Optimised Maintenance Medium of *Acetobacter xylinum* for Bacterial Cellulose Production

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ABSTRACT

The production of bacterial cellulose (BC) is very important nowadays because of its potential application in many fields. The maintenance medium was optimised to obtain a suitable medium for Acetobacter xylinum with high production of BC. Two sets of medium composition are being compared which are known as the crude medium and the derived medium. The crude medium uses natural substrate as a carbon source called coconut water. The composition of the derived medium is based on a modified H-S medium using sucrose as a carbon source. Both media were cultivated for three days at 30°C under static fermentation. Analysis was done by measuring the thickness of the gel forming on the surface of the liquid medium. The result shows that the growth of cells in the crude medium was observed at as early as 24 hours of the incubation time. The approximately 4-fold production in the crude medium as compared to the derived medium was observed at 3 days' cultivation time. It was concluded that the crude medium was the optimised maintenance medium for bacterial cellulose production by Acetobacter xylinum.

Keywords: *Acetobacter xylinum, bacterial cellulose, crude medium, derived medium*

Introduction

Cellulose is normally derived from trees and is the main constituent of paper. However, certain strains of bacteria excrete cellulose as an extra-

cellular polysaccharide. This cellulose is relatively pure and does not contain the lignin and hemi-cellulose associated with wood. The latest resulting material by microbial cellulose has many potential applications and could be used in many fields. Bacterial cellulose shows vast potential as a novel wound healing system (Czaja et al., 2005). Due to the great potentials and applications, microbial cellulose will be a new source for wood, paper, textiles, food, health-care and speciality products. Therefore, it is challenging and meaningful for us to look for optimised maintenance medium for bacterial strain to grow. The proposed maintenance medium should be efficient in cost, easy to supply, cheap and from a natural source.

Literature Review

With regards to carbon sources used in bacterial cellulose production, many substrates were analysed for its possibility to produce cellulose by *Acetobacter xylinum*. A carbon source is a nutrient (such as sugar) that provides carbon skeletons needed for synthesis of new organic molecules (anabolism) (*Carbon Source*, 2004). In this report, the main comparison of both media was on the source of carbon. Sucrose was used as a carbon source for the derived medium, while coconut water was used for the crude medium. Sucrose is a carbon source from the disaccharides group which could produce cellulose yield up to 33% relative to the D-glucose medium (Masaoka et al., 1993). The cells were cultivated for 3 days in the standard medium with an initial carbon source concentration of 0.3 g/flasks.

Carbon sources, which are useful for the production of cellulose, may be used as monosaccharide or mixtures thereof such as glucose and fructose, disaccharides such as sucrose and mixtures of monosaccharide and disaccharides. In addition to single carbon sources, mixtures of glucose and fructose or glucose and sucrose in various ratios and comprising 2.0% of Hestrin & Schramm (HS) medium were examined. The cellulose production and final pH of the culture broth were determined after 6 d in shaking cultures (Yang et al., 1997). The amount of cellulose produced after six day-cultures in shaking flasks show the lowest rate by 0.55 g/l compared to the overall results.

Budhiono et al. (1999) studied the effects of a natural media named coconut-water to the production of bacterial cellulose by *Acetobacter xylinum* in static culture. Glucose was the only saccharide found in the

coconut water stored for 3 days (Budhiono et al., 1999). Others are sucrose and fructose which are unstable unless sterilised. In tropical countries, coconut-water is obtained from the copra process and trace as a waste. It will become valuable if the efficiency to produce bacterial cellulose is verified. Besides that, sugar and nitrogen-containing compounds were added to the medium to complete the nutrient needed for cell growth.

Several groups of researchers concentrated on the thickness of gel to trace the yield of cellulose (Borzani & de Souza, 1995; Yamanaka et al., 1989; Masaoka et al., 1993). It is understood that, after the initial stage, the cellulose is generated only in the vicinity of the surface, because the bacterium is an aerobic one. As long as the system is kept unshaken, the disc-shaped gel is suspended by the cohesion to the interior wall of the vessel and slides steadily downwards as it thickens.

Methods and Materials

The *Acetobacter xylinum* used was a stock supply by the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. The experimental procedures were conducted in two stages which involved cultivation using the crude medium and cultivation using the derived medium. All sets of experiments were performed triplicate. The reference culture composition for the crude medium was obtained from MARDI. Besides that, modifications were made to the medium by changing the glacial acetic acid with acetic acid. The usage of glacial acetic acid, which is harmless in food preparation, is focused on preparation of the nata-de-coco, one type of popular food in tropical countries (Budhiono et al. 1999; Roberfroid, 1993). The derived medium was obtained from Schramm and Hestrin (1954).

The composition of the crude medium: coconut water, 200 mL; sugar, 4 g; ammonium sulphate, 1 g; inoculum usage by ratio 1 mL inoculum to 10mL medium, and acetic acid (to check pH to 4.5). The coconut-water obtained from matured fruits and stored for 3 days was used in this preparation. 200 mL of coconut water was weighed and sieved. 4 g of sugar and 1 g of ammonium sulphate were weighted and mixed with the coconut water. The mixture was stirred until all of the chemicals were dissolved. Acetic acid was added to get pH 4.5.

The composition of the derived medium: sucrose, 20 g/L; peptone (Difco Bactopeptone), 5.0 g/L, yeast extract, 5.0 g/L; disodium phosphate anhydrous, 2.7 g/L; citric acid monohydrate, 0.115 g/L; pH adjusted to 6.0 with HCl or NaOH. The mixture was cleared by filtration and sterilised by autoclaving.

For each medium, the mixture was divided into 4 flasks with each of the flasks containing 50 mL of the mixture. Then, the sterilisation process was done for 15 minutes at 121°C and left overnight for cooling down to the room temperature. After that, the inoculation process was done by putting 5mL of inoculum in each flask. All flasks were put into the oven incubator at 30°C for 3 days. After harvesting the gel from the vessel at day three of the cultivation time, the thickness of the gel performed in each flask was measured. Before that the excess liquid in the vessel was wiped-off and the gel was removed from the flask carefully to avoid any changes in the shape of the gel. Then, the thickness of the wet gel was measured using vernier calipers. The value of mean and total mean for each run was recorded.

Results and Discussion

Table 1 and Figure 1 summarise the results of the study by averaging the thickness value of the two different medium applied in this study and the gel thickness trend for the triplicate run. The total average for the crude medium shows 3.708 mm gel thickness for 3 days' cultivation time where the derived medium only obtained 1.0 mm for the same number of days of incubation time.

Table 1: The Average of Gel Thickness for the Crude Medium and the Derived Medium

Medium	Flask (mm)				Average (mm)	Total Average (mm)
	1	2	3	4		
Crude 1 Run	3.5	4.0	3.5	4.0	3.750	3.708
Crude 2 Run	3.5	3.5	3.5	4.0	3.625	
Crude 3 Run	4.0	4.0	3.5	3.5	3.750	
Derived 1 Run	1.0	1.0	1.5	1.5	1.250	1.000
Derived 2 Run	0.5	1.0	1.0	0.5	0.750	
Derived 3 Run	0.5	1.0	1.5	1.0	1.000	

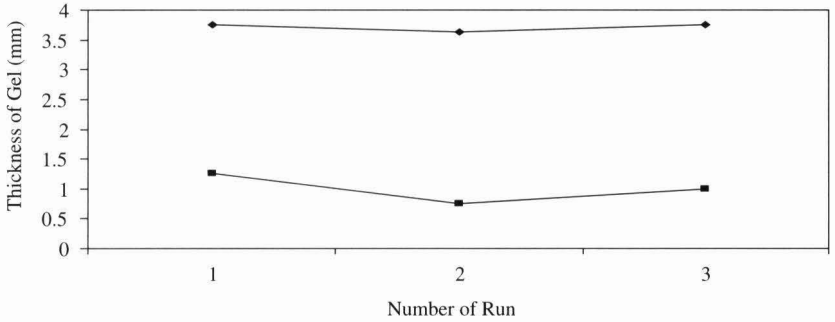

Figure 1: The Comparison of Gel Thickness for the Crude Medium (Diamond) and the Derived Medium (Square)

The vast growth in the crude medium was approximately 4-folds compared to the derived medium suggests that the carbon source is a very important parameter to be considered in conducting the experiment. As mentioned earlier by Budhiono et al. (1999), glucose was the only saccharide found in the coconut water stored at 3 days. The simplest sugar present in the medium enhanced the probability of strain to produce bacterial cellulose by the pathway shown in Figure 2 below:

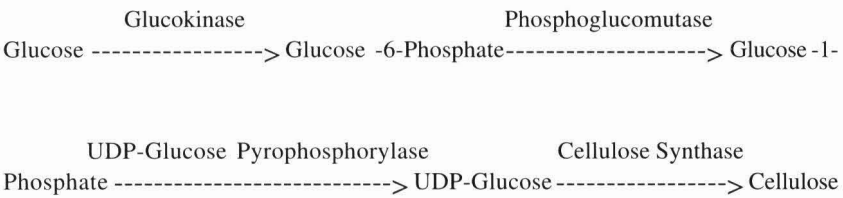

Figure 2: Biochemical Pathway for Cellulose Synthesis in *Acetobacter xylinum* Proposed by Cannon and Anderson (1991)

Schramm et al. (1957) and Weinhouse & Benziman (1974) reported the possibility of gluconate as a carbon source. During the degradation process of glucose, it undergoes partially the reaction sequence glucose to glucanolactone to gluconate, with the respective enzymes glucose dehydrogenase and gluconolactonase. Otherwise, no production of gluconate would be possible. Thus gluconate can serve as a carbon source as well as an intermediate product of the metabolism.

In comparing the different medium used in producing biocellulose, the product shows the ability of strain in converting the medium to biocellulose although it is in a different composition especially with the

carbon source. It is well known that *A. xylinum* has the ability to synthesise cellulose from a wide variety of substrates, but there have been conflicting reports about what type of carbon source would give the best production rate. In one investigation (Masaoka et al., 1993), glucose gave the highest yield of cellulose over a wide variety of carbon sources. In another study (Embuscado et al., 1994), fructose turned out to be the sugar of choice producing as much as 7 times more cellulose than from glucose. Such a difference in the results can be attributed to the different strains used in each study.

Conclusion

The results show that the optimised medium of *Acetobacter xylinum* for bacterial cellulose production was obtained in the crude medium. The findings will give better chances to natural substrate to be a source of carbon for efficient bacterial cellulose production. The low cost of raw material with good production performance will instigate more challenging research in the future.

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