African Journal of Biotechnology Vol. 5 (17), pp. 1519-1523, 4 September 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

Full Length Research Paper

Bacterial Cellulose Production from Beet Molasses

Sherif M. A. S. Keshk[1]*, Taha M. A. Razek[1] and Kazuhiko Sameshima[2]

[1]Basic Environmental Sciences Department, Institute of Environmental Studies and Research, Ain Shams University, Abbassia 11566, Cairo, Egypt.
[2]Kochi University, Faculty of Agriculture, Department of Forest Science, Nankoku, Kochi 783-8502, Japan.

Accepted 17 July, 2006

Bacterial friendly cellulose is produced from beet molasses using *Gluconacetobacter xylinus* ATCC 10245. The yield of the bacterial cellulose (BC) produced from beet molasses was higher than that using glucose as a sole carbon source. The structure of BC produced in presence of beet molasses was studied using IR spectroscopy and X-ray diffractometry. IR spectra show the relative absorbance of C-O-C ether linkage (at 1120 cm^{-1}) in BC using glucose has a relatively lower value than that from molasses. This indicates that BC produced from glucose has a relatively higher degree of polymerization. From X-ray pattern, no remarkable differences in crystallinity index of cellulose between the two media were recorded.

Key words: Bacterial cellulose products, beet molasses, *Gluconacetobacter xylinus*, IR spectroscopy, X-ray diffractometry.

INTRODUCTION

Environmentally friendly or green products are those that use less environmental resources, emit less pollutant to the different environmental media, use a substitute for genuine resources, utilize waste in the production of materials (resource recovery), and save energy used for industrial processes (Miller, 1992; Chiras, 1994; Kirkwood and Longley, 1995; Williams, 1998). Cellulose for industrial purposes is usually obtained from wood plant resources using different pulping processes. These pulping processes discharge different pollutants to the different environmental media (air, water and soil). These pollutants are polychlorinated dioxins, furans, and persistent organics (a result of bleaching processes using chlorine) and are accompanied by air emissions (sulfur oxides, nitrogen oxides, and carbon monoxide) with the generated solid waste (boiler fly ash, and bottom) (Freeman, 1995). On the other hand, resource recovery is a new trend to achieve sustainable development. Wastes are considered secondary material resources and at the same time are renewable material resources. The production of cellulose using bacteria (BC) has been achieved. *Gluconacetobacter xylinus*, one of the best bacterial species for large-scale cellulose production, accepts a wide variety of substrates. BC is chemically pure, free of lignin and hemicellulose (there is no need for chlorine chemical bleaching) and has high polymer crystallinity and high degree of polymerization that distinguishes it from other forms of cellulose (Yamanaka et al., 1989; Yoshinaga et al. 1997). There are many commercial applications for BC such as “Nata” a food product of the Philippines, audio headphone diaphragm and artificial skin for scalded or wound healing (Keshk 2002). In general, glucose has been used as a carbon source for cellulose production from *G. xylinus*. It has been reported, that cellulose was also synthesized from other carbon sources such as 5- or 6-carbon monosaccharides (Hestrin et al., 1954; Masaoka et al., 1993; Oikawa et al., 1995). Gas chromatographic analysis showed that, the polymer composition (cellulose and other by-product) produced from xylose as a carbon source was 80% glucose and 20% other sugars. Also, there were no big differences in the degree of polymerization (Romano et al., 1989). However, time initiation of BC production was different from different carbon sources. The production of BC from sucrose as a carbon source results in only half than those from glucose, which can be attributed to a low activity of sucr-

*Corresponding author. E-mail: sherifkeshk@yahoo.com

ase in *G. xylinus*. Tajima et al. (1995) have succeeded in enhancing BC production from sucrose by the co-cultivation of two different types of *G. xylinus* (ATCC 10245 and NCI 1005).

BC is relatively expensive to produce, and as such is unlikely to replace traditional sources of cellulose. One way to reduce the cost of producing BC is to use cheap and readily available substrates. In Egypt, beet molasses comprises about 5.5% of the beet weight used for sugar production. It is estimated that three beet sugar factories produce 250,000 tons of beet sugar and about 15,000 tons of beet molasses as by-product. This amount of beet molasses was partially used in the production of fodder concentrates, while the rest is exported.

The objective of this work was to produce low cost environmentally friendly cellulose utilizing beet molasses as a sole cheap carbon source in Scharmm-Hestrin medium.

MATERIAL AND METHODS

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* (10245). IR spectra were recorded on Horiba FT-210 infrared spectrometer.

Determination of reducing sugars

Molass hydrolysate was analyzed for its reducing sugar content. The dinitrosalicylic acid method was used (Miller, 1959).

Total sugars

Total sugars for raw molass and molass hydrolysate were determined according Dubois et al. (1956).

Organic nitrogen

Organic nitrogen determination was carried out for raw molass and molass hydrolysate according Csuros (1997).

Bacterial strain and culture conditions

All cultures were incubated statically at 28°C, in liquid or solid medium; the medium compositions are shown in Table 2. Wilds type *G. xylinus* ATCC 10245 was activated for pellicle production in Schramm-Hestrin (SH) medium (Schramm and Hestrin, 1954).

Pellicles production and purification

A 0.5 ml aliquot of the growing cultures (in SH glucose or the molass hydrolysate medium) was inoculated into 15 ml of SH glucose or a mixture medium (Table 1) and incubated statically at 28°C. The pellicles produced at the surface of the mediums were harvested after 5-7 of incubation days, and immersed into 2% aqueous solution of sodium dodecyl sulfate (SDS) and washed under ultrasonication in 1% (w/v) aqueous NaOH followed by neutralization by the addition of 1% (v/v) AcOH. Finally, the pellicles were rinsed extensively with distilled water.

Table 1 .Chemical Characterization of Beet Molasses

Parameters	Percentage (%)
Prix (Total dissolved solids)	80-81
Purity	57-58
Protein	11.0 –12.0
Organic Nitrogen	8.0 - 8.5
Reducing Sugar	0.1
Total Sugars	57.0

FT-IR spectroscopy

The thin samples of BC were prepared according to Keshk and Kai (1999). FT-IR spectra were recorded on Horiba FT-210 infrared spectrometer. The reproducibility of the spectra was verified on three-sample preparation; from 64 to 100 scans were taken with a resolution of 0.5 cm^{-1}. The crystallinity index of different samples was measured according to the absorption ratio (A_{1430}/A_{900}) (Nada et al., 2000). The relative absorbance of the subscript wave number to the absorbance of the wave number at 1328 cm^{-1} which corresponding to the CH rocking of the ring, and the mean strength of the H-bonds (MBHS) (it was calculated as the ratio of A_{OH}/A_{CH}, where A is the absorbance of the stretching vibration of subscript groups) were calculated (El-saied et al., 1990). Moreover, the asymmetry index (defined as the ratio of the band width at 4000-3000 cm^{-1} region) on the low and high frequency side of the maximum at half band absorbency was calculated (El-saied et al. 1990).

Wide angle X-ray diffractometry

The thick samples of BC were prepared according to Kai and Keshk (1999). The diffractogram of the samples was recorded at room temperature with RIGAKU PRINT 2200V series using Ni-filtered CuK_α radiation (λ =1.54Å). 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} was the maximum intensity of the lattice diffraction and I_{am} was the intensity at 2θ =18°.

Viscosity measurement

The viscosity of different dried and finely divided BC samples from both media was measured as centipoises (cP), using cupriethylendiamine (CED) as a solvent and a capillary viscometer (TAPPI Test Method T 230 om-89). From viscosity values, the average degree of polymerization (DP) of the BC samples was calculated from the following equations:

$DP^{0.905}$ = 0.75[954 log (X) – 325], where X = TAPPI viscosity in cP.

RESULTS AND DISCUSSION

Beet molasses contains about 50% sugar comprising an important, cheap and renewable carbon source. As

Table 2. Composition of Medium Components

Components of Medium	Concentration (w/v %)					
Medium	**SH Glucose Medium**	**SH Mixture**				
Glucose	2.00	1.8	1.4	1.0	0.6	0.0
Molasses	0.00	0.2	0.6	1.0	1.4	1.8
Bacto Peptone	2.0	-	-	-	-	-
Yeast Extract	0.50	-	-	-	-	-
Disodium Hydrogen	0.50	-	-	-	-	-
Phosphate	0.27	-	-	-	-	-
Citric Acid	0.12	-	-	-	-	-

The initial pH value of all media is 6.0. SH= Schramm-Hestrin Medium.

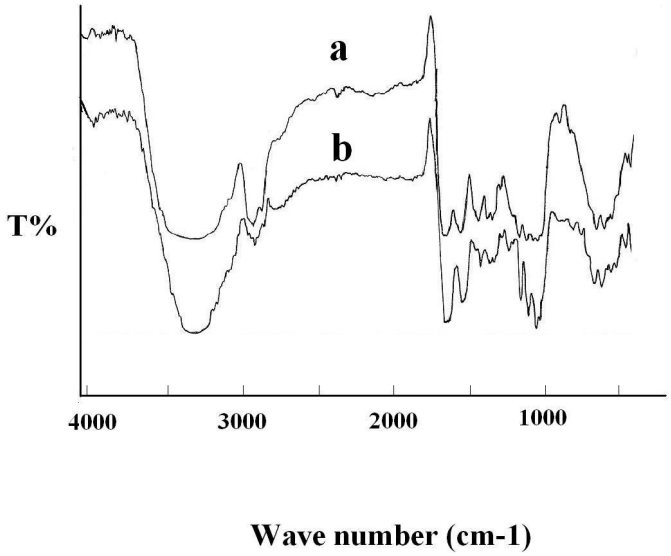

Figure 1. IR spectra of cellulose produced by *Gluconacetobacter xylinum* ATCC 10245 from a. glucose medium b. molass medium.

shown in Table 2, it is clear that beet molasses is rich in protein (12.8%) and organic nitrogen (2.05%). Also, it contains appreciable amount of sulfur results from the color reduction using sulfites or sulfur dioxide. As it is noted, nitrogen and sulfur constitutes are major requirements as bacterial nutrients, this results in higher growth rate which is shown in the pellicle yield recorded for the recipe formulated with higher concentration of molasses. So, beet molasses was chosen as a sole carbon source for further study to optimize the culture conditions of growing *G. xylinus* in the SH medium. Table 3 shows the yield of a pellicle from *G .xylinus* ATCC 10245 per 250 ml of medium. The yield of pellicles produced from glucose and beet molasses based media was increased by the reduction of glucose content from 100 to 0%. These result matches with the observation that the addition of a commercial sulfite pulping waste fraction into culture medium remarkably enhanced the efficiency for the pellicle production (Premjet et al., 1995). So in case of beet molasses, the sulfur and nitrogen content play an important factor to increase the yield of BC. Moreover, the physical properties of the BC produced from both wild type and molasses were remarkably unchanged as deduced from FT-IR spectra (Figure 1). Table 4 shows the maximum absorption band of stretching vibration of OH groups (A_{OH}), MHBS, asymmetry index and C.I of BC and BC from molasses. From Table 4, the asymmetry index reveals that the hydroxyl groups of the BC are not free but entering into different modes of hydrogen bonds rather than BC from molasses.

Also, MHBS in case of BC is relatively stronger than that from molasses; this was confirmed by increasing in crystallinity index (Table 4). Whereas, the relative absor-

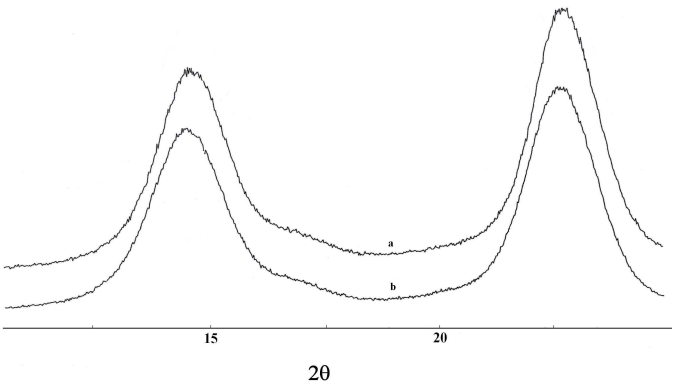

Figure 2. X-ray pattern of cellulose produced by *Gluconacetobacter xylinum* ATCC 10245 from a. glucose medium b. molass medium.

Table 3. Bacterial cellulose Productivity and Degree of polymerization of Gluconacetobacter xylinus ATCC 10245 in the Medium of HS and HS with Molasses.

Glucose/g	Molass/g	Pellicle weight	DP
2.0	0.0	1.34	2695
1.8	0.2	1.40	2593
1.4	0.6	1.44	2578
1.0	1.0	1.50	2510
0.6	1.4	1.56	2490
0.2	1.8	1.60	2425
0.0	2	1.75	2398

Table 4. Infrared Spectroscopy Properties of BC Produced From Different Sources.

M. O. %	C. I.	MBHS	Asymmetry index	A ether linkage/ACH_2	A1°OH/ACH_2
0	5.0	6.60	1.00	3.00	0.6
10	4.8	6.40	0.92	2.70	0.8
30	4.6	6.30	0.84	2.50	1.0
50	4.3	6.10	0.63	2.30	1.3
60	4.0	5.80	0.41	2.20	1.4
80	3.9	5.50	0.35	1.90	1.5
100	3.8	5.20	0.30	1.80	1.7

bance of ether linkage in BC from glucose has a lower value than that from molasses, which means that BC from glucose has higher DP. The relative absorbance of primary OH at 1035 cm^{-1} of BC from molasses has a lower value than that from glucose, which can be attributed to the higher DP (Table 4). These results are confirmed by measuring the DP experimentally as listed in Table 3. The X-ray diffractograms of BC samples in both media revealed a typical cellulose pattern (Figure 2). The usage of molasses instead of glucose has not decreased the crystallinity idex remarkably from 88% in glucose medium to 84% in molasses medium, which confirms FT-IR results. From these results, Beet molasses support substantial production of BC using *Gl. xylinus* ATCC 10245.

ACKNOWLEDGEMENTS

Financial support of this work (Project no. 187/2, 15 December 2002) by General Management of Environmental Projects, Ain Shams University, Cairo, Egypt, is gratefully acknowledged. Professor Dr. Magdy Madkour, Agriculture Department, Institute of Environmental studies and research, Ain Shams University is really appreciated for providing the bacterial strain.

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