Effect of Acetic Acid and Ethanol as Additives on Bacterial Cellulose Production by *Acetobacter xylinum*

Y E Agustin and K S Padmawijaya

Chemical Engineering Department, University of Surabaya, Indonesia
Email : yuana@staff.ubaya.ac.id

Abstract. Bacterial cellulose was the result of fermentation by *Acetobacterium xylinum*. Fermentation medium that used in this research is is HS (Hestrin – Shramm) media with $MgSO_4$ as cofactor and also with 5% (v/v) glycerol and 0.8% (w/v) urea as additional carbon and nitrogen sources. This research focus is to determine the effect of acetic acid and ethanol as additives agent in the production of bacterial cellulose. Addition of additives to the fermentation medium aims to improve the characteristic of bacterial cellulose. Variable that used in this research is additive concentration (0.5%, 1%, 1.5%, and 2% v/v). Synthesized bacterial cellulose will be physically and mechanically characterize using dry thickness measurement, water capacity measurement, swelling ability, tensile strength and elongation at break measurement. Morphology measurement will be known through SEM (Scanning Electron Microscopy) and FTIR (Fourier Transform Infra Red) analysis. SEM analysis showed that addition of 1.5% (v/v) acetic acid to the fermentation medium gave more fiber on bacterial cellulose than addition of 1.5% ethanol, this result also supported by FTIR analysis. Rated of moisture content and swelling ratio of bacterial cellulose using 1.5% (v/v) ethanol higher when compared with 1.5% acetic acid on fermentation medium. Tensile strength and elongation at break analysis showed that bacterial cellulose has the highest tensile strength and the lowest elongation at break value with addition of 1.5% (v/v) acetic acid to the fermentation medium.

1. Introduction

Biodiesel is one of the best choices that have the potential to effectively reduce the dependence on petroleum. Glycerol is the major by-product from biodiesel production and it is one major concern regarding biodiesel production. In general, biodiesel production generates 10% crude glycerol (w/w) during transesterification of triglycerides with an alcohol, most frequently methanol. Crude glycerol generated during biodiesel production contains impurities such as alcohol, salts, heavy metals, and water, it is must be purified before use in further applications. Many processes already used to purify crude glycerol from biodiesel industries. However, many techniques lack sufficient selectivity and yield to make them commercially viable [1]. A wide variety of microorganisms can utilize glycerol. When considering the microbial conversion of glycerol (as the sole carbon source) into value- added products, however, the options are somewhat limited. Crude glycerol could be an economic carbon and nutrient source for bacterial cellulose production.[2] [3]

Bacterial cellulose (BC) produced by gram negative bacteria, *Acetobacter xylinum*, is a pure cellulose nanofiber with a diameter of approximately 50 nm. Bacterial cellulose presents very interesting properties such as high purity, unique physical and mechanical properties that arise from its tridimensional and branched nano and micro-fibrillar structure and biocompatibility. These singular characteristic triggered considerable interest on BC, especially in the biomedical area. Some examples of applications are as wound healing membranes for substituting natural skin, cirurgical implants but

also other high added value applications such as membranes for audio devices and optically transparent nanocomposites [4][5][6]. This study focuses on the effects of acetic acid and ethanol as additives on bacterial production from glycerol by *Acetobacter xylinum*. Furthermore, the physical and mechanical properties of the bacterial cellulose were determination.

2. Material and methods

Glycerol was provided by PT. Brataco Chemica with purity 87% (in weight). While strain of *Acetobacter xylinum* was provided by Agrotekno Sarana Industri. Modified Hestrin and Scharmm (HS) media with Magnesium Sulphate ($MgSO_4$) as a cofactor were used as fermentation media in this research. HS media was modified by replacing D-glucose with glycerol as an additional carbon source and also urea was added as an additional nitrogen source. Fermentation media contains 2%w/v glucose, 0.5%w/v peptone, 0.5%w/v yeast extract, 0.5%w/v disodium phosphate, 0.115w/v citric acid, 5% v/v glycerol, 0.8% w/v urea and 0.115%w/v magnesium sulphate. Concentration of additive (acetic acid and ethanol) that used in this research was 0.5%, 1 %, 1.5%, and 2% v/v.

2.1. Cultivation media and conditions

Inoculum was cultured for 24 hours in Mc. Cartney bottle containing fermentation media at 26^{o}C and 150 rpm orbital speed. For BC production, static incubations were performed in Erlenmeyer's flasks for 14 days. To purified BC production, pellicles were rinsed with water to remove the culture medium, and then boiled in 1N NaOH solution at 90^{o}C for 20 minutes in order to eliminate the bacteria cells from the cellulose matrix. Then, pellicles were washed with distilled water till neutralization. Dry weight was measured after drying the films at 50^{o}C till constant weight.

2.2. Analytical method

Bacterial cellulose films were characterized for Scanning Electron Microscopy (SEM) analysis, Fourier Transform Infrared Spectroscopy (FTIR) analysis, thickness and mechanical properties as described below.

2.2.1 Scanning Electron Microscopy

The surface and morphology of bacterial cellulose films were analyzed by Scanning Electron Microscopy in Sepuluh Nopember Institute of Technology. The freeze- dried samples were coated with gold.

2.2.2 Fourier Transform Infrared Spectroscopy

The chemical structure of bacterial cellulose films were analyzed by FTIR (Bruker). The bacterial cellulose produced by *Acetobacter xylinum* from our research was mixed well with potassium bromide (KBr) powder and press into small tablet.

2.2.3 Thickness measurement

Thickness of each bacterial cellulose film was measured at eight different positions by a thickness gauge, and the values were averaged.

2.2.4 Tensile Strength and Elongation at break

Mechanical properties of the films were investigated using autograph in University of Airlangga, Surabaya. The dimensions of the test specimen were 7 cm x 2 cm. each test was performed in duplicate.

3. Results and discussion

3.1. Bacterial cellulose production

Acetobacter xylinum formed a cellulose layer on the fermentation media containing HS- $MgSO_4$ medium with glycerol as an additional carbon source and urea as an additional nitrogen source at the end of 14 days incubation at 26^{o}C. NaOH and water were used to purify the pellicles of bacterial cellulose. After purification bacterial cellulose was dried at 50^{o}C. Two additives, acetic acid and ethanol, were added to the fermentation media to control the growth of bacterial cellulose and also to increase mechanical properties of bacterial cellulose. Acetic acid was added to control the acidity level

of fermentation media and optimized the production of bacterial cellulose. Ethanol was added to increase the growth rate of bacterial cellulose, ethanol can act as energy source on bacterial cellulose fermentation (Rani et al., 2011)

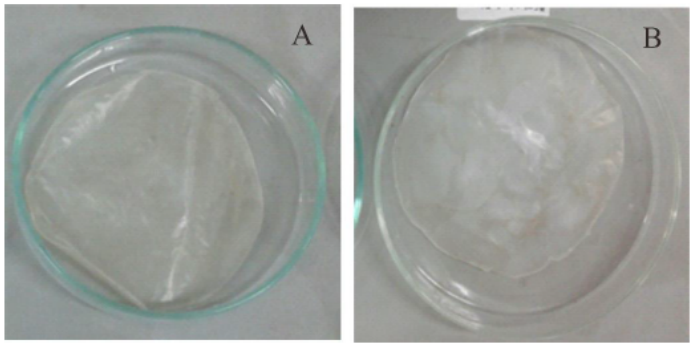

Fig 1. Bacterial cellulose production with addition of (a) 1.5% v/v ethanol (b) 1.5%v/v acetic acid

3.2. Characterization of bacterial cellulose (BC)

3.2.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR spectra obtained from bacterial cellulose films are shown in Table 1. The result showed that bacterial cellulose from this research have O-H bond at 3530-3430 cm^{-1}. The peaks at 1542-1528 cm^{-1} were attributed to bending O- H group. Another intense peak located at 1600 cm^{-1} was attribute to C-O (β-glycosidic bond) group.

Table 1. Bacterial cellulose functional group from FTIR analysis

Functional Group	Wavelength, cm^{-1}		
	Bacterial cellulose	Bacterial cellulose with 1.5% v/v ethanol on fermentation media	Bacterial cellulose with 1.5% v/v acetic acid on fermentation media
O-H	3433.41	3527.92	3446.91
O-H Bend	1541.18	1529.6	1527.67
β-1, 4- glycosidic	1163.11	1163.11	1159.26

3.2.2 Scanning Electron Microscopy (SEM) Analysis

Surface morphology of bacterial cellulose that produced by different fermentation media was shown in Figure 2. Bacterial cellulose from HS-$MgSO_4$ media has compact surface morphology with loose fibril structure (Fig 2a). Bacterial cellulose from modified HS-$MgSO_4$ media with 1.5%v/v ethanol as an additive has random assembly of fibril and also porous structure (Fig 2b). The structure of bacterial cellulose produced in modified HS-$MgSO_4$ media with 1.5% v/v acetic acid has more compact surface morphology with lots of fibril (Fig 2c).

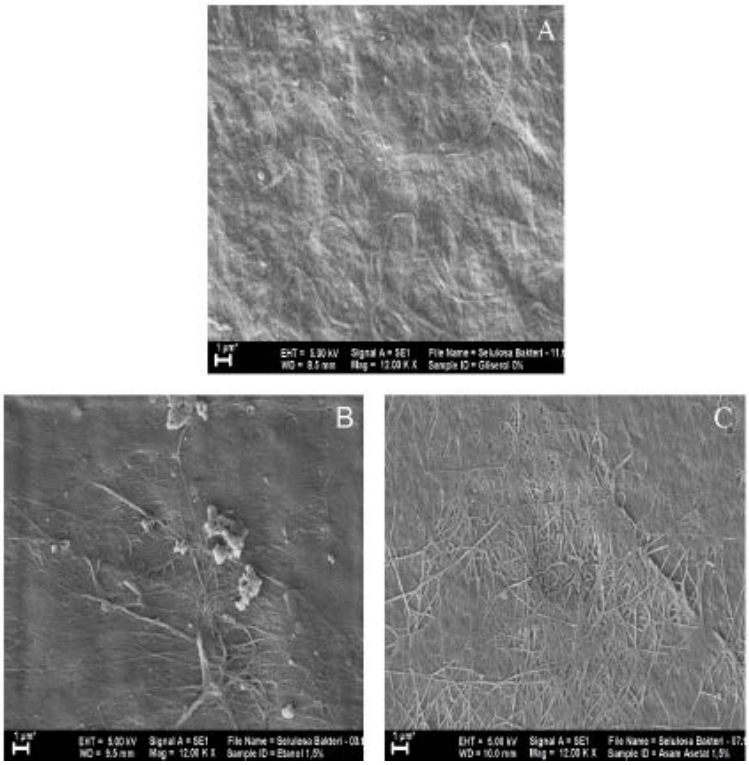

Fig 2. SEM images of the top view of bacterial cellulose (BC) produced in (a) HS-$MgSO_4$ media (b) Modified HS-$MgSO_4$ media with 1.5% v/v of ethanol as an additive (c) Modified HS-$MgSO_4$ media with 1.5% v/v of acetic acid as an additive

3.3. Thickness measurement

Fig 3 showed the dry thickness of bacterial cellulose produced on this research using two different additives at modified HS-MgSO4 media. The maximum thickness of bacterial cellulose was produce on modified HS-$MgSO_4$ media with 1.5% v/v ethanol concentration. Hence, HS-$MgSO_4$ media with additional of 5% and 1% of acetic acid did not form bacterial cellulose, maybe because *Acetobacter xylinum* not perfectly oxidized acetic acid from fermentation media and the pH of fermentation media was not support for bacterial cellulose fermentation .

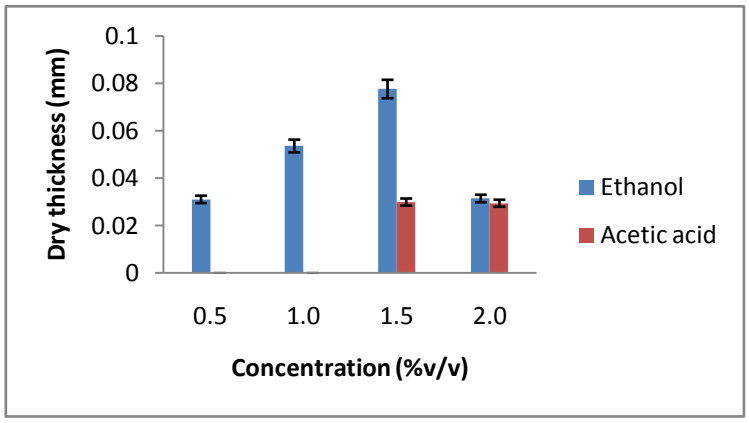

Fig 3. Bacterial cellulose thickness at various additive concentrations

3.4. Mechanical test

The mechanical properties of bacterial cellulose at various media were presented in Table 2. Increasing of ethanol as an additive on fermentation media will decrease tensile strength but increase the elongation of break of bacterial cellulose formation. Ethanol was increase the elasticity of bacterial cellulose. Ethanol with 1.5% v/v produced bacterial cellulose with tensile strength was about 68.978 MPa and the elongation at break was about 2.83. Bacterial cellulose formation with 1.5% v/v acetic acid addition give higher tensile strength, it was about 153.637 Mpa, but with lower elongation at break, only 0.9429. Bacterial cellulose with acetic acid as additive has rigid characteristic.

Table 2. Mechanical properties of bacterial cellulose at various media

Additive	Concentration (%v/v)	Young Modulus (N)	Tensile Strength (Mpa)	Elongation at break (100%)
Ethanol	0.5	94.34	152	1.7000
	1.0	161.81	151	2.6286
	1.5	107.089	68.978	2.8300
	2.0	34.814	55.259	3.6286
Acetic Acid	0.5	-	-	-
	1.0	-	-	-
	1.5	92.183	153.637	0.9429
	2.0	57.859	98.066	2.0429

4. Conclusions

Bacterial cellulose composite was successfully prepared in this research. Bacterial cellulose produced with 1.5%v/v of acetic acid addition has better characteristic and mechanical properties compared with addition of 1.5%v/v ethanol (higher tensile strength). These results showed that glycerol can be used as a potential substrate for bacterial cellulose production.

5. References

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