

*Full Length Research Paper*

# Evaluation of different carbon sources for bacterial cellulose production

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**The production of bacterial cellulose (BC) using *Gluconacetobacter xylinus* (= *Acetobacter xylinum*) ATCC 10245 from three categories of carbon sources (Monosaccharide, disaccharide and alcohol) was examined. Glycerol gave the highest yield, followed by glucose, fructose, inositol and saccharose. The highest production efficiency of glycerol might be due to the rate of its consumption (45.4%), which is lower than that of glucose (97%). These results showed that the pH is not the only factor that affects the efficiency of BC production. The rate of consumption of carbon sources in the medium might play another important factor. From X-ray diffractometer, the crystallinity index of bacteria cellulose produce in presence of glucose and fructose have the highest values than those from other carbon sources.**

**Key word:** Bacterial cellulose, *Gluconacetobacter xylinus*, Hestrin-Schramm's medium, crystallinity index.

## INTRODUCTION

The production of bacterial cellulose (BC) is receiving great attention since they can be used in many fields including pulp and paper industry because of its wide application possibilities (Keshk, 2002). One of the BC application problems in industry is its low productivity. Therefore, researchers have tried to increase the productivity of cellulose from *Gluconacetobacter xylinus* using various biochemicals (Ross et al., 1991). Recently different carbon sources, such as monosaccharides, oligosaccharides, alcohol and organic acids, were used to develop the BC production (Masaoko et al., 1993; Ishihara et al., 2002). It has been observed that xylose is not metabolized by *Acetobacter xylinum* and the BC production was unsuccessful using this substrate, whereas the productivity of BC from arabitol was more

than 6 times as much as of glucose (Toda et al., 1995). These studies have not explained the reason of the efficiencies or deficiencies of the carbon substrates, except reference to the pH effect (Masaoko et al., 1993; Ishihara et al., 2002).

Many extensive biosynthetic investigations have been made, but they are mostly centered on the polymerizing mechanism of UDP-glucose and the structure of the cellulose produced (Kai et al., 1989; Kai and Keshk, 1998, Kai and Keshk, 1999). In the case of glucose substrate, the inefficiency of the cellulose production comes from the gluconic acid formation; this prevents the development of large-scale fermentation system. It has been demonstrated that the bacterial cellulose production from glucose was enhanced by the addition of lignosulfonate (Premjet et al., 1996). We described here the production of the BC from three categories of carbon sources and examined their efficiencies to produce the BC. The relationships between the BC yield and the final pH of the culture media during and after the incubation period were examined to explore the effect of pH on the

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BC production. Furthermore, the crystal structure of the BC from various carbon sources will be studied.

## MATERIALS AND METHODS

### Culture media and conditions

The chemicals used throughout this work were purchased from Sigma and Aldrich Chemical Co. American Type Culture Collection (ATCC) is the supplier of the *Gluconacetobacter xylinus* (*G. xylinus*) ATCC 10245.

In the Hestrin-Schramm's medium culture (Hestrin and Schramm, 1954), which contains (% w/v): carbon source 1.0, peptone 0.5, yeast extract 0.5, disodium phosphate 0.27, and citric acid 0.115. The prepared 30 ml culture medium in 100 ml Erlenmeyer flasks were sterilized by autoclaving and were inoculated from the solid agar culture of *G. xylinus*.

### Pellicles production and purification

The inoculated media were incubated at 28°C for 7 days using 90-mm (i.d.) petridishes. After incubation, the pellicles produced on the surface of each media were harvested and washed with water, 1% NaOH at 90°C for 15 min., neutralized with 1% acetic acid and washed with distilled water, successively. It was then dried on a Teflon plate to measure both the weight and X-ray diffractometry.

### The consumption of carbon sources

Consumption of carbon sources was measured using high performance liquid chromatography (HPLC). The HPLC system consisted of a pump (HITACHI, Type L-6000) and an injection valve of 20  $\mu$ L HEODYNE, USA), and a refractive index detector (HITACHI, Type D-2500). The packed column A-603 (Yamamura Chemical Laboratories, S-5 120A NH<sub>2</sub>) was used at ambient temperature. The solvent was a mixture of acetonitrile and water (3:1) at flow rate of one ml/min.

### Wide angle x-ray diffractometry

The diffractogram of the samples was recorded at room temperature with RIGAKU PRINT 2200V series using Ni-filtered CuK $\alpha$  radiation ( $\lambda = 1.54\text{\AA}$ ). The operating voltage and current were 40 Kv and 30 mA, respectively. Crystallinity was calculated from the diffracted intensity data using the method of Segal et al. (1959), where the crystallinity index, Cr.I. =  $(I_{002} - I_{am})/I_{002}$ ;  $I_{002}$  is the maximum intensity of the lattice diffraction and  $I_{am}$ , the intensity at  $2\theta = 18^\circ$ .

## RESULT AND DISCUSSION

### Production of cellulose membrane in different glucose concentrations

The change in yield is listed in Table 1 and shown in Figure 1 as the function of the percentage of the glucose concentration. The maximum yield was obtained at 1% concentration whereas, the minimum yield was observed at both 2% and 3% concentrations. From these results, we

decided to use the 1% concentration for the eighteen carbon sources from the three categories.

### Production of cellulose membrane in the cultures of various carbon sources

It has been known as early as 1950, that *G. xylinus* is capable of producing cellulose from a variety of inexpensive carbon substrates, which include glucose, sucrose, and fructose. The cellulose production mechanism from these varieties of substances, however, has not been adequately investigated because most of the investigations of the cellulose synthesis have been done on glucose culture medium. In this study, various carbon substrates were examined for their efficiencies in the production of cellulose membrane. The relationship between final pH values and the cellulose membrane yield from various carbon substrates is listed in Table 1. The glucose culture gave the lowest pH followed by xylose and ethanol. Ethanol gave slightly higher cellulose yield than that of the blank culture, while the other sugars (arabinose, galactose and mannose) had little difference when compared to that of the blank culture. The pH changes of the culture might be the indicator of the side reactions taking place in the cellulose production culture. The final pH of the ribose medium was comparative to that of the inositol culture (pH= 5.3). The fructose culture showed only slight decrease in pH as in case of alcoholic category except ethanol, which has sharp pH drop. Five substrate cultures (rhamnose, sorbose, cellobiose, lactose and methanol) showed negligible deviation from the initial pH of 6.0 and the cellulose membrane yields were comparable to that of the blank cultures. Although the ethanol culture gave a little higher membrane yield than that of the blank culture, the pH was substantially lower than that of initial pH. It is obvious that the decrease of pH is not the only factor determining the efficiency of cellulose production. It might be possible that the efficient production of cellulose by the bacteria lies on its ability to synthesize glucose from various carbon substrates and glucose polymerization to cellulose.

### The consumption of substrates in the culture media

Glucose, fructose, inositol and glycerol (the highest yield) were selected to measure the consumption during and after the incubation period according to their yield (Table 1). As shown in Figure 2, glucose was consumed rapidly in the early stage of incubation and almost completely (97%) after 7 days incubation. Inositol was consumed as completely as glucose (94.7%) but most of the consumption began after 4 days of incubation. Glycerol and fructose have the same final consumption percentage (<50%) as shown in Figure 2. The pH changes of the four culture media were shown

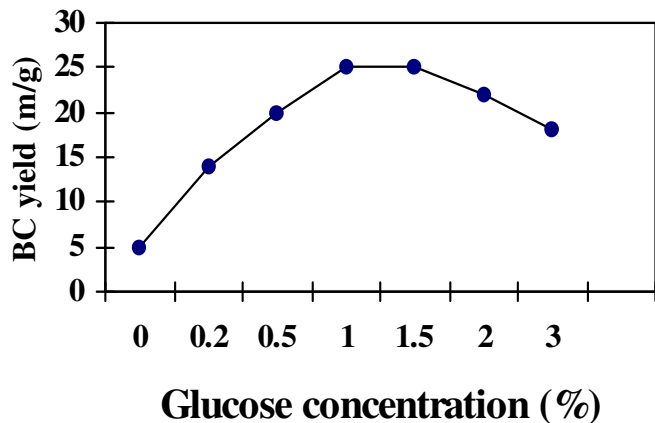
**Table 1.** Bacterial cellulose productivity of various carbon sources from *G. xylinus*.

Carbon source	Final pH	Yield (%)*	Consumption (%)	Cellulose Yield (%)**	Production Efficiency (%)***	Crystallinity Index (%)
Blank	6.3	22	-	-	-	-
Galactose	5.1	24				
Glucose	3.9	100	<b>97.0</b>	<b>8.4</b>	<b>8.7</b>	<b>88</b>
Fructose	5.6	95	<b>51.9</b>	<b>7.9</b>	<b>15.3</b>	<b>86</b>
Mannose	4.7	24				
Ribose	5.4	42				
Rhamnose	5.8	22				
Sorbose	5.7	23				
Xylose	4.6	38				
Lactose	6.3	22				
Trehalose	6.0	52				
Saccharose	5.9	69				
Maltose	6.1	25				
Ethanol	4.1	25				
Methanol	6.3	22				
Inositol	5.3	85	<b>94.7</b>	<b>7.4</b>	<b>7.8</b>	<b>75</b>
Glycerol	5.5	155	<b>45.4</b>	<b>13.0</b>	<b>28.7</b>	<b>78</b>

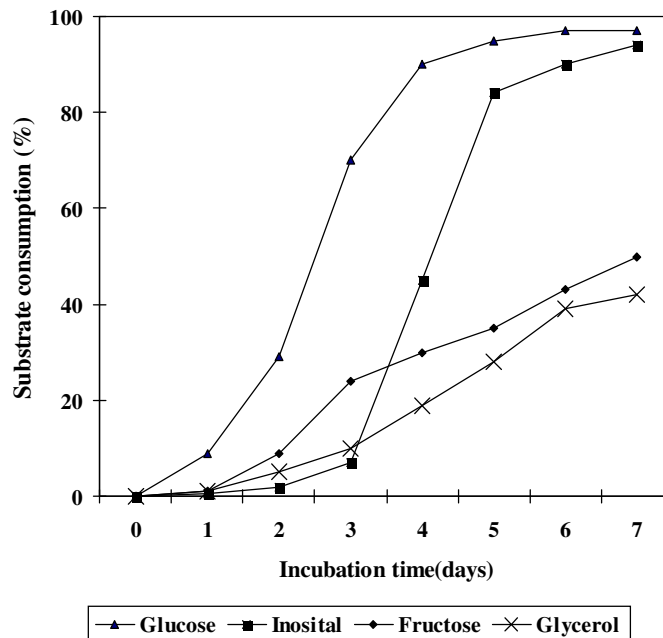
\* % of BC yield in comparison to that of 1% glucose (25mg/30ml).

\*\* Calculated from the dry weight of BC and the weight of carbon source added (300mg/30ml).

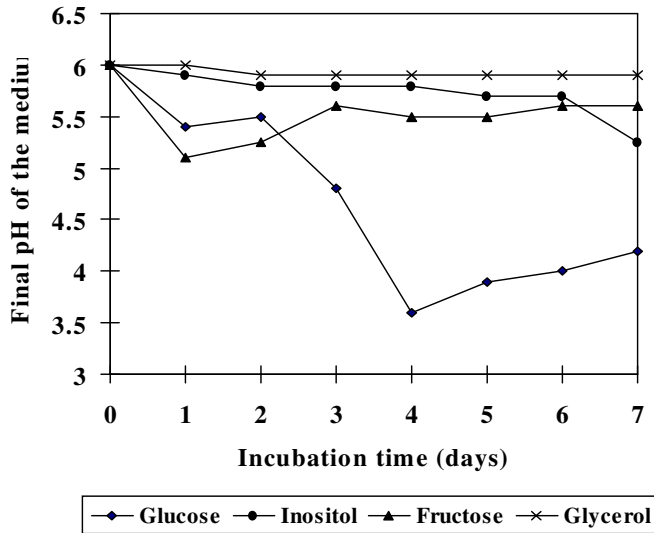
\*\*\* Calculated from the dry weights of BC and the weight of consumed carbon source.



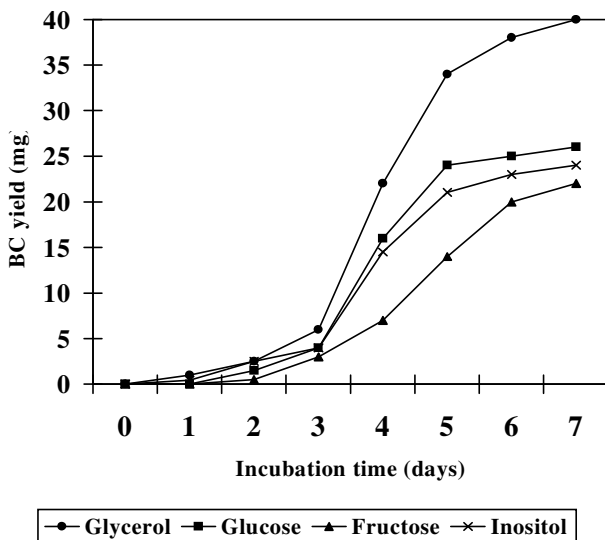
**Figure 1.** BC yields from glucose culture at various percentages.



**Figure 2.** The consumption curves during the cultivation period of glucose, fructose, inositol and glycerol substrates.



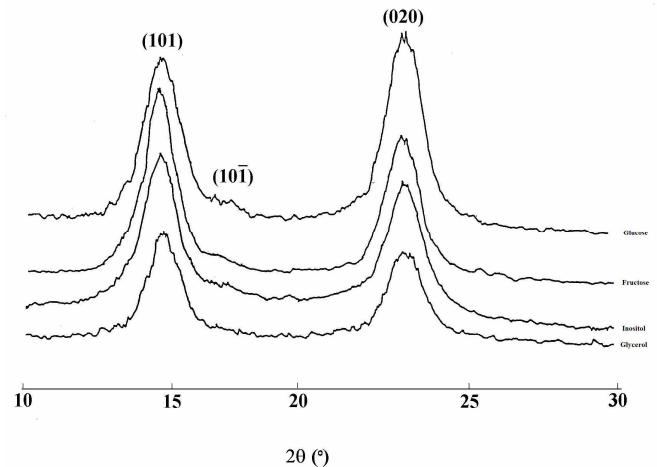
**Figure 3.** The pH change during the cultivation period of glucose, fructose, inositol and glycerol substrates.



**Figure 4.** Change in BC yield during cultivation period of glucose, fructose, inositol and glycerol substrates.

in Figure 3. Glycerol and inositol media showed a negligible drop in pH, but glucose and fructose media showed a sharp drop at the beginning of culture, which increased again on the second and /or fifth day of incubation. In inositol medium the pH decrease occurred at the end of incubation period. The BC yields from these four carbon sources were shown in Figure 4. The glycerol medium showed the highest cellulose yield.

Whereas the other three substrates (Glucose, inositol, and fructose) produced similar yields which was much lower than that of glycerol. These results indicate that the resulted BC is the product of very complicated reactions. The pH changes of the culture media reflect the complexity of the reactions. The pH change of the glucose medium might be the reflection of the formation and consumption of gluconic acids (Fujiwara et al., 1989).



**Figure 5.** X-ray diffraction pattern of the BCs from top to bottom, Glucose, fructose, inositol and glycerol.

#### Determination of the cellulose production efficiency

From the consumption percentage and the yield of the cellulose membrane after seven days incubations, the BC production efficiencies have been calculated for the four main substrates as shown in Table 1. The glycerol is the best substrate for cellulose production with the efficiency of 28.7% on the added substrate weight. Ishihara et al. (2002), studied the utilization of D-xylose as carbon source for the production of cellulose membrane (Toda et al., 1995), and deduced that xylose is not well metabolized by any bacterial strains that exhibited high cellulose production in glucose medium. Whereas, sucrose, glucose and mannitol were found to be suitable for optimum levels of cellulose production (Ramana et al., 2000). On the other hand, there are no high differences in crystal structure among the hexoses (Figure 5). Hexose substrates have the best crystallinity index among the other sources of carbon.

#### CONCLUSION

The final pH values of the monosaccharides media were lower than that of their initial cultures, the lowest pH was that from glucose followed by xylose. Whereas fructose

and disaccharides showed only slight decrease in pH values after incubation, their BC yield were comparable with that from glucose. In the alcoholic category, inositol gave yield comparable to glucose and fructose. Glycerol gave the highest yield of BC without the sharp drop of pH during the incubation.

Furthermore, the crystallinity index of the BC from hexose is higher than that from other carbon sources.

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