THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Effect of Culture Conditions on the Properties of Bacterial Cellulose Produced by *Gluconacetobacter xylinus* BTCC B796

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Abstract:
The objective of this work was to look at the effects of temperature and agitation on the properties of bacterial cellulose produced by Gluconacetobacter xylinus BTCC B796. Gluconacetobacter xylinus was grown in HS medium at 23, 25, 27, and 30 ^{o}C at static conditions for 7 days. Other experiment was carried out at room temperature at different degree of agitation, i.e.: 0, 50 rpm for 7 days. The strain was also grown at static condition at room temperature, and samples of cellulose produced were drawn after 4,7,10,14 days. The cellulose produced was analyzed for crystallinity index, cellulose morphology, tensile strength, water holding capacity (WHC), and rehydration ratio. Cellulose produced at lower temperature gave lower crystallinity index and tensile strength, but the WHC and the rehydration ratio were higher. Cellulose produced using agitation gave lower crystallinity index and tensile strength compared to static conditions but WHC and rehydration ratio were higher. Bacterial cellulose produced gave the highest crystallinity index and tensile strength at 7 days but then decreased with increasing fermentation time. Water Holding Capacity and rehydration ratio of bacterial cellulose increased after 7 days fermentation.

Keywords: *Bacterial Cellulose, Gluconacetobacter xylinus, agitation, growth times*

1. Introduction

Cellulose is a type of dietary fiber is beneficial for health. Diet sufficient cellulose can prevent constipation and obesity. Dietary fiber diet also noted an effect on blood cholesterol levels (Waloya *et al*., 2013). One source of cellulose is cellulose produced by bacteria called bacterial cellulose. Bacterial cellulose is also used as a thickener, stabilizer, and texture modifiers on food (Shi *et al.,* 2014).
Bacterial cellulose is a polysaccharide that is synthesized by bacteria through fermentation. *Gluconacetobacter xylinus* is a bacteria that is widely used to synthesize bacterial cellulose because of its higher productivity (Chawla *et al*., 2007). Bacterial cellulose has very small fibril diameter (1.5 nm) and a degree of polymerization between 1000-6000 (Khan *et al*., 2007). Bacterial cellulose has a high crystallinity, and have high ability to bind water as well as high mechanical strength (Chen *et al*., 2010). With these properties, the bacterial cellulose is used in the field of food also applied to the field of health (dressings), electronics (audio speaker diaphragm), paper and composites manufacture.

Bacterial cellulose has a high crystallinity index (above 60%). High crystallinity is not desired for food such nata de coco because it will affect the texture and sensory properties. High crystallinity of bacterial cellulose tends to reduce its water holding capacity. Thickness of nata influences the water holding capacity which in turn has an effect on its physical textural and organoleptic properties (Jagannath *et al*., 2008). Bacterial cellulose also exhibits poor rehydration after drying because of its high crystallinity (Huang, *et al.,* 2010; Lin *et al*., 2009). This property limits the application of dried bacterial cellulose for stabiliser or thickening agent.

Crystallinity of bacterial cellulose able to changes depending on media and culture condition (Ruka *et al*., 2012; Zhou *et al*, 2007), such temperature, growth times and agitation. In the production of bacterial cellulose, crystalline arragement synthesized by bacteria through aggregation and crystallization phases of sub elementary fibrils to form pellicle. In forming the crystalline structure requires free movement of bacterial cells that cellulose produced gave ordered arrangement. The difference in temperature influence on the speed of movement of bacteria and further lead to changes in the composition of bacterial cellulose produced (Hirai *et al*., 1997). Movement of the bacterial cell will be affected by mechanical forces of agitation so that disrupt the process of aggregation and crystallization of bacterial cellulose (Moon *et al*., 2006; Yan *et al*., 2008). The time difference fermentation can also lead to changes in the degree of crystallinity of bacterial cellulose. The longer the fermentation period, bacterial cellulose formed denser that restrict the movement of bacterial cells to form the crystalline arrangement of the cellulose structure. These changes may result in changes to other the physical properties of bacterial cellulose (Sheykhnazari *et al*., 2011).

Research on the influence of fermentation conditions to the changes in crystallinity and futhermore in other physical properties is still very limit, so as to obtain the characteristics of cellulose in accordance with its use still requires further research. This research studied the effect of temperature and agitation on the degree of crystallinity of bacterial cellulose produced. Furthermore, we will study the relationship between bacterial cellulose crystallinity to other physical properties.

2. Materials and Methods

2.1. Microorganism and Medium

Gluconacetobacter xylinus BTCC B796 obtained from Biotechnology Centre of LIPI Culture Collection.Media used was Hestrin Schramm (HS) medium with containing 2.0% D-glukosa, 0.5% peptone, 0.5%, yeast extract,0.27% Na_2HPO_4 and 0.115% citric acid (Hestrin and Schramm,1954). Glacial acetic acid was used to lower pH medium to 5.0. Polysaccharides were added to the medium, i.e.: sodium alginate (at 0%, 0.04%, 0.07%, 0.1%), agar (at 0%, 0.05%, 0.01%, 0.15%), and starch (at 0%, 0.5%, 1.5%, 2%).

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 ^oC 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 of HS medium and incubated at 23, 25, 27, and 30 ^oC at static conditions for 7 days. Other experiment was carried out at room temperature at different degree of agitation, i.e.: 0 and 50 rpm for 7 days. The strain was also grown at static condition at room temperature, and samples of cellulose produced were drawn after 4,7,10, and 14days. 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 and analyzed for X-ray diffraction, tensile strength, water holding capacity, and rehydration ratio.

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 et.al.(1959) method.

2.4. Tensile Strength

Tensile strength of dried cellulose have been measured using Universal Testing Machine ZWICK Z.05-type mechanical tester.

2.5. Water Holding Capacity (WHC)

WHC was determined using Jiang *et al*. (1985). Nata was cut into cubes of equal dimensions, wrapped in filter paper and centrifuged at 5,000 g for 10 min. During centrifugation the water released is absorbed by the filter paper. The percentage ratio of the moisture in the centrifuged nata to the original moisture content provided the WHC.

2.6. Rehidration Ratio

This parameter was measured by following the method, proposed by Bodhibukkana et al. (2006). Dried BC was weighted (W-dry) and immersed in deionized water (w/v = 1:2) until the weight of the rehydrated sample (Wrwet) constant (12 hours) . The rehydration ratio, represented the degree of the removal water and was replaced by deionized water, which was calculated as:

Rehydration ratio(%) = (Wrwet-Wdry)/(Wwet - Wdry) x 100%

3. Result and Discussion

3.1. Crystallinity Index

The condition of fermentation affects the crystallinity of bacterial cellulose (table 1,2). Cellulose produced at lower temperature, using agitation and fermentation more than seven day gave lower crystallinity index. Bacterial cellulose produced using agitation have decreasead crystallinity index 61.46% from 76.90 at static condition. Bacterial cellulose produced at 23 ^{o}C resulted in bacterial cellulose with a lowest crystallinity index, 59.25%, while cellulose production for 7 days and static condition resulted in bacterial cellulose with a high crystallinity index of 73, 26 and 76.96%.

Fermentation conditions	Crystallinity Index (%)	Dry cellulose (g)
Suhu 23 ^{o}C	59.25 ± 2.47	4.61
Suhu 25 ^{o}C	61.50 ± 2.77	7.41
Suhu 27 ^{o}C	65.84 ± 3.70	10.25
Suhu 30 ^{o}C	70.22 ± 3.23	17.83

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

Kondisi Fermentasi	Crystallinity Index (%)	Dry cellulose (g)
4 days	66.11 ± 2.40	4.37
7 days	73.26 ± 5.37	11.98
10 days	61.60 ± 5.28	7.06
14 days	64.05 ± 5.90	7.20

Table 2: Crystallinity index of cellulose bacterial produced by G. xylinus with different time fermentation.

At low temperature, bacterial cells tend to be inactive, whereas to produce bacterial cellulose with crystalline structures, bacterial cells must be active so they can move freely and produce crystalline cellulose. According to Hirai *et al*. (1997), the using low temperature than the optimum temperature of bacterial growth can lead to slow motion of the molecule, bacteria tend to motionless and affect the resulting crystalline structure of cellulose. Other studies have shown the higher the fermentation used by the cellulose descending crystallinity index (Zeng *et al.,* 2011).

The crystallinity index also decreased when using agitation compared to static condition. Bacterial cellulose produce crystalline structure requires free cell movement so that cellulose can be arrange ordered structure. The movement of the cell will be affected by mechanical forces from agitation and disturb aggregation and crystallization of cellulose (Yan *et al.*, 2008). Moon *et al.* (2006), also suggested that using of agitation can cause intermoleculer hydrogen bonds discontinued and decreased the crystallinity index.

The longer fermentation times also affects the bacterial cellulose crystallinity index. At the beginning of the fermentation the cell movement is still free and produces crystalline structure, but with the longer cellulose fermentation time generated and forming a dense 3-dimensional network thus inhibiting the movement of bacterial cells and crystallinity tends to decreased (Sheykhnazari *et a*l. 2011, Zeng *et al*., 2011).

3.2. Tensile Strength

Bacterial cellulose using different temperature and time produces bacterial cellulose with different tensile strength. Production cellulose bacterial with agitation was not carried out by tensile strength testing because bacterial cellulose produced, the forms were different. The use of lower temperatures resulted in a decrease in the tensile strength of bacterial cellulose, whereas the longer fermentation time resulted in a decrease in tensile strength, but in fermentation to 7 tensile strengths but decreased again in the 10th fermentation (Table 3). The use of 23 ^{o}C temperature produces bacterial cellulose with the lowest tensile strength while seven day of fermentation produces bacterial cellulose with the highest tensile strength. The change of tensile strength may be related to the decrease of bacterial cellulose crystallinity index, the lower the crystallinity index of bacterial cellulose, tensile strength also decreases. Decreasing the crystallinity index results in a less compact and porous cellulose structure. The number of void or less dense structures as indicated by SEM observations can lead to decreased tensile strength (Kitdpondpattara *et al*., 2015). Decreasing in tensile strength can be attributed to a decrease in crystallinity (Watanabe *et al.,* 1994). According to Sheykhnazari *et al.,* (2011), the index of crystallinity and microfibril tissue reached a maximum of 7 days, after which the crystallinity decreased, but in the study, did not explain its effect on physical properties.

Fermentation Conditions	Tensile strength (MPa)
Suhu 23 ^{o}C	9,64 ± 4,02
Suhu 25 ^{o}C	15.65 ± 4.01
Suhu 27 ^{o}C	21.41 ± 7.30
Suhu 30 ^{o}C	24.89 ± 2.36
4 days	25.73 ± 9.95
7 days	31.66 ± 7.88
10 days	21.45 ± 3.88
14 days	28.43 ± 5.11

Table 3: Tensile strength of cellulose bacterial produced by G. xylinus with different temperature and time fermentation.

3.3. Water Holding Capacity (WHC)

Water retained on bacterial cellulose that forms a 3-dimensional network is an important property for food and non-food applications (Jagannath *et al.,* 2008). Bacterial celluloce produced using different temperature, agitation and fermentation time have different effect on WHC. WHC tends to decrease with the higher fermentation temperatures used (Figure 1). Bacterial cellulose produced using agitation results higher WHC bacterial cellulose than static conditions. The WHC of the cellulose produced was 85.30% and it increased to 88,70% when using agitation 50 rpm. WHC on 4th and 10th day fermentation showed higher WHC than other fermentation time. The use of agitation results in a relatively higher WHC compared to temperature and time variations.

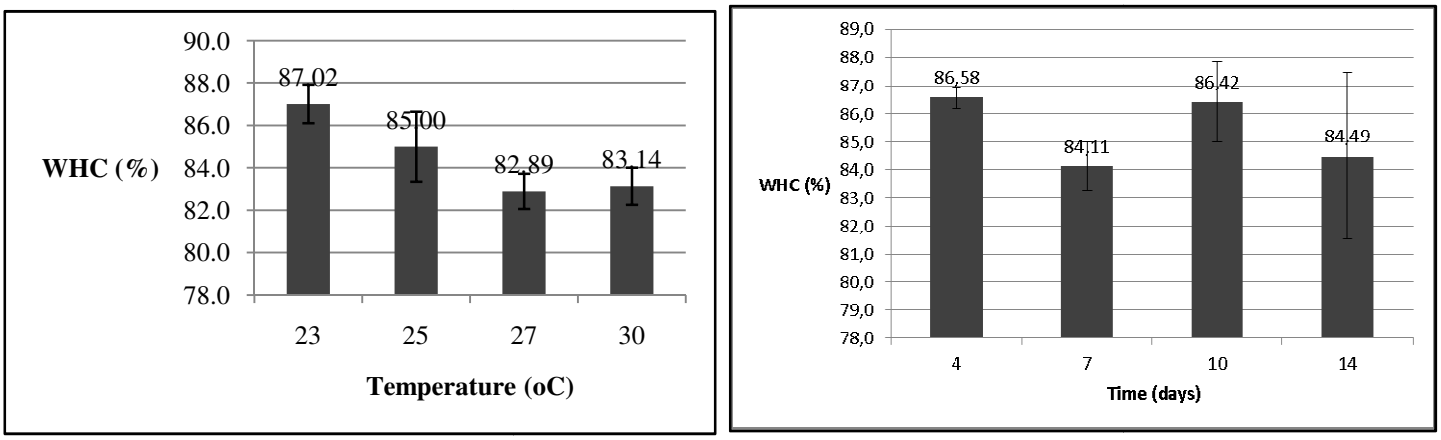

Figure 1: Water Holding Capacity (WHC) of bacterial cellulose produced by Gluconacetobacter xylinus with different temperature, and time fermentation

The WHC level is associated with the structural arrangement of bacterial cellulose. The amount of water absorbed by bacterial cellulose is strongly influenced by the 3-dimensional matrix of bacterial cellulose. The more empty or porous space in the cellulose the more water can enter and be absorbed in the material matrix (Lin *et al*, 2009; Huang *et a*l., 2010; Dalman, 2009; Islam *et al*., 2012). Chang *et a*l., (2012) also suggested that larger amounts of amorphous cellulose accelerate and encourage the absorption of water into cellulosic tissue resulting in larger WHCs.

The movement of the cell will be affected by mechanical forces when using agitation thus disrupting the aggregation and crystallization process of cellulose. Changes in cellulose composition due to decreased crystallinity result in more amorph structure resulting in a lot of pores. Increased pores make more bacterial cellulose or are able to absorb water so that the WHC increases. The higher the speed of agitation resulting in bacterial cellulose regrouping to form a denser period. According to Bae and Shoda (2004), Zhou, *et al* (2007), the use of agitation is able to increase WHC compared to static conditions.

3.4. Rehydration ratio

Bacterial cellulose produced using different temperature, agitation and fermentation time gave different effect on rehydration ratio (Fig. 2). The use of increasing temperatures results in increased rehydration of bacterial cellulose. Bacterial cellulose produced using agitation (50 rpm) increased rehydration ratio of bacterial cellulose, while bacterial cellulose produced with different time fermentation shows different rehydration ratio. In the old fermentation 4 and 10 days showed greater rehydration than any other time.

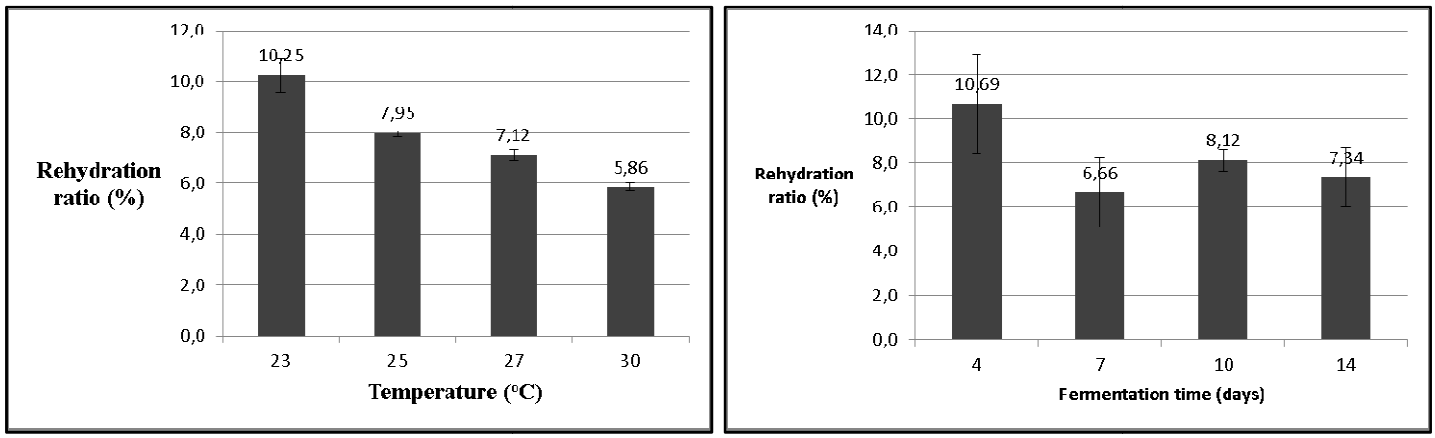

Figure 2: Rehidration ratio of bacterial cellulose produced by Gluconacetobacter xylinus with different temperature and time fermentation

The rehydration ratio at seven days fermentation have decreased may be related to crystallinity. Increased rehydration properties of cellulose are associated with decreased crystallinity of bacterial cellulose (Lin et al., 2009). The 3-dimensional network arrangement of bacterial cellulose having many pores will increase the space for water to enter into the bacterial cellulose matrix (Huang, et al., 2010; Lin et al.2009; Chang et al., 2012). Sheykhnazari *et al.,* (2011) suggests the index of crystallinity and microfibril tissue to reach a maximum of 7 days, then after which the crystallinity becomes decreased. The longer the cultivation time will decrease cellulose crystallinity, this is because cellulose that forms a dense tissue will limit the movement of bacteria along with increasing cultivation time (Zeng *et al*., 2011). The influence of agitation can change the crystalline region to be more amorph so that water is absorbed more during rehydration takes place. This result is in accordance with the results of previous studies (Huang, *et al*., 2010; Lin *et al*.2009). The ability of bacterial cellulose to absorb water (WHC, rehydration ratio) is an important characteristic because it deals with its sensory properties (Jagannath, et al., 2008) and its functional properties as dietary fiber and is potentially applied as a thickener and food stabilizer (Shi *et al.*, 2014).

4. Conclusion

The fermentation conditions gave different effects on crystallinity and other cellulose physical properties. Decreased fermentation temperature can decrease the crystallinity of bacterial cellulose and further result in decrease of tensile strength, increase of WHC and rehydration ratio. Bacterial celluloce using agitation gave decreased the crystallinity of bacterial cellulose compared to static conditions. The use of agitation leads to WHC and the rehydration ratio increases. Bacterial cellulose produced gave the highest crystallinity index and tensile strength at 7 days but then decrease with increasing fermentation time. Water Holding Capacity and rehydration ratio of bacterial cellulose increased after 7 days fermentation.

5. Acknowledgements

This work was financed by Ministry of Research Technology and Higher Education (Kemenristekdikti).

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