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Physico-chemical Properties of Bacterial Cellulose Produced by Newly Strain *Gluconacetobacter xylinus* ANG-29 in Static and Shaking Fermentations

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Physicochemical properties of bacterial cellulose produced by newly acetic acid bacterial strain *Gluconacetobacter xylinus* ANG-29 in different fermentation methods were compared using SEM and XRD techniques. Production of cellulose by *G. xylinus* ANG-29 with static fermentation method, shaking at 50 rpm, 100 rpm and 150 rpm were 1.59±0.08 g/100 ml, 0.17±0.02 g/100 ml, 0.11±0.00 g/100 ml and 0.21±0.01 g/100 ml, respectively. The degree of crystallinity determined by XRD method in static fermentation was 93%, shaking fermentation at100 rpm was 51% and shaking fermentation at150 rpm was 65%. Static fermentation method produced bacterial cellulose in the sheets form, while shaking fermentation produced fragmented cellulose with predominantly spherical shape. The observation of surface structure of bacterial cellulose by SEM showed that the static fermentation method generated woven densely of cellulose microfibrils. Shaking fermentation caused the woven microfibrils become more loose and formed a larger holes. Bacterial cellulose produced from both fermentation methods had their own advantages depending on the application.

Keywords : *Gluconacetobacter xylinus*, bacterial cellulose, static fermentation, shaking fermentation.

Cellulose is the most abundant biopolymer on earth and is a major component of plant biomass[1]. Plant cellulose is usually not pure because it is mixed with lignin and hemicellulose[2], making it difficult to develop applications in the industrial world because it requires purification before use. Therefore, it is necessary to develop alternative sources of producing the more pure cellulose, which are microbial cellulose mainly bacterial cellulose[2]. The unique properties of bacterial cellulose, especially its purity, has attracted many researchers to apply the bacterial cellulose in a variety of applications such as the manufacture of paper[3], membrane[4,5], food industry[6] and as biomaterials for medical applications[7]. In addition to its purity, bacterial cellulose has high crystallinity index, degree of polymerization, tensile and water absorption[8,9].

Some genera of bacterial strains members are known as a producer of bacterial cellulose, among which *Acetobacter, Aerobacter, Azotobacter, Agrobacterium, Archromobacter,*

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Gluconacetobacter, Rhizobium , Sarcina and *Salmonella*[9,10,11]. Strain members of the genera *Gluconacetobacter* and *Acetobacter* have the diverse ability to produce cellulose and most widely studied because of the quantity and quality of the resulting cellulose,[9,12] so they are used as a model organism in the study of cellulose producing bacteria. Both of these genera are members of acetic acid bacteria (AAB) and classified in the familia *Acetobactericeae* are generally isolated from natural substrate containing glucose, acid and alcohol[13]. For example, the ripe fruits, fermented products,[14] vinegar, liquid plant sap, alcoholic beverages, flowers, beer, wine, acid fruit juice[13] and honey[15].

The ability to produce bacterial cellulose among species members of the genera *Gluconacetobacter* and *Acetobacter* are vary. For example, *G. xylinus* subsp. *sucrofermentan* (mutants resistant to sulfaguanidin) was capable to produce cellulose of 9.7 g/L,[16] *G. hansenii* PJK was able to produce cellulose of 2.7 g/L in medium containing 1 % ethanol and 5.47 g/L in the basal medium SMRs[17]. *Acetobacter* sp. KCTC was able to produce cellulose in media containing glucose and xylose 4.16 g/L and 3 g/L, respectively.[9]

One of the factors that influence the production of bacterial cellulose was the method of production. The method used in the cellulose production of industrial scale are static[18] and agitated.[18-20] Static method of fermentation in industrial scale production have been proved to be very low due to the formation of gluconic acid.[18,19] Meanwhile, agitated fermentation decrease the production of cellulose as closely associated with resulting negative mutant.[18,20-22] Fermentation method or culture condition also affects the macroscopic morphology of bacterial cellulose produced ,[23,24] whereas the difference in morphology of cellulose between static and agitated culture contribute to variations in the degree of crystallinity and the difference of crystalic size.[23] Therefore, in the effort to increase the quantitative productivity of bacterial cellulose, it was also important to assess the qualitative aspects such as physico-chemistry characters to know its potential as an industrial raw material.

This research evaluated the ability of production, morphology and physicochemical properties of cellulose produced in different methods fermentation (static and shaking) by *G. xylinus* ANG-29, which was a newly bacterial strains isolated from rotten grapes in the region of Yogyakarta, Indonesia.

MATERIALS AND METHODS

Microorganism

Newly acetic acid bacterial isolate *G. xylinus* ANG-29 used in this study was isolated from rotten grapes in Yogyakarta, Indonesia. Previous research using samples of tropical fruit obtained some potential isolates among which ANG-29 with cellulose production of 3.4 g/L in standard medium Hestrin- Schramm (HS) and 12 g/L in coconut water based medium. The production capability of this isolate had been higher than ever reported in the literature, namely 9.7 g/L by sulfaguanidin resistant mutant strains *Acetobacter xylinum* subsp. *Sucrofermentan*.[16]

Media and culture conditions

G. xylinus ANG-29 was taken from stock cultures and grown on standard HS liquid medium composed of D-glucose 2.0 % (w/v), Peptone 0.5 % (w/v), Yeast extract 0.5 % (w/v), Na_2HPO_4 0.27 % (w/v) and citric acid 0.115 % (w/v). Production medium used was coconut water-based medium with supplementation carbon source, nitrogen source and glacial acetic acid to adjust the acidity of medium to be 5.

Production and harvesting of bacterial cellulose

Production medium that used was coconut water-based with supplementation of 5% (w/v) granulated sugar, 0.5% (w/v) ammonium sulfate and the pH of medium was adjusted to 5 in 100 ml of production scale. Production medium was sterilized by autoclaving at 121°C at a pressure of 2 atmosphere for 15 minutes. The medium was inoculated with 10% (v/v) culture starter and then incubated at 30°C for 7 days by static and shaking fermentation methods.

Harvesting of bacterial cellulose was conducted by a modification of the method developed by Ishihara.[25] Bacterial cellulose gel was harvested and cleaned using cold water to remove residual medium, then boiled in boiling water for approximately 15 minutes. After wards, it was washed with cold water and then oven-dried at 60° C for 24 hours.

Observation the surface structure of bacterial cellulose by SEM

For observation of the surface structure of cellulose in each treatment Scanning Electron Microscope (SEM) was used. First bacterial cellulose film samples were oven-dried to a zero water content. Furthermore, a small specimen was sliced to about 0.5 cm^2 and placed in a specimen holder and coated with gold metal thickness of 200 Å, and then observed with SEM instrument JEOL JSM-6360LA type. The images of SEM were taken at the power voltage of 30 kV and a magnification of 10.000 times.

Measurement of crystallinity by X - ray difractometry (XRD)

Bacterial cellulose thin film samples were prepared by the method developed by Kai and Keshk.[26] Diffractogram of the samples were recorded at room temperature by a Shimadzu XRD-7000 series Maxima-X using Ni - filtered CuKα radiation (» = 1.54 ú). The voltage and current used were 40 KV and 30 mA, respectively. The diffraction data were taken at a scan angle range 2¸ of 5 to 30 degrees, using continuous scan mode at scan speed of 4 degrees per minute and the sampling pitch of 0.02 degrees. The crystallinity calculated from diffraction intensity data using the method of Segal,[27] where the index of crystallinity (Cr.I.) = $(I_{002} - I_{am}) / I_{002}$; I_{002} is the maximum intensity of the diffraction grating, while I_{am} is the intensity at an angle 2¸ = 18°.

RESULTS AND DISCUSSION

Production of bacterial cellulose

As can be seen from Fig.1 that the production of cellulose by isolate ANG-29 highly influenced by fermentation method. In the shaking treatment, the cellulose productivity of *G. xylinus* ANG-29 decrease significantly. It is clear that the production of cellulose for this isolate was more effective to be done in a static fermentation condition. The decrease in bacterial cellulose production in isolate *G. xylinus* ANG-29 occured along with the increase of shaking speed, but the production slightly increased again in shaking speed of 150 rpm.

Table 1. Strongest peaks, intensity and crystallinity index of bacterial cellulose produced by *G. xylinus* ANG-29 determined by X-ray diffractogram

Frementation	Peak angle (degree)			Intensity			crystallinity
methods	1	2	3	1	2	3	index (%)
Static	22.71	14.49	16.76	1260	373	130	90
100 rpm	22.82	22.62	22.50	141	136	130	51
150 rpm	22.88	23.16	22.56	249	220	202	65

Production of bacterial cellulose is not only dependent on the type of microorganism, but is also influenced by the production methods. In this research there were two methods for producing bacterial cellulose, namely static and shaking fermentation methods. Although the shaking method was able to increase the diffusion of oxygen in the fermentation medium, this process could lead to the emergence of mutant cells that lose the ability to produce cellulose and thus causing a decrease in the production of cellulose as a whole.[28]

The morphology of bacterial cellulose

Production of bacterial cellulose by static and shaking fermentation method generated the bacterial cellulose with the different morphology and properties. Cellulose produced by static fermentation method in this research was a thick sheet of cellulose, while shaking fermentation produced fragmented cellulose with predominantly spherical shape (Fig.2). This was consistent with results of previous research that treatment of agitation produced spherical cellulose.[39,30] According to Krystynowicz,[31] stationary or static culture bacterial cellulose will form shape like a sheet of cellulose mats and texture of surfaces such as gelatin in liquid culture medium, in which the bacterial cells trapped in the webs of cellulose fibers. In the agitated or shaking culture conditions, no pellicle sheet formed and cellulose in a form of irregular granules, stellate and fibrous strands, well dispersed in culture broth.

The observation of the surface structure of bacterial cellulose by SEM showed that cellulose microfibrils formed as a woven ribbon. In static fermentation method cellulose microfibrils were densely woven look. The change from a static to shaking fermentation causes the surface structure changed. Some changes had been observed which were woven into a stretchable microfibrils or not solid and the formation of larger cracks webbing between cellulose microfibrils, as shown in Fig.3.

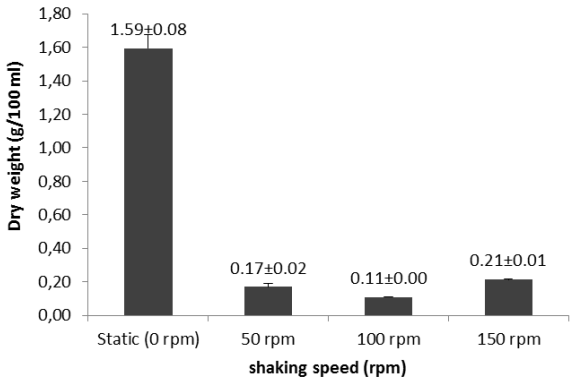
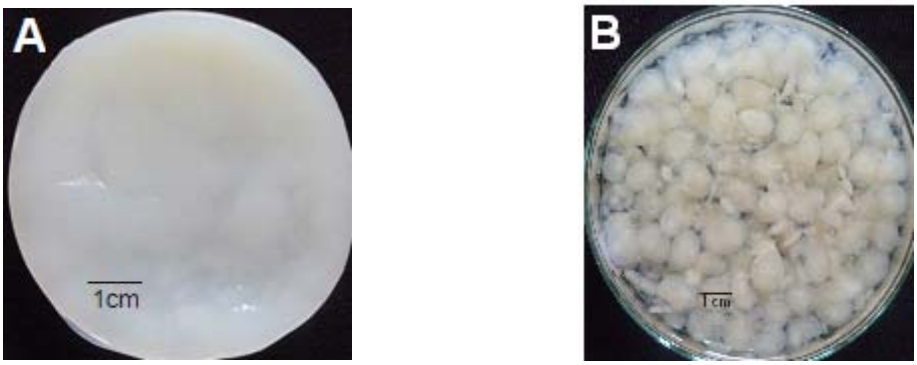
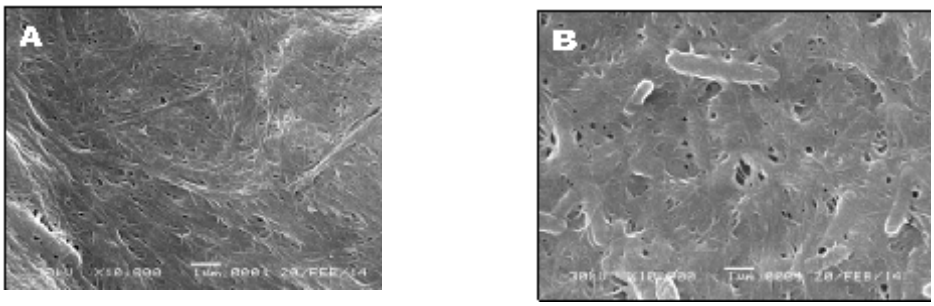


Fig. 1. Production of bacterial cellulose by *G. xylinus* ANG-29 in static and shaking fermentation methods



(A) a sheet of cellulose from static fermentation; (B) spherical granules of cellulose from shaking fermentation

Fig. 2. Morphology of bacterial cellulose produced by *G. xylinus* ANG-29 in static and shaking fermentation methods



(A) static fermentation (B) shaking fermentation

Fig. 3. The surface structure of bacterial cellulose produced by *G. xylinus* ANG-29 using SEM techniques

Stretching the cellulose microfibrils in the treatment of shaking fermentation method can be understood because the shaking treatment during the fermentation process disrupt the formation of microfibrils woven into a regular braid. Shaking caused the stretching bands of cellulose and the formation of larger holes between woven cellulose microfibrils.

Bacterial cellulose crystallinity index

X-ray diffraction patterns of bacterial cellulose produced by *G. xylinus* ANG-29 in different fermentation method is presented in Fig. 4.

The peak value in the X-ray diffraction and bacterial cellulose crystallinity index values produced by the static and shaking fermentation methods are shown in Table 1.

Fig. 4 and table 1 showed that the crystallinity index of the cellulose produced by static fermentation method is higher than that of shaking. This is consistent with research result conducted by Watanabe[23] and Moon[32] that the bacterial cellulose produced by the static fermentation method generates higher crystallinity index compared to that produced by the agitating or shaking method. The process of agitation or shaking during fermentation causes the hydrogen bonds between the microfibrils are broken and produce the shorter microfibrils. The breaking of hydrogen bonds between the microfibrils causes a low crystallinity index.[32] Increasing crystallinity index causes the higher tensile strength of cellulose fibers.[33,34]

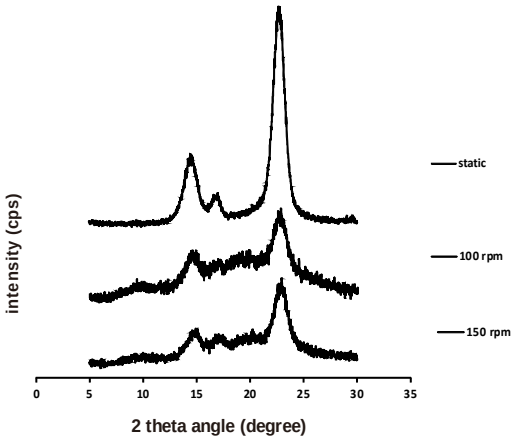


Fig. 4. X-ray diffraction pattern of the bacterial cellulose produced by *G. xylinus* ANG-29 on static and shaking Fermentation methods

Bacterial cellulose produced by *G. xylinus* ANG-29 in both fermentation methods have their own advantages useful depending on the application. Cellulose derived from the static fermentation that has sheets form, more dense woven microfibrils and more high crystallinity index suitable for use as biomaterials in industries that require cellulose as a raw material. While cellulose derived from shaking fermentation was more suitable for use as raw materials in the food industry because it has a spherical shape, more tenuous woven microfibrils and lower crystallinity index so that a more lenient.

CONCLUSION

Productivity and properties of bacterial cellulose produced by acetic acid bacteria *G. xylinus* ANG-29 was affected by the use fermentation method. Shaking fermentation method caused a decrease in the amount of production and the crystallinity index of the cellulose produced. Static fermentation method produced bacterial cellulose in the sheet formed, while shaking fermentation method produced fragmented cellulose with predominantly spherical shape. Shaking caused woven cellulose microfibrils become more loose and formed a larger hole. Bacterial cellulose produced by the fermentation of both methods had their own advantages depending on the application.

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