Glycerol as an additional carbon source for bacterial cellulose synthesis

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Y E Agustin, K S Padmawijaya, H F Rixwari and V A S Yuniharto

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

Abstract. Bacterial cellulose, the fermentation result of *Acetobacter xylinus* can be produced when glycerol was used as an additional carbon source. In this research, bacterial cellulose produced in two different fermentation medium, Hestrin and Scharmm (HS) medium and HS medium with additional $MgSO_4$. Concentration of glycerol that used in this research were 0%; 5%; 10%; and 15% (v/v). The optimum conditions of bacterial cellulose production on each experiment variations determined by characterization of the mechanical properties, including thickness, tensile strength and elongation. Fourier Transform Infra Red Spectroscopy (FTIR) revealed the characterization of bacterial cellulose. Results showed that the growth rate of bacterial cellulose in HS-$MgSO_4$-glycerol medium was faster than in HS-glycerol medium. Increasing concentrations of glycerol will lower the value of tensile strength and elongation. Elongation test showed that the elongation bacterial cellulose (BC) with the addition of 4.95% (v/v) glycerol in the HS-$MgSO_4$ medium is the highest elongation value. The optimum bacterial cellulose production was achieved when 4.95% (v/v) of glycerol added into HS-MgSO4 medium with stress at break of 116.885 MPa and 4.214% elongation.

1. Introduction

Bacterial cellulose (BC) is a strong and ultrapure form of cellulose produced naturally by several species of *Acetobacteraceae*. Its high strength, purity, and biocompatibility make it of great interest to material science. It is currently used to create materials for tissue engineering, medicine, electronics, paper manufacture, food industry and fabrics. The enhanced mechanical properties of BC occur due to the uniform, continuous and nano-scalar network of cellulosic fibers. These properties are affected by various factors, such as culture conditions, the microorganism and the fermentation media employed. [1][2][3]

In microbial fermentations, the cost of substrate normally accounts up to 50-65% of the total cost of production. Despite its enormous potential in various applications, the high cost of BC production is the main drawback that hinders industrial implementation [4]. The utilization of industrial wastes and by-product streams as fermentation media could improve the cost-competitiveness of BC production. In recent years, many studies have focused on developing cost-effective fermentation media for BC production, such as pineapple peel juice, sugarcane juice, coffee cherry husk extract (a by product from the coffee processing) and corn steep liquor (a by product from the starch processing industry) as less expensive sources of carbon and nitrogen [5][6][7].

In the current work, production of bacterial cellulose by *Acetobacter xylinus* used glycerol (to represent by-product from biodiesel production) as carbon sources. Aim of this research is formulating a general, simple, and inexpensive medium to produce BC. Furthermore, the physical and mechanical properties of the bacterial celluloses produced from different fermentation media were determined.

2. Materials and Methods

Glycerol p.a was provided by PT. Brataco Chemica with purity 87% (in weight). While strain of *Acetobacter xylinus* was provided by Agrotekno Sarana Industri. Two different fermentation media were used in this research, Hestrin and Scharmm (HS) media [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] and HS-$MgSO_4$ [0.115%w/v magnesium sulphate]. Culture media used for BC production were HS modified by repleacing D-glucose with other carbon source, such as glycerol. Concentration of glycerol that used in this research was 0%; 5%; 10%; 15% and 20% (v/v).

2.1. Cultivation media and conditions

Inoculum was cultured for 24 hours in Mc. Cartney bottle containing fermentation media [HS-glycerol or HS-Glycerol-$MgSO_4$] 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 Fourier transform infrared spectroscopy (FTIR), thickeness and mechanical properties as described below.

2.2.1 Fourier Transform Infrared Spectroscopy

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

2.2.2 Thickness measurement

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

2.2.3 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 Discussions

3.1. Bacterial cellulose production

Acetobacter xylinus formed a cellulose layer on the fermentation media containing HS medium with glycerol as an additional carbon source at the end of 14 days incubation at 26^{o}C. NaOH and water were used to purified the pellicles of bacterial cellulose. After purification bacterial cellulose was dried at 50^{o}C. Two different media were used for bacterial cellulose production in this research, such as HS with various concentration of glycerol (Fig 1) and HS-$MgSO_4$ with various concentration of glycerol (Fig 2).

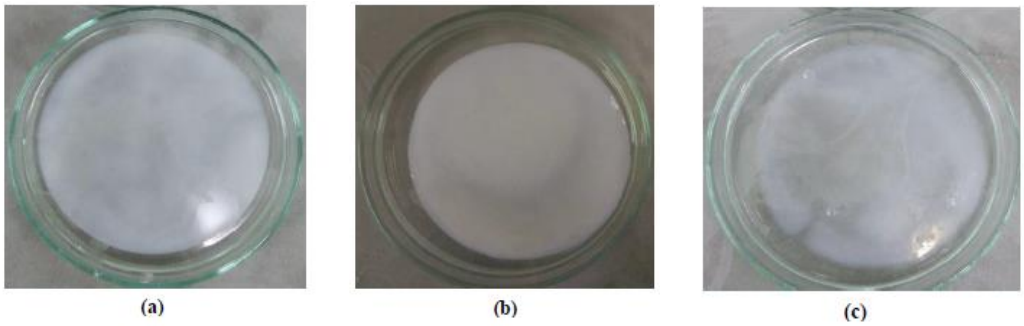

Fig 1. Bacterial cellulose production from HS media with addition of (a) 5%v/v(b) 10%v/v(c) 15% v/v glycerol

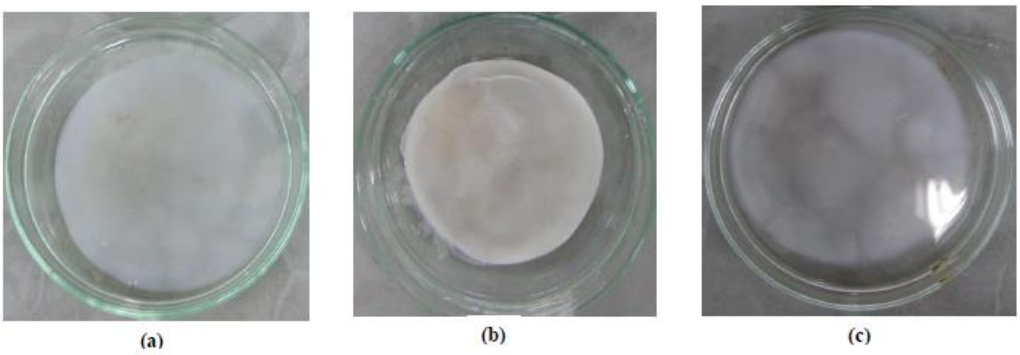

Fig 2. Bacterial cellulose production from HS-$MgSO_4$ media with addition of (a) 5%v/v (b) 10% v/v (c) 15% v/v glycerol

3.2. Characterization of bacterial cellulose (BC)

FTIR spectra obtained from bacterial cellulose films are shown in Fig 3. There were 3 main groups in bacterial cellulose, such as O-H group, pyranose cyclic group and C-O (β-1,4 glycosidic bond) group. The result showed that bacterial cellulose from this research have O-H bond at 3450-3400 cm^{-1}. The peaks at 1640-1504 cm^{-1} were attributed to pyranose cyclic. Another intense peak located at 1000 cm^{-1} was attribute to C-O (β-glycosidic bond) group. Moreover, some additional peaks appeared in the spectrum. The peaks include 3000-2900 cm^{-1} (C-H group) and 1480-1400 cm^{-1} (bending O-H) group.

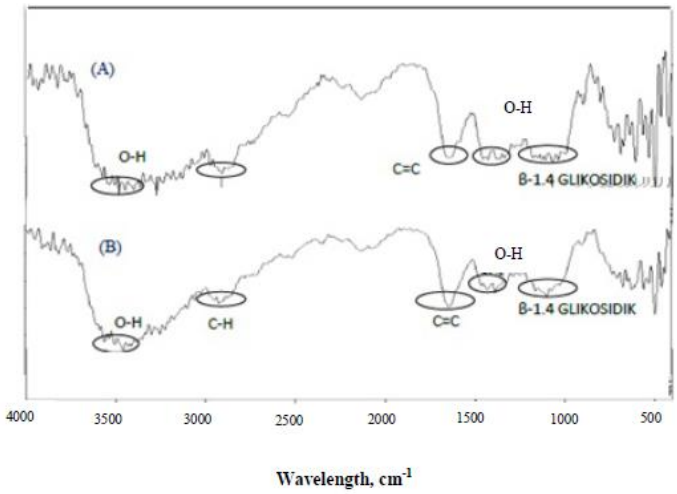

Fig 3. Fourier transform infrared (FTIR) spectra of bacterial cellulose on (a) HS (b) HS-$MgSO_4$ media with addition of 5% v/v glycerol

3.3. Thickness measurement

Fig 4 showed the thickness of bacterial cellulose produced on this research using two different media at various glycerol concentrations. The maximum thickness of bacterial cellulose was produce on HS-$MgSO_4$ media with 5% v/v glycerol concentration. Hence, HS-$MgSO_4$ media with additional of glycerol as a carbon source gave maximum thickness when compared with HS media with addition of glycerol. MgSO4 act as a nutrient that can increased growth rate of *Acetobacter xylinus*.

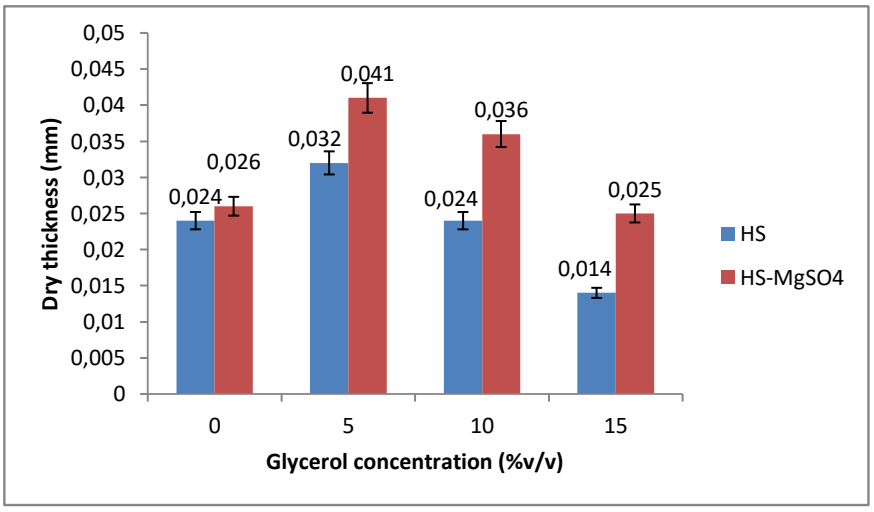

Fig 4. Bacterial cellulose thickness on HS and HS-$MgSO_4$ media at various glycerol concentrations

3.4. Mechanical test

The mechanical properties of bacterial cellulose at various media were presented in Table 1. For pure BC, the tensile strength was about 99 MPa and the elongation at break was about 3.5%. The value of bacterial cellulose vary depend on situations such as culture time, medium supplement.

Table 1. Mechanical properties of bacterial cellulose at various media

Material	Glycerol concentration (%v/v)	Young's modulus (N)	Tensile strength (MPa)	Elongation at break (100%)
HS	0	48.045	99.06	3.514
	5	87.755	86.458	2.157
	10	23.042	85.3398	2.14
	15	4.420	10.43	0.567
HS-$MgSO_4$	0	50.50	97.107	2.429
	5	103.443	116.885	4.214
	10	54.418	105.665	3.876
	15	8.534	21.086	1.934

4. Conclusions

Bacterial cellulose composite was successfully prepared in this research. HS-MgSO4 media with addition of glycerol produced thicker films compared to HS media. Bacterial cellulose from HS-MgSO4 with addition of glycerol also produced films with better mechanical properties (higher tensile strength and higher elongation of break number). These results showed that glycerol can be used as a potential substrate for bacterial cellulose production.

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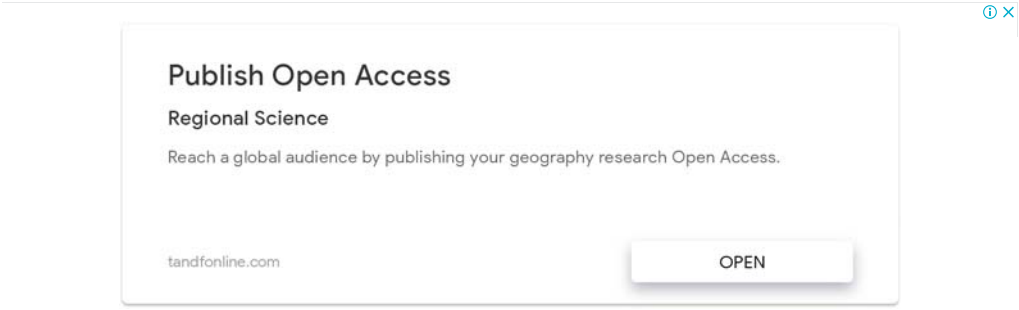

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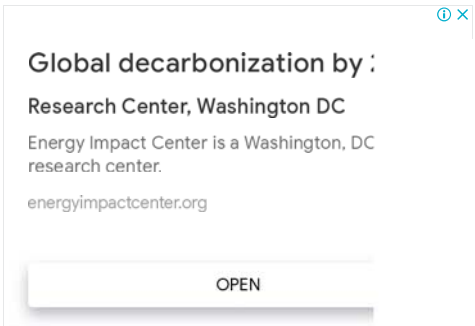

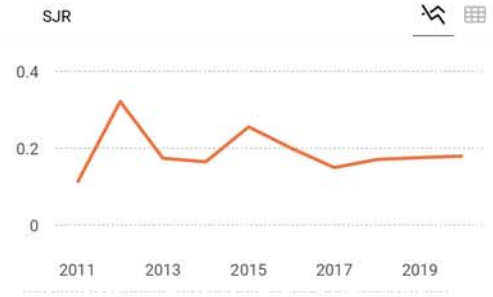

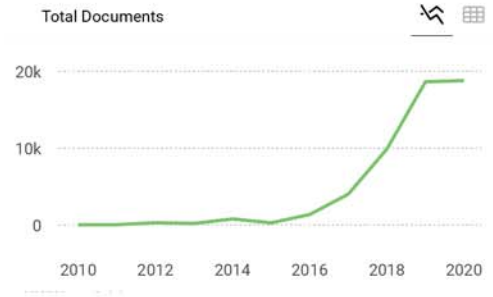

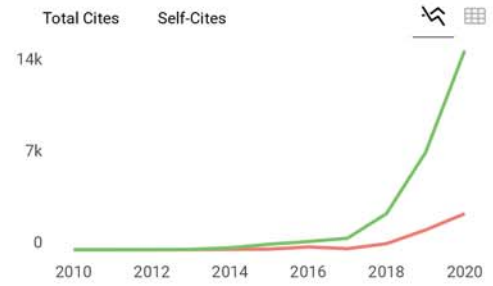

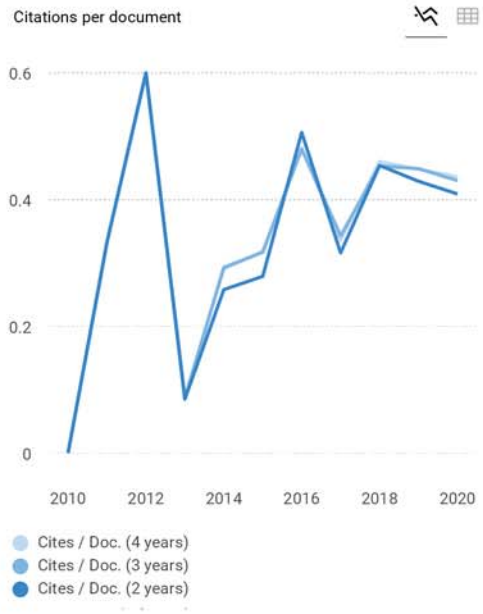

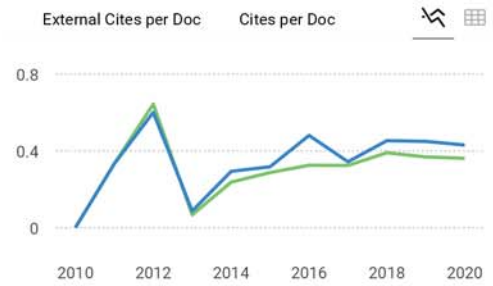

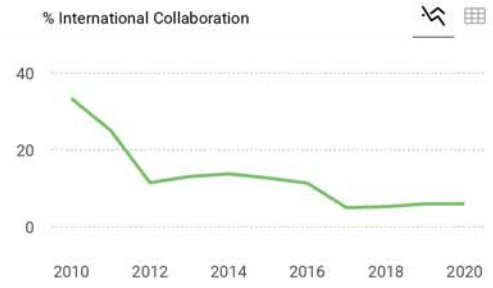

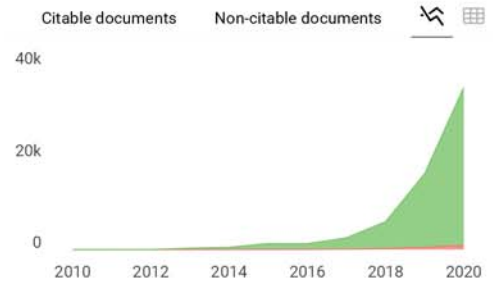

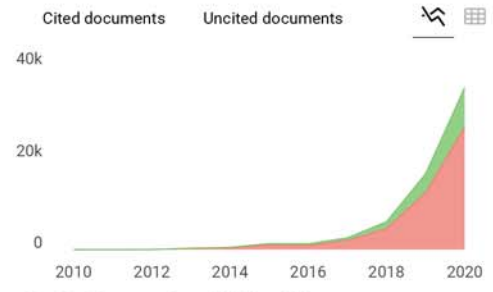

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