperature. At temperatures lower than the optimum temperature of its growth can lead to slow motion of the molecule, bacteria tend to be silent and the cellulose that is synthesized crystallinity decreases. At 28 ° C, Acetobacter xylinum ATCC 23769 cells produce cellulose I (crystalline) and when the cultivation temperature is lowered to 4 ° C the cell produces cellulose II (less crystalline) [8]. Previous research also indicating the higher the fermentation temperature used (25-30 ^{o}C), the crystallinity of cellulose increased [9].

According to previous research, effect of agitation and temperature differences on crystallinity and other physical properties still limit. This research has a purpose to know the effect of fermentation condition (agitation and different temperature) on the index of crystallinity, functional group, and morphology of bacterial cellulose.

2. Research Method

2.1 Materials

Gluconacetobacter xylinus BTCC B796 obtained from biotechnology centre of LIPI culture collection. Media used was Hestrin Schramm (HS) medium with containing 2.0% (w/v) D-glukosa, 0.5% (w/v) peptone, 0.5% (w/v) yeast extract,0.27% (w/v) Na_2HPO_4 and 0.115% (w/v) citric acid [10] . Glacial acetic acid was used to lower pH medium around pH 5.0.

2.2 Growth Conditions

Seed culture from HS agar was inoculated to 10 ml HS broth for three days at 30 oC under static conditions. The resulting culture was shaken vigorously to release cells from the cellulose pellicle and cell suspension was inoculated into 100 ml HS medium in 500 ml conical flask at a concentrations of 5% (v/v). Cultures was incubated for 3 days at 30 ^{o}C under static conditions. The resulting culture was shaken vigorously to release cells from the cellulose pellicle. 5% (v/v) cell suspension was inoculated into 2 L HS medium and incubated using agitation 50 rpm for 7 days at room temperature. The strain was also grown at 23, 25, 27 and 30 ^{o}C static for 7 days. The resulting pellicle was removed from cultures. Pellicles was rinsed with water and boiled in 0.1 N NaOH for 20 min to remove any residual media. The pellicle

produced are washed repeadly/thoroughly using water until the pH of water became neutral.

2.3 X-ray Diffraction

X-ray pattern measurement was carried out to analyze the change in crystallinity of the BC by shimadzu-6000 diffractometer with using Ni-filtered CuK radiation (k = 1.54 Å). The XRD operating voltage and current were 40 kV and 30 mA, respectively. The crystallinity index (CrI) was calculated from diffracted intensity data using Segal method [11].

2.4 FTIR

The FTIR spectra of the BC was measured at wave numbers ranging from 4000 to 400 cm^{-1} using spectrometer Shimadzu-8201 PC. Corp.,Japan) and utilizing the KBr (Potassium Bromide) technique for BC preparation.

2.5 Scanning Electron Microscopy (SEM)

SEM (JEOL JSM6510 LA) was used to observe the topography of the surface and matrix of pellicle. Bacterial Cellulose was dried and coated with palladium and then observed with a scanning electron microscope.

3. Result and Discussion

3.1 Crystallinity Index

The fermentation conditions may affect the crystallinity of bacterial cellulose and have subsequent effects on other physical properties. In this study the use of agitation and different tempetarure able to change on the cristallinity index of bacterial cellulose (Table 1).

Table 1: Crystallinity index of cellulose bacterial produced by *G. xylinus* with agitation dan different fermentation temperature

Fermentation conditions	Crystallinity Index (%)
0 rpm	78,10
50 rpm	60.13
23 ^{o}C	57.50
25 ^{o}C	59.54
27 ^{o}C	69.56
30 ^{o}C	71.00

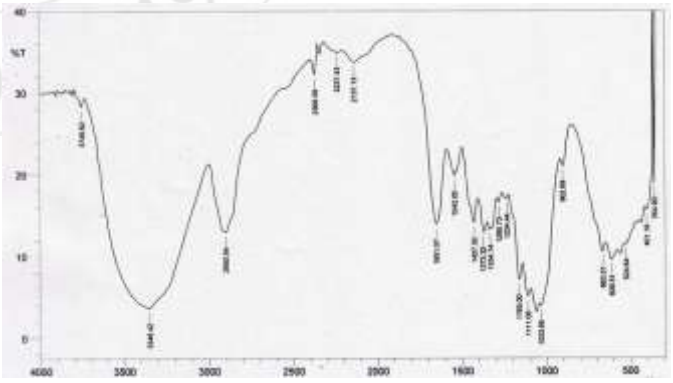
Fermentation by using agitation leads to a decrease in the crystallinity index compared to static conditions. Fermentation with 50 rpm agitation has decreased the crystallinity index from 78.10% to 60.13 %. The use of 23 ^{o}C temperature produced bacterial cellulose with a low crystallinity index of 57.50% and has significant difference compared to cellulose production at its ophthalmic growth conditions at 30 ^{o}C, static condition for 7 days produces bacterial cellulose with a high crystallinity index of 71,00. In this study, the cellulose crystallinity index decreased due to the use of agitation compared to static conditions. Preparation of a structure that is crystalline by bacteria requires free cell movement so that cellulose can be arranged regularly. In the use of agitation, the movement of the cell will be affected by mechanical forces resulting in aggregation and crystallization of disturbed cellulose to alter the intermolecular and intramolecular arrangements of bacterial cellulose [5]. Other research also suggested the use of agitation may result in hydrogen bonds connecting the fibrils to be discontinued resulting in a decrease in the crystallinity index[12].

Decreasing the crystallinity index due to the low use of fermentation temperature is likely to cause bacterial cells to tend to be inactive, whereas to produce bacterial cellulose structures that crystalline bacterial cells must be active so they can move freely and produce crystalline cellulose. Changes in temperature lower than the optimum temperature of growth of bacteria can lead to slow motion of the molecule, bacteria tend to be silent and affect the resulting crystalline structure of cellulose[8]. Other studies have shown the higher the fermentation used by the cellulose descending crystalline index [9].

The decrease of crystallinity of bacterial cellulose due to the addition of polysaccharides is also supported by observations using FTIR and SEM.

3.2. FTIR

The observation using FTIR showed that bacterial cellulose has characteristic of functional group in the form of peak which appears in peak 3600-3000 cm-1 is OH group of bacterial cellulose (Figure 1, Figure 2). Peak at 2500-3000 represents the CH group [13], whereas peaks in 1765-1715 cm-1 show the presence of C = O stretching [14], [15]. Absorption at about 900 cm-1 may be derived from β-glycoside bonds in cellulose, and a peak at 895.86 cm-1 indicates the presence of bacterial cellulose[15]. Among the typical peaks of bacterial cellulose, peak that is often used as a basis for changes in cellulose structure is the OH group because it is associated with hydrogen bonding in cellulose intermolecule bonds.



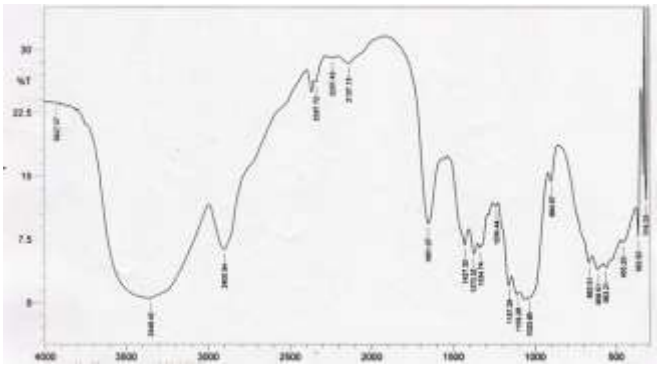


Figure 1: FTIR test results of bacterial cellulose produced by Gluconacetobacter xylinus at (A) 0 rpm (B) 50 rpm

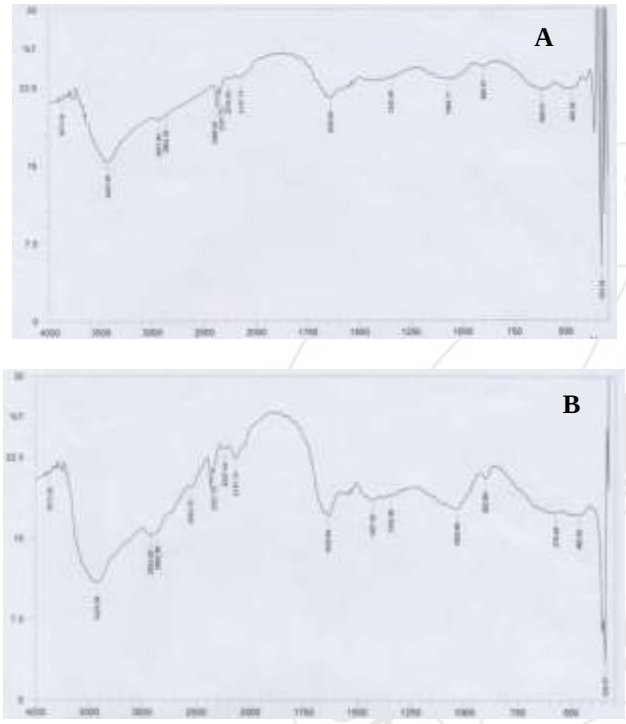


Figure 2: FTIR test results of bacterial cellulose produced by *Gluconacetobacter xylinus* at (A) 23 ^{o}C (B) 30 ^{o}C

The increasingly sloping peaks indicate the change of hydrogen bonds from bacterial cellulose and result in decreased crystallinity of bacterial cellulose [16]. Other research suggests that crystalline structures are formed by the presence of hydrogen bonds so that cellulose is able to form sheets[17].

Based on FTIR observations showing the use of temperature variation and agitation give change to peak OH. At 23^{o}C the temperature is more gentle than the temperature 30^{o}C, while the use of agitation shows 50 rpm more gentle than the static condition. The gentle peak OH indicates that the hydrogen bonds in the molecular or intra molecular interfractions are weak and show that their crystallinity decreases[5]. These results support the results of testing using XRD showing bacterial cellulose produced at the use of 50 rpm and 23 ^{o}C able to reduce crystallinity of bacterial cellulose.

3.3. Morphology of bacterial celluloce

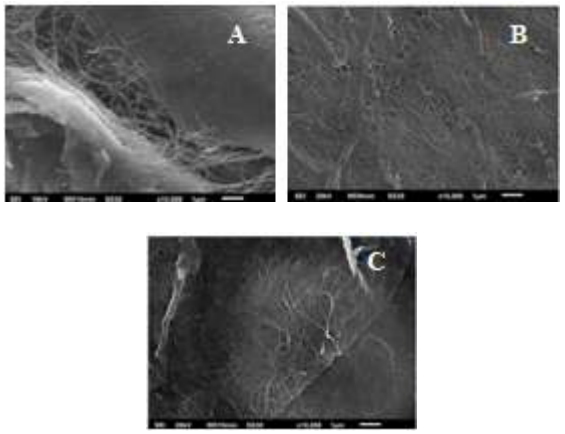


Figure 3: Scanning Elektron Microscopy (SEM) dari selulosa bakterial yang diproduksi oleh *Gluconacetobacter xylinus* pada (A) 23^{o}C , (B) 30 ^{o}C, dan (C) agitation 50 rpm

Observations using SEM show the use of 23 ^{o}C temperature produces bacterial cellulose that has a larger pore than the temperature of 30 ^{o}C (Figure 3). The observations using SEM support data showing the use of 23 ^{o}C fermentation temperature to produce bacterial cellulose with low crystallinity index. The use of agitation shows a tenuous and irregular array of fibrils compared to static conditions.

Changes in the cellulose structure are thus looser / less compact and have many pores supporting evidence of crystalline cellulose structural changes with denser structural features. Further cellulose structural changes affect the physical properties of cellulose[13], such as tensile strength, WHC and rehydration ratio. The ability of bacterial cellulose to absorb water (WHC, rehydration ratio) is an important characteristic because it deals with its sensory properties [18] and its functional properties as dietary fiber and is potentially applied as a thickener and food stabilizer.

4. Conclusion

The use of agitation and decreasing temperature can reduce the index of crystallinity of bacterial cellulose, and shown by evidence of changes in hydrogen bonding. Decreasing crystallinity index of bacterial cellulose furthermore resulted in more voids and loose cellulose arrangement.

5. Acknowledgement

The author gave an acknowledgements to Ministry of Agriculture of the Republic of Indonesia, and Ministry of Research Technology and Higher Education (Kemenristekdikti).

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