CHAPTER 3

RESULTS

1. Evaluation of bacterial cellulose properties

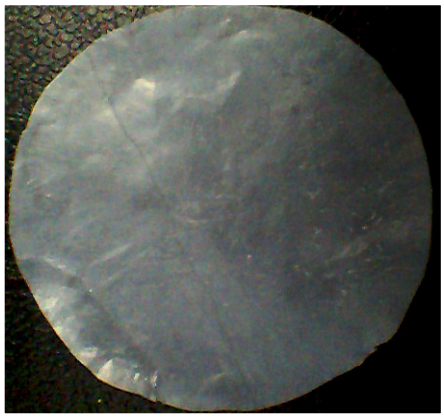
1.1 Dry-weight, thickness and percent yield of bacterial cellulose dry films

After cultivation of *A. xylinum* TISTR 975 in modified HS medium containing 8% w/v of different carbon sources i.e, glycerine, mannitol, sucrose, glucose, fructose, lactose and arabinose in static condition for 3 days at 30°C, the cellulose wet films or pellicles were produced from the first five carbon sources but not from lactose and arabinose. After washing and drying, the BC dry films were obtained as shown in Figure 1. The BC dry films were look like translucent paper. They were then tested for dry-weight, thickness and %yield. The results are presented in Table 1. The weight of BC dry film (area 63.58 cm^2) was between 113.85 and 202.99 mg and the highest weight was from glycerine (202.99 mg) and the lowest one was from fructose (113.85 mg) The highest thickness of BC dry film was BC produced from glycerine (33.17 µm) followed by mannitol (32 µm). Fructose gave the lowest thickness (21.50 µm). Concerning about the %yield of BC dry film produced from different carbon sources, the results showed that glycerine and mannitol gave 2.54 and 2.14 %yield, respectively. Sucrose, glucose and fructose gave 1.89, 1.70 and 1.42 %yield, respectively.

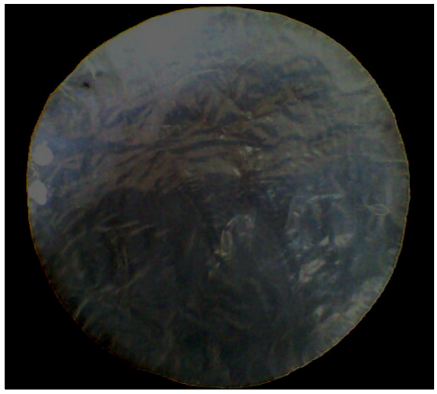
(A) glycerine

(B) mannitol

(C) sucrose



(D) glucose



(E) fructose

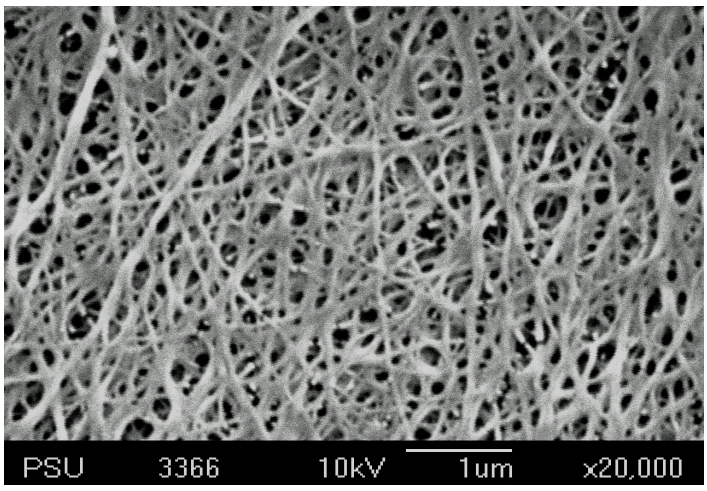
Figure 1. Bacterial cellulose dry films from different carbon sources: (A) glycerine; (B) mannitol; (C) sucrose; (D) glucose and (E) fructose

Table 1. Dry-weight, thickness and percent yield of bacterial cellulose dry films from different carbon sources (area 63.58 cm^2) (mean ± SD, n = 6)

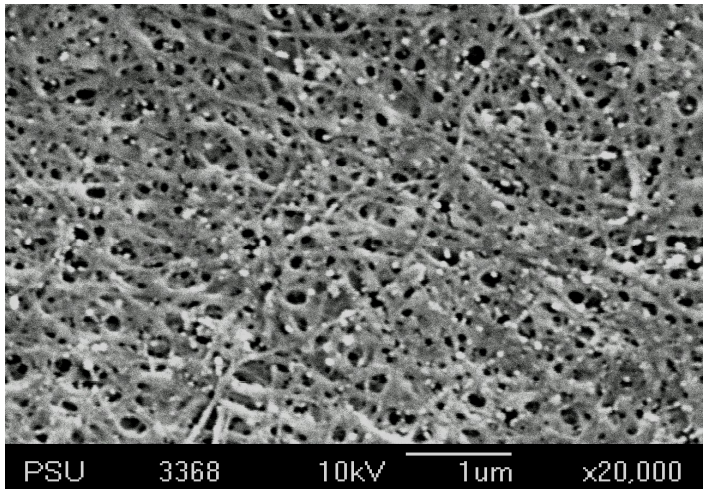
Carbon sources	Dry-weight (mg) mean ± SD	Thickness (µm) mean ± SD	Percent yield (%) mean ± SD
Glycerine	202.99 ± 4.80	33.17 ±1.94	2.54 ± 0.06
Mannitol	170.82 ± 1.19	32.00 ± 2.76	2.14 ± 0.01
Sucrose	151.57 ± 0.95	26.50 ± 7.87	1.89 ± 0.01
Glucose	135.87 ± 4.00	25.33 ± 2.42	1.70 ± 0.05
Fructose	113.85 ± 3.42	21.50 ± 2.81	1.42 ± 0.04
Lactose	-	-	-
Arabinose	-	-	-

1.2 Observation of bacterial cellulose dry films under scanning electron microscope (SEM)

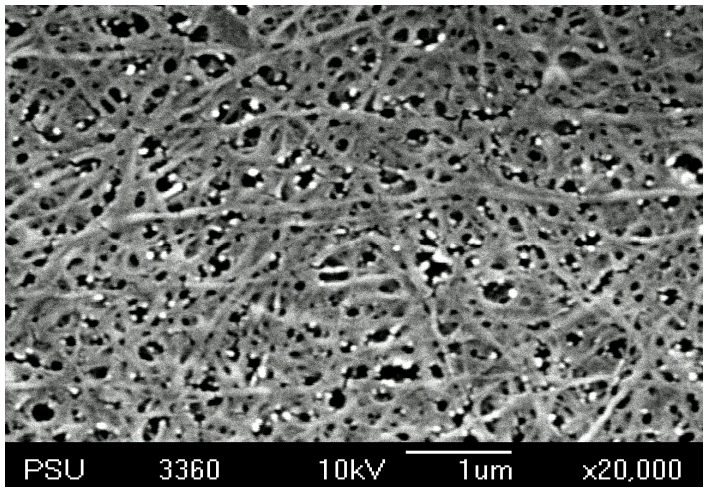
Figure 2. shows the electron micrographs of BC dry films produced by *A. xylinum* TISTR 975 from HS medium containing different carbon sources, i.e., glycerine, mannitol, sucrose, glucose and fructose. The bundles of fibrils from all sources of carbon had diameter approximately 100-200 nm and they were woven to each other into networks. They formed tutorial pores between fibrils with diameter approximately 50-200 nm. Normally, the bacterial cells embedded in the fibril networks but they were destroyed while immerging in NaOH during washing process. When focusing on fibrils from each carbon source, fibrils from glycerine and fructose were bigger bundle than fibrils from other sources. The fibrils from glycerine had smooth surface and the bundles of fibril are not broken while the fibril from mannitol, sucrose, glucose and fructose showed rough surface and some of them are broken. The fibril networks from glucose are dense and less porosity when compared with others.



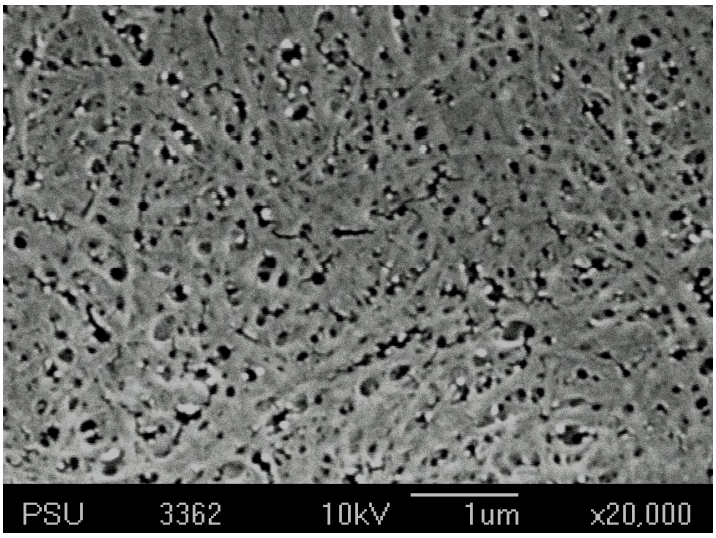
(A) glycerine



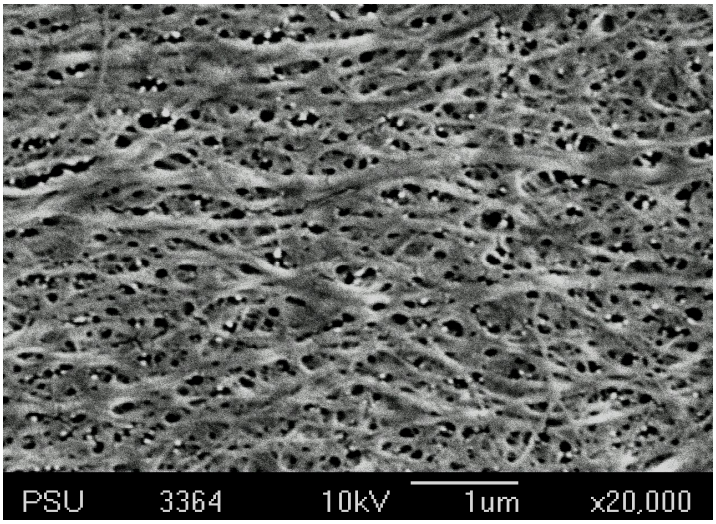
(B) mannitol



(C) sucrose



(D) glucose



(E) fructose

Figure 2. Scanning electron micrographs of bacterial cellulose dry films from different carbon sources: (A) glycerine; (B) mannitol; (C) sucrose; (D) glucose and (E) fructose

1.3 Crystallinity of bacterial cellulose dry films by X-ray diffractometer (XRD)

XRD patterns of BC dry films produced by *A. xylinum* TISTR 975 from different carbon sources are presented in Figure 3. There were apparently to each other. Each of them show two diffraction dominant peaks at A_1 and A_2, At A_1 peak located at between 11.03° to 19.38° and A_2 peak located at between 19.38º to 25.98°. The % crystallinity of BC dry films was calculated as the ratio of the area of the resolved crystalline peaks to the total area of a diffraction profile. The results are presented in Table 2. Concerning about the % crystallinity of BC dry films produced from different carbon sources, the result showed that mannitol and sucrose gave 84.38 and 75.81%, respectively. Glucose, fructose and glycerine gave 67.95, 66.60 and 64.84%, respectively. The highest crystallinity of BC dry films was BC produced from mannitol (84.38%) while glycerine gave the lowest crystallinity (64.84%).

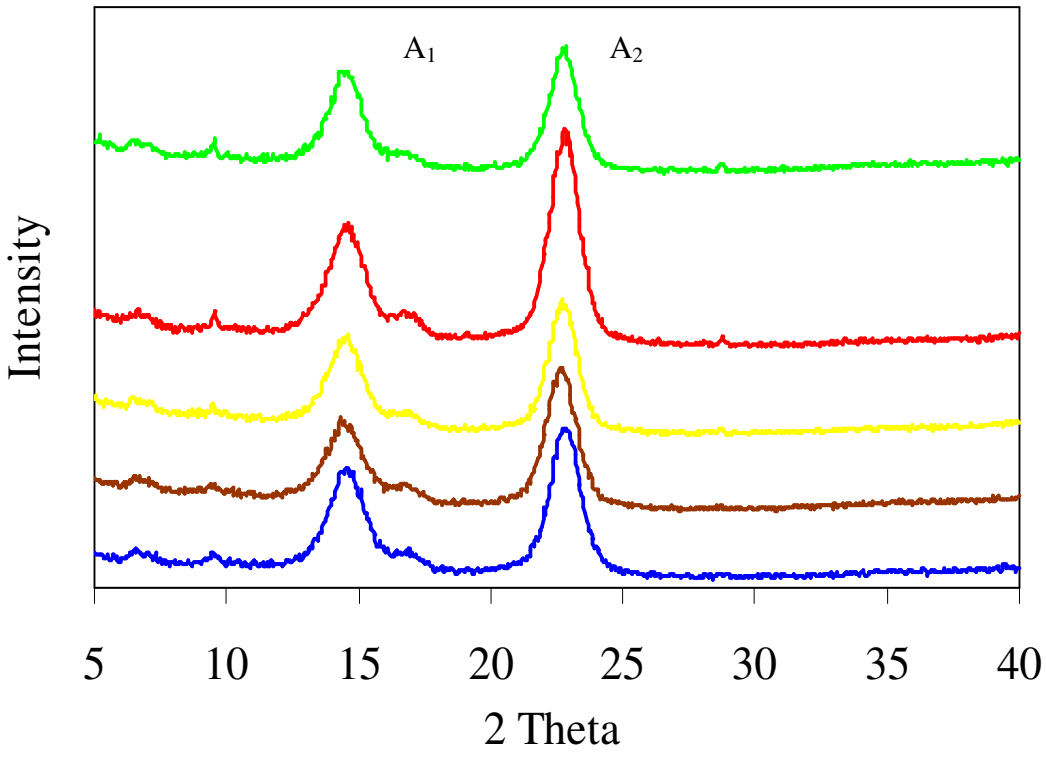


Figure 3. X-ray diffraction patterns of bacterial cellulose dry films from different carbon sources: (—) glycerine; (—) mannitol; (—) fructose; (—) glucose and (—) sucrose

Table 2. Percent crystallinity of bacterial cellulose dry films from different carbon sources

Carbon sources	The area of the resolved crystalline peaks		The total area of a diffraction profile	(A1-A2)	Percent crystallinity
	A1	A2	At		
	11.03°-19.38°	19.38°-25.98°	5.03°- 39.93°		
Glycerine	1677.77	1629.15	5022.70	3256.92	64.84
Mannitol	2131.29	2832.10	5881.99	4963.39	84.38
Fructose	1579.19	1761.99	5016.73	3341.18	66.60
Glucose	1472.46	1896.67	4957.98	3369.13	67.95
Sucrose	1915.33	2164.30	4079.63	5381.49	75.81

1.4 Tensile strength of bacterial cellulose films

Maximum Load, Extension at Break and Tensile strength of BC films produced by *A. xylinum* TISTR 975 different carbon sources i.e., glycerine, mannitol, sucrose, glucose and fructose are presented in Table 3. The Maximum Load of BC films was range between 10.07 to 56.98 N. and the highest Maximum Load from mannitol 56.98 N and the lowest from fructose 10.07 N. The Extension at Break of BC films was range between 2.600 to 8.563 mm and the highest Extension at Break from glycerine 8.563 mm and the lowest from fructose 2.6 mm. The tensile strength was range between 22.37 to 126.63 kN/m^2 and the highest tensile strength from mannitol 126.63 kN/m^2 and the lowest from fructose 22.37 kN/m^2.

Table 3. Maximum Load, Extension at Break and Tensile strength of bacterial cellulose films from different carbon sources (mean ± SD, n = 5)

Carbon sources	Maximum Load (N) mean ± SD	Extension at Break (mm) mean ± SD	Tensile strength (kN/m^2) mean ± SD
Glycerine	11.31 ± 1.31	8.563 ± 3.068	26.26 ± 2.91
Mannitol	56.98 ± 15.35	6.718 ± 2.897	126.63 ± 34.12
Sucrose	38.68 ± 5.55	6.777 ± 1.234	85.96 ± 12.33
Glucose	25.00 ± 2.93	7.638 ± 1.886	57.77 ± 6.59
Fructose	10.07 ± 4.39	2.600 ± 1.582	22.37 ± 9.76

1.5 Nitrogen adsorption isotherm and pore size distributions of bacterial cellulose films

Figure 4. shows the nitrogen adsorption isotherms measured on BC films produced by *A. xylinum* TISTR 975 from HS medium containing different carbon sources i.e., glycerine, mannitol, sucrose, glucose and fructose. The adsorption isotherms of BC films from five carbon sources were similar to each other in shape. The nitrogen adsorption volume of BC film produced from glycerine was the highest followed by BC films from glucose, mannitol, fructose and sucrose, respectively. According to the BET classification, these kinds of isotherms belong to the isotherms of type II describing the process of physical adsorption of nitrogen (Zhao, 2005). The pore volume and pore diameter were calculated using the Barrett-Joyner-Halenda (B.J.H.) method. The results are presented in Figure 5. The pore diameters of all BC films distributed from under 6 nm to over 80 nm. At the pore size diameter 20-80 nm, all BC films showed the highest pore volume. BC films from glycerine gave the highest pore volume (0.06995 ml/g) followed by glucose (0.05666 ml/g), Mannitol (0.03836 ml/g), fructose (0.03546 ml/g) and sucrose (0.03221 ml/g).

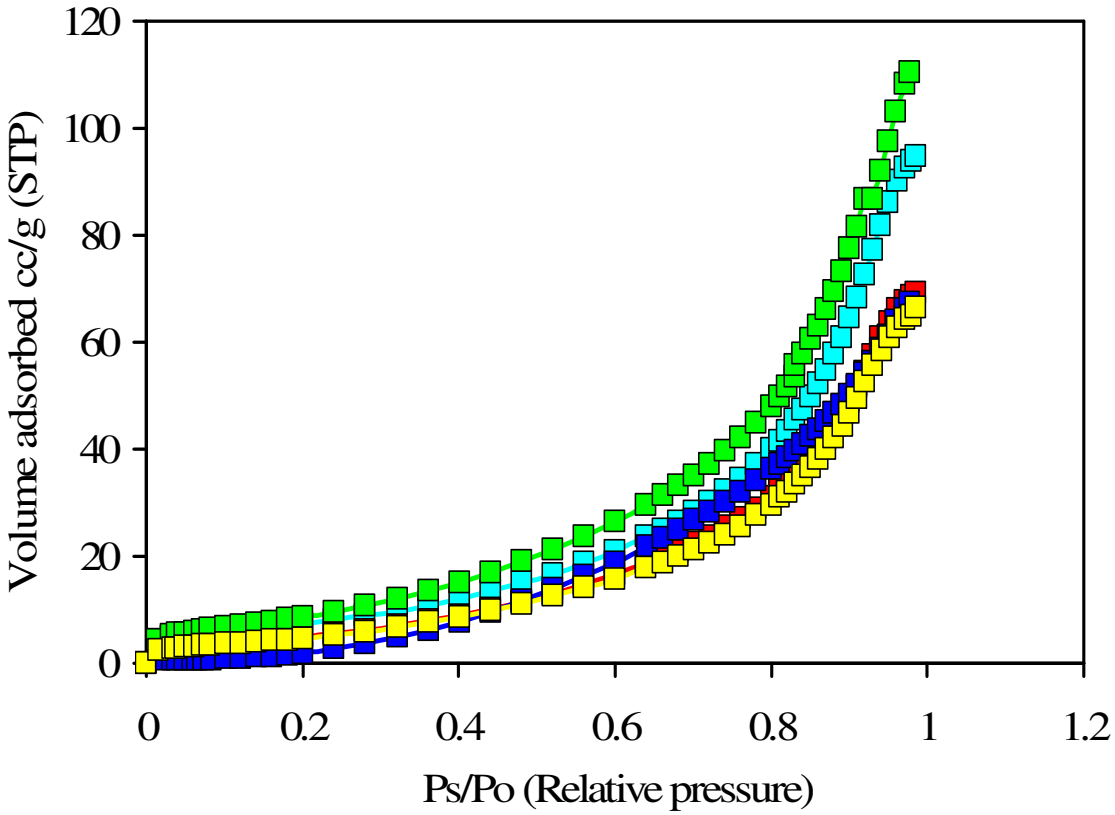
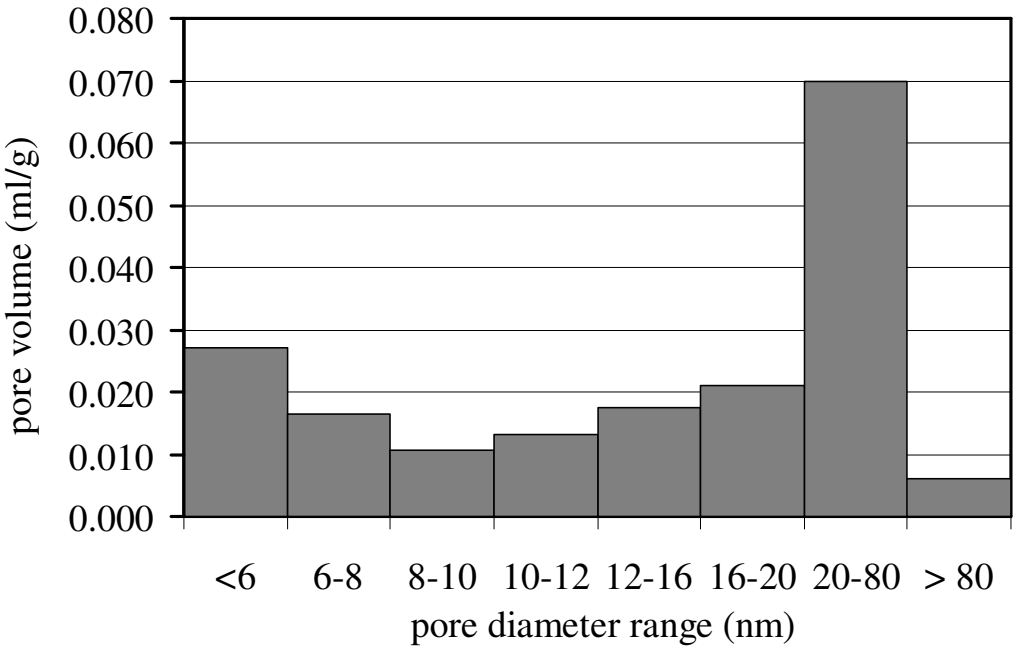
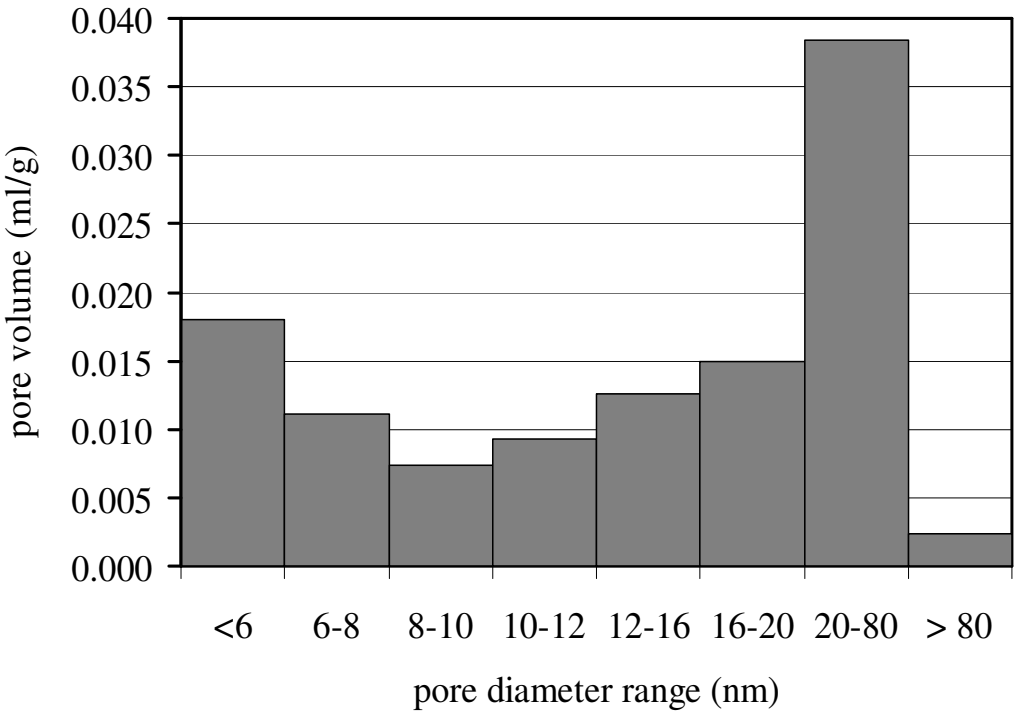


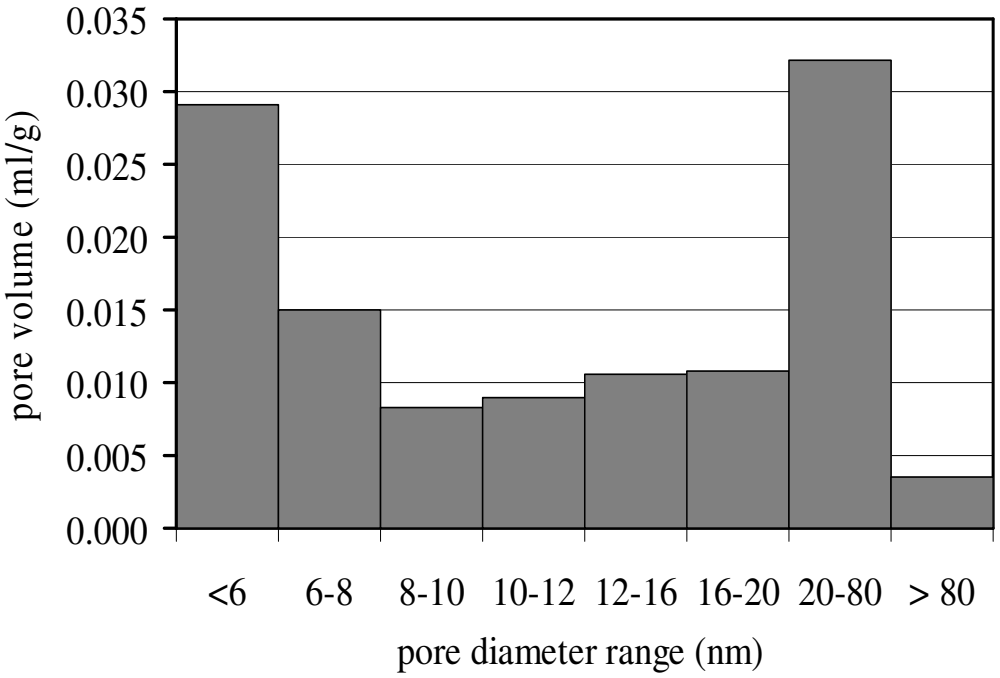
Figure 4. Nitrogen adsorption isotherms of bacterial cellulose films from different carbon sources**:** (■) glycerine; (■) mannitol; (■) sucrose; (■) glucose and (■) fructose



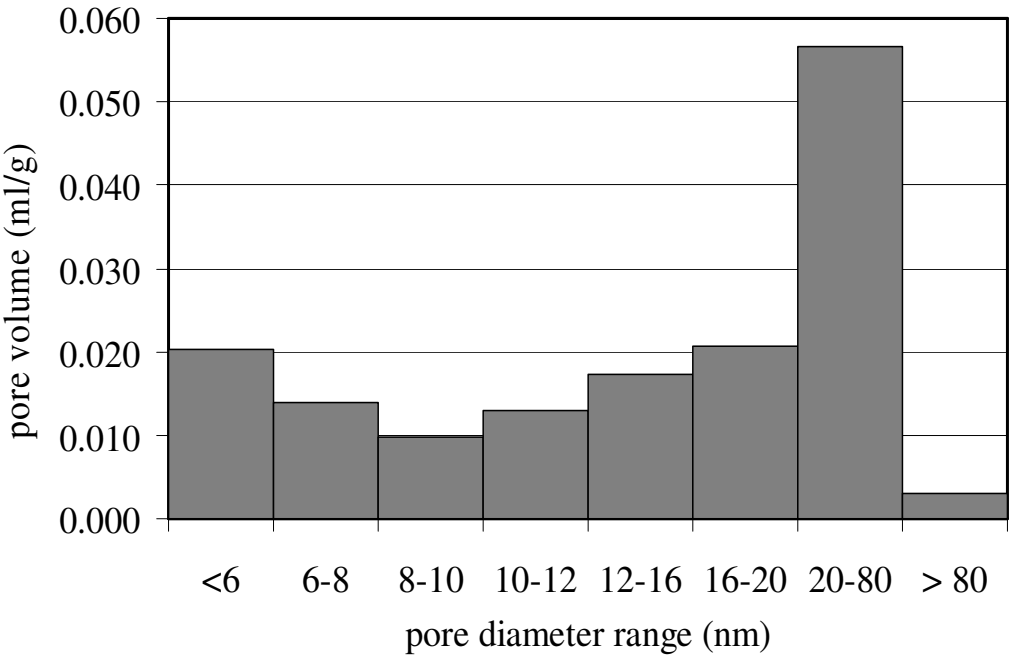
(A) glycerine



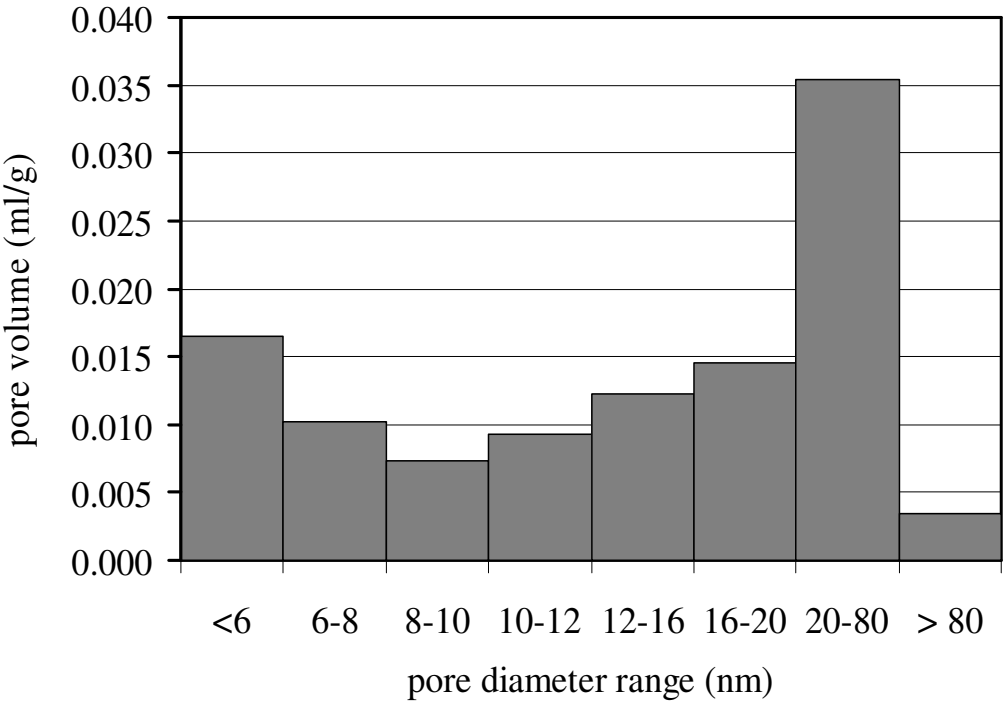
(B) mannitol



(C) sucrose



(D) glucose



(E) fructose

Figure 5. Pore-size distributions of bacterial cellulose films from different carbon sources: (A) glycerine; (B) mannitol; (C) sucrose; (D) glucose and (E) fructose

1.6 Percent of water loss and percent water readsorption of bacterial cellulose wet film

When BC from culture was collected, it adsorbs a lot of water. It needs to be removed a certain amount of water and checked for its ability to readsorp water at different drying state. In this study BC films from glycerine was used as the representative of the films from other carbon sources. Percent water loss and % water readsorption of BC after drying at 50°C for 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 h are presented in Table 4. The % water loss of BC wet films increased with the time of drying. From 2-8 h, % water loss of BC wet films gradually increased from 31.84 to 91.68%. After 8 h until 24 h, % water loss of BC wet films was almost constant. On the other hand, % water readsorption was observed. The % readsorption of water of BC wet films gradually increased from 84.05 to 348.92% after 2 to 8 h of drying. After 8 h to 10 of drying, % water reasorption decreased from 348.92 to 117.56%. During12-24 h of drying, the % water readsorption was almost constant at 81.38-89.33%. The results indicated that at temperature 50^{o}C and 8 h of drying, the BC films give the highest % water readsorption 348.92%.

Table 4. Percent of water loss and percent of water readsorption of bacterial cellulose wet films after drying at 50ºC for 2, 4, 6, 8, 10, 12, 14, 16, 20 and 24 h (mean ± SD, n = 6)

Periods of time (h)	Percent of water loss (mean ± SD, n = 6)	Percent of water readsorption (mean ± SD, n = 6)
2	31.84 ± 2.42	84.05 ± 3.80
4	53.01 ± 3.33	147.45 ± 3.24
6	67.75 ± 5.69	190.53 ± 3.55
8	91.68 ± 6.33	348.92 ± 4.90
10	99.97 ± 0.01	117.56 ± 2.61
12	99.65 ± 0.50	86.14 ± 2.45
14	99.95 ± 0.03	89.04 ± 4.83
16	99.98 ± 0.06	88.03 ± 2.47
20	99.94 ± 0.01	81.38 ± 2.37
24	100.04 ± 0.03	89.33 ± 6.24

1.7 Moisture adsorption isotherm of bacterial cellulose at various relative humidity (RH)

Figure 6. shows the % moisture absorption of BC films at various RH i.e., 6, 32, 51, 62, 73 and 97%. The percent moisture absorption was calculated at 2, 4, 6, 8, 10, 12 and 24 h of incubation in each RH atmosphere. All the data showed that the more moisture in atmosphere the higher % absorption of all kinds of BC films. After 2 h of incubation, BC films from glycerine showed the highest average % moisture absorption (16.95%) in any RH atmospheres, followed by film form sucrose (14.06%), fructose (9.66%), glucose (6.89%) and mannitol (6.35%), respectively. After that % moisture absorption of all BC films gradually increased and almost stable at 12 h. At 24 of incubation all of them showed not much higher water absorption than that of 12 h. BC film form glycerine after 24 h of incubation in any RH atmospheres gave the highest average % moisture absorption (33.18%) followed by the films from sucrose (32.08%), fructose (31.98%), glucose (26.58%) and mannitol (26.50%), respectively.

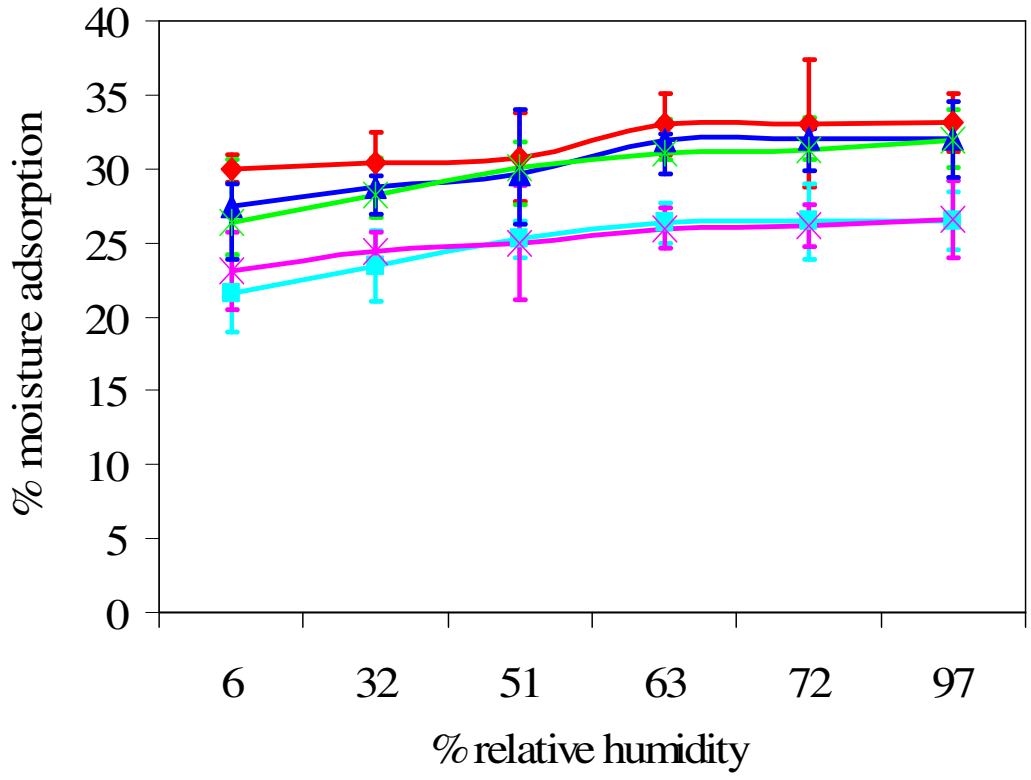


Figure 6. Percent moisture absorption of bacterial cellulose films from different carbon sources: (—) glycerine; (—) mannitol; (—) sucrose; (—) glucose and (—) fructose at various relative humidities.

2. Antimicrobial activity of chlorhexidine digluconate

The result of antimicrobial activity of chlorhexidine digluconate against bacteria and yeast is shown in Table 4 and Figure 7. It can inhibit Gram-negative, Gram-positive bacteria and *C. albicans* NCPF 3153. Form the paper disk containing 4.04 μg chlorhexidine digluconate, it gave the inhibition zone to *E. coli* ATCC 25922 is 17.5 ± 0.08 mm. The inhibition zones against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 12228 and *C. albicans* NCPF 3153 are 15.35 ± 0.17, 22.76 ± 0.10, 20.05 ± 0.23 and 18.02 ± 0.32 mm, respectively.

The result of antimicrobial activity of bacterial cellulose disk containing chlorhexidine digluconate against bacteria and yeast is shown in Table 5 and Figure 8. BC films from glycerine were tested for antibacterial activity by agar diffusion method. Form the BC disk containing chlorhexidine digluconate 42.05 μg/disk, it gave the inhibition zone to *E. coli* ATCC 25922 is 14.5 ± 0.09 mm. The inhibition zones against *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 12228 and *C. albicans* NCPF 3153 are 15.35 ± 0.15, 18.42 ± 0.21, 17.02 ± 0.16 and 13.6 ± 0.13 mm, respectively.

Table 5. Antimicrobial activity of chlorhexidine digluconate (mean ± SD, n = 3)

Microoganisms	Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
E. coli ATCC 25922	17.5 ± 0.08	2	2
S. aureus ATCC 25923	15.35 ± 0.17	4	4
P. aeruginosa ATCC 27853	22.76 ± 0.10	2	32
S. epidermidis ATCC 12228	20.05 ± 0.23	2	4
C. albicans NCPF 3153	18.02 ± 0.32	2	2

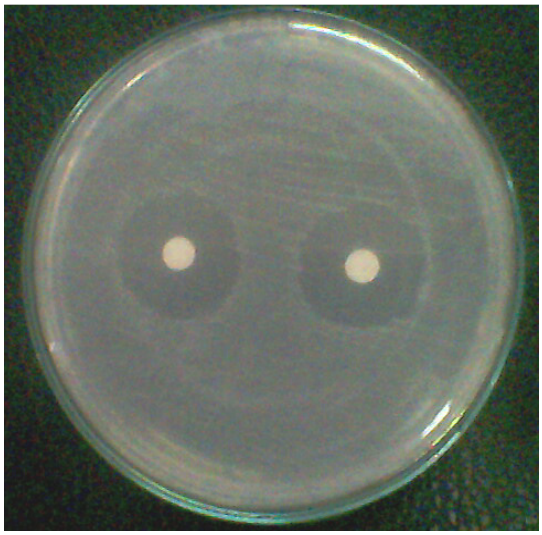


Figure 7. The inhibition zone of chlorhexidine digluconate to *Pseudomonas aeruginosa* ATCC 27853

Table 6. Antimicrobial activity of bacterial cellulose disk containing chlorhexidine digluconate (mean ± SD, n = 3)

Microoganisms	Inhibition zone (mm)
E. coli ATCC 25922	14.5 ± 0.09
S. aureus ATCC 25923	15.35 ± 0.15
P. aeruginosa ATCC 27853	18.42 ± 0.21
S. epidermidis ATCC 12228	17.02 ± 0.16
C. albicans NCPF 3153	13.6 ± 0.13

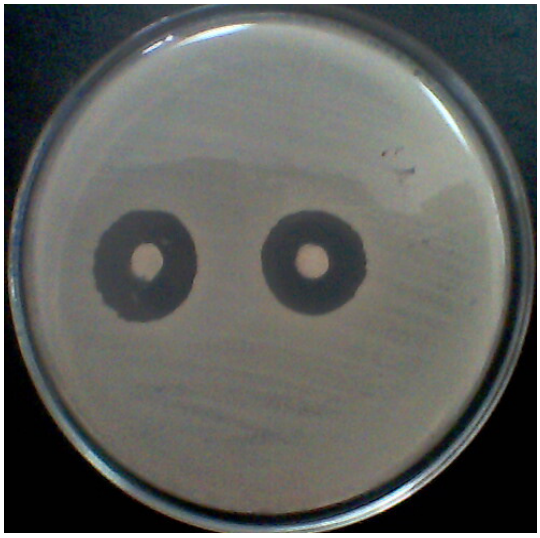


Figure 8. The inhibition zone of bacterial cellulose disk containing chlorhexidine digluconate to *Pseudomonas aeruginosa* ATCC 27853

3. Chlorhexidine digluconate content and percent accumulative of chlorhexidine digluconate released from bacterial cellulose films

BC films were evaluated for chlorhexidine digluconate content. Chlorhexidine digluconate contents of BC film from glycerine, mannitol, sucrose, glucose and fructose are 87.79, 84.79, 76.74, 79.57, and 82.56%, respectively.

Releasing of chlorhexidine digluconate from BC films preparations were examined *in vitro* using a Frantz-type diffusion cell. The released drug was collected and analyzed at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h of study. The % accumulative of released chlorhexidine digluconate is presented in Figure 10. BC film from glycerine showed the highest drug release profile followed by films from sucrose and mannitol, which had the same drug release profile, film from glucose and fructose, respectively. After 24 h of study, the total drug release from BC films from glycerine, sucrose, mannitol, glucose and fructose were 98.07, 80.07, 80.03, 73.1 and 49.73%, respectively, compared with drug content of each film.

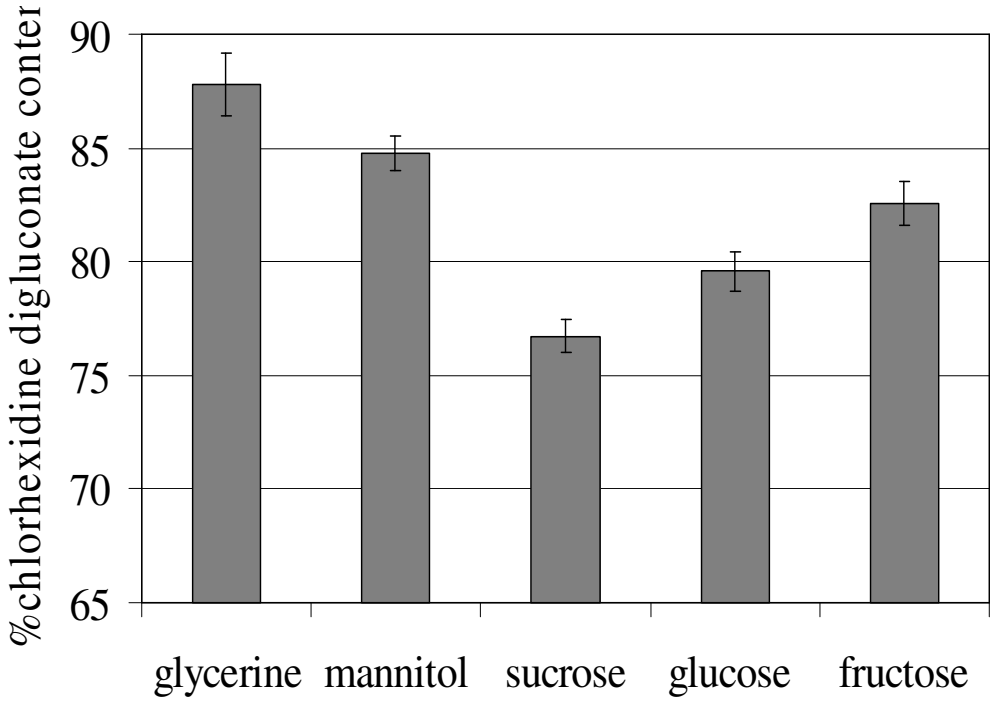


Figure 9. The content of chlorhexidine digluconate on bacterial cellulose films from different carbon sources (surface area 1 cm^2) (mean ± SD, n = 3)

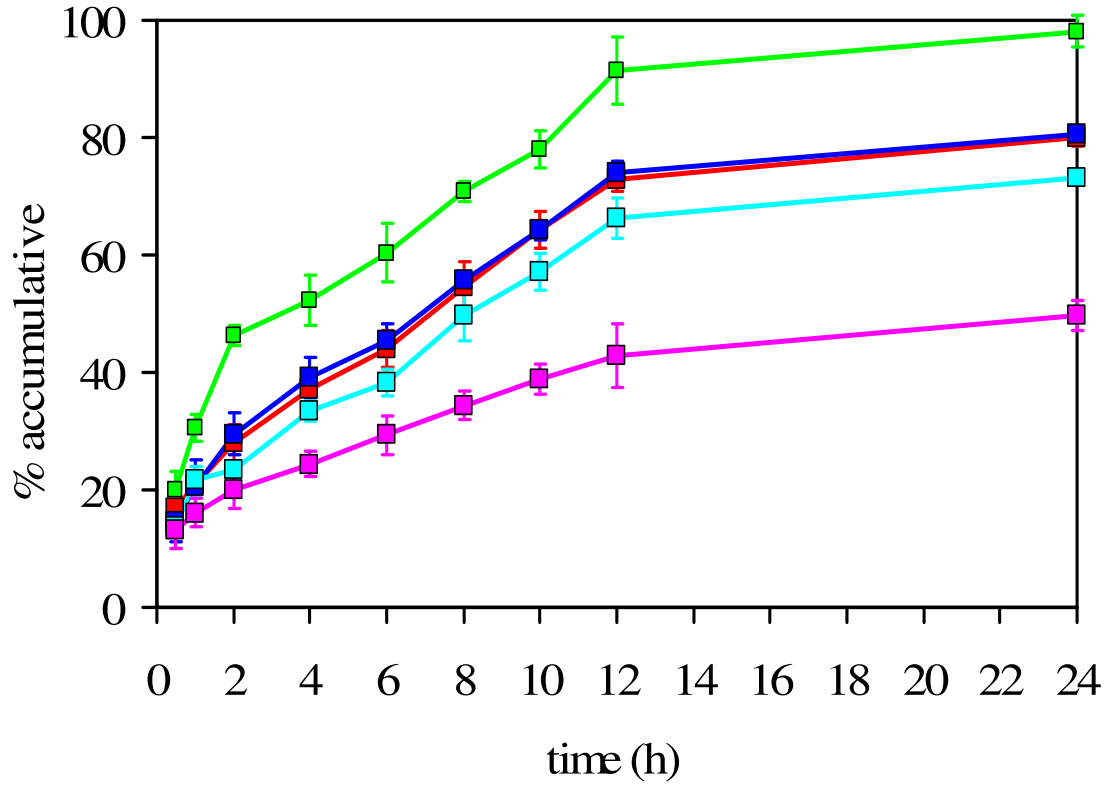
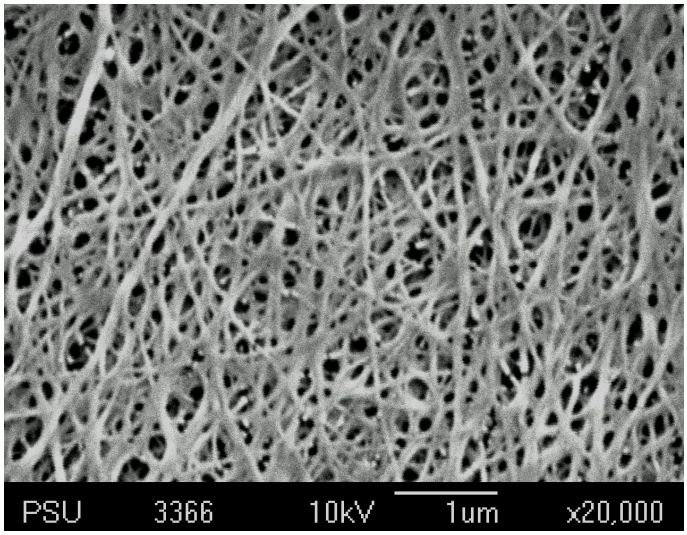


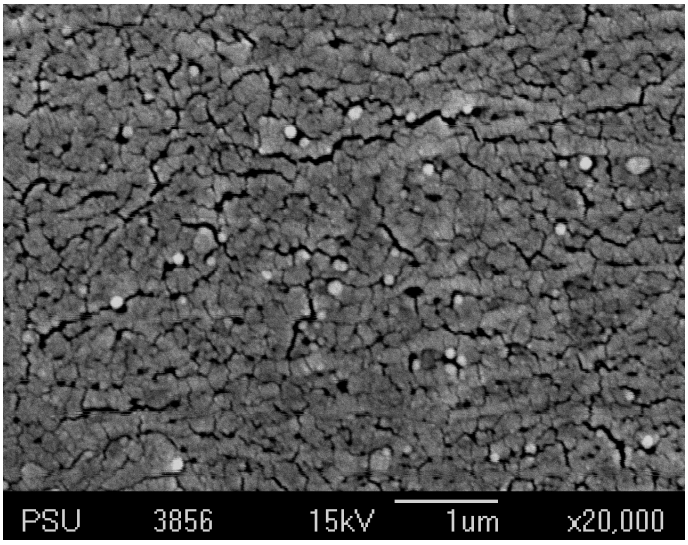
Figure 10. Percent accumulative of chlorhexidine digluconate released from bacterial cellulose films produced from different carbon sources: (■) glycerine; (■) mannitol; (■) sucrose; (■) glucose and (■) fructose, respectively. (mean ± SD, n = 6)

4. Scanning electron microscope of bacterial cellulose film containing chlorhexidine digluconate

Figure 11a showed SEM micrograph of as-prepared BC film with network structure and interconnected pores. When BC film was added with chlorhexidine digluconate, the entire BC surface appeared to be covered with chlorhexidine digluconate as shown in Figure 10b. The deposits were uniformity distributed throughout the BC fabric and interconnected pores were filled with drug.



(A)



(B)

Figure 11. The scanning electron micrograph of (A) BC film from glycerine; (B) BC film from glycerine containing chlorhexidine digluconate