PRODUCTION AND CHARACTERIZATION OF ECONOMICAL BACTERIAL CELLULOSE

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The present study investigates the economical production of bacterial cellulose (BC) by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) in 250 ml Erlenmeyer flasks cultivated under static conditions. The fermentation media used contained food industrial by-product liquors, such as black strap molasses solution and corn steep liquor (CSL), which represents some of the most economical carbon and nitrogen sources. However, because of the presence of undesirable components in molasses (such as coloring substances, heavy metals, and other compounds) that may act as inhibitors, and in order to eliminate them, crude molasses has been treated with an acid, as an attempt to increase BC productivity. The amount of BC produced using these carbon and nitrogen sources was determined and compared to that produced using previously reported fermentation media. The characterizations of the bacterial cellulose (BC) pellicles obtained using either conventional or by-product media were studied by thermal and spectral techniques and compared to those of plant-derived cellulose such as cotton linter, viscose pulp, and microcrystalline cellulose.

Keywords: Gluconacetobacter subsp. Xylinus (ATCC 10245)*(BC); Corn steep liquor (CSL); Sugar cane molasses solution; Physiological studies; Plant-derived cellulose; Thermal and I.R analyses.*

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INTRODUCTION

Biopolymers, which have becoming increasingly valuable as they are now used in many applications, are superior to the polymers derived from petrochemicals in being biocompatible, biodegradable, and friendly to the environment. Cellulose is one of the most important biopolymers and is mainly produced by higher plants. However, some bacterial genera such as *Acetobacter*, *Rhizobium*, and *Agrobacterium* can also produce cellulose, hence called bacterial cellulose (BC) (Yamamoto et al. 1989; Fontana et al. 1991; Shirai et al. 1994; Yamada et al. 1997; Jonas and Farah 1998; Klemm et al. 1999, 2001; Iguchi et al. 2000; El-Saied et al. 2004). BC has been found to have a chemical formula similar to that of plant cellulose but with unique physical properties such as high crystallinity, high degree of polymerization, high tensile strength, high purity, and high water absorbing capacity. These improved BC properties are due to the reticulated network of fine fibers, the diameter of which (0.1 μm) is about one hundredth that of plant-derived fibers and therefore, makes the BC far superior to its counterparts from plant origin. Consequently, BC is nowadays used in many special applications such as the

pervaporation of aqueous organic mixtures (Bubey et al. 2002), as a scaffold for tissue engineering of cartilages (Svensson et al. 2005) and blood vessels (Yamanaka et al. 1990, Klemm et al. 1999, 2001), as well as for artificial skin for temporary covering of wounds as mentioned by Krystynowicz et al. (2000).

Moreover, BC is also distinguished over plant-derived cellulose in being ecologically safe, since the latter is usually associated with hemicellulose and lignin, the removal of which was found to cause environmental hazards (Yoshinaga et al. 1997; Watanabe et al. 1998).

Unfortunately, the current price of BC remains too high compared to that of plant cellulose. Therefore, the present work was undertaken in order to optimize the economic production of BC using by-product carbon and nitrogen sources as alternatives to the expensive conventional ones as well as the study of the properties of the produced BC pellicles.

EXPERIMENTAL

Materials and Methods

Plant-derived celluloses

Three types of plant-derived cellulose were selected in this work. Cotton linters were kindly provided from Chemical Industries Co. at Abou-Zaable-Cairo, Egypt, Viscose wood pulp was from Miser Rayon Co., Kaffer El-Dawar, Egypt. Microcrystalline cellulose (Avicel®) was purchased from Merck.

Microorganism and culture media

Gluconacetobacter subsp. Xylinus (ATCC 10245) used in this study was purchased from the American Type Culture Collection (ATCC), Manassas, VA, USA.

Three constitutive media were tested for the preliminary study of BC production. The compositions of these media were as follow (g/l):

- Medium No. 1: Mannitol (M) medium (Oikawa et al., 1995): mannitol, 25.0; yeast extract, 5.0; and peptone, 3.0.
- Medium No. 2: Corn steep liquor (CSL)-glucose medium (Hwang et al. 1999): CSL, 80 mL(total solid content 32.49%); glucose, 20.0g; Na_2HPO_4, 2.7g; and citric acid monohydrate, 1.15g .
- Medium No. 3: Scharmm Hestrin (SH) medium (Ishihara et al., 2002): glucose, 20.0; yeast extract, 5.0; peptone, 5.0; Na_2HPO_4, 2.70 and citric acid, 1.15.

The pH of the above mentioned media was adjusted to 5-6. The sugars and organic acids were autoclaved separately before their addition to the media under aseptic conditions.

The Schramm Hestrin medium was modified by the addition of 10 mL ethanol or by the substitution of its glucose content by 20 mL of fresh coconut milk in media 4 and 5, respectively. The compositions of these media were as follows (g/l):

- Medium No.4: Modified Scharmm Hestrin medium (Krystynowicz et al. 2002): glucose, 20.0; yeast extract, 5.0; peptone, 5.0; Na_2HPO_4, 2.70; citric acid, 1.15 and ethanol, 10 ml.
- Medium No.5: Modified Scharmm Hestrin medium (Budhiono et al., 1999): yeast extract 5.0; peptone 5.0; Na_2HPO_4 2.70; citric acid 1.15 and coconut milk 20.0 ml.

Moreover, in order to economically produce BC pellicles with improved properties, other media were tested. The compositions of these media were as follows:

- Medium No. 6: Modified CSL medium: Mannitol, 25.0; Na_2HPO_4, 2.70; citric acid, 1.15 and CSL, 80.0 ml.
- Medium No. 7: CSL-sugar cane treated molasses medium (Bae and Shoda, 2005): H_2SO_4-heat treated sugar cane molasses, 110.0; Na_2HPO_4, 2.70; citric acid, 1.15 and CSL, 80.0 ml.
- Medium No. 8: Modified mannitol medium: H_2SO_4-heat treated sugar cane molasses, 110.0; yeast extract, 5.0 and peptone, 3.0.

Media No. 1, 3, 4, and 5 were designated as conventional media, since they are composed of carbon and nitrogen sources commonly used in the fermentation media. However, media No. 2, 6, 7, and 8 were designated as by-product media, since they contained food industrial by-product liquors such as CSL and sugar cane molasses. The former is obtained as a by-product of corn wet-milling industry, while the latter is obtained as a by-product after the final crystallization stage in sugar production.

Treatment of molasses

The molasses solution used in this study was kindly supplied by the Sugar and Integrated Industries Corporation, Al-Howamdia, Egypt. It consisted of about 24.91% total solid and 50% of this solid represented total sugar.

Unless otherwise stated, the crude molasses was treated according to the method of Bae and Shoda (2005), in which crude molasses was diluted five-fold (w/v) with distilled water and then centrifuged at 6000 rpm for 20 min to separate solid materials. The supernatant, designated as molasses solution, was adjusted to pH 3.0 using 4N H_2SO_4 and then heated at 120ºC for 20 min. The obtained solution (termed as H_2SO_4-heat treated molasses) was kept overnight at room temperature and then centrifuged.

Determination of total solid content of corn steep liquor

The CSL used in this study was obtained from the Starch and Glucose Company-Torah-Cairo. The total solid content of this CSL was 32.49% (w/v). The latter was calculated according to the following method:

A certain volume of CSL was taken in a weighing bottle and dried at 103-105°C for 12 hours. The obtained oven-dry solid was weighed, then the percent of total solid content was calculated as follows:

$$\% \text{ of total solid content} = \frac{X-Y}{Z} \times 100 \text{ (g \%)} \quad (1)$$

where

X = Sum of weighing bottle and weight of oven dry tested volume of CSL (g)
Y = weight of empty weighing bottle (g)
Z = tested volume of CSL (ml)

Inoculation and cultivation conditions

The fermentation media were divided into 40 mL volume triplicates in 250 mL Erlenmyer flasks, and each flask was inoculated using a 48 hours old slant of *Gluconacetobacter subsp. Xylinus* (ATCC 10245)**.**

The inoculated flasks were incubated at about 30ºC either for 72 or 144 hours under static conditions.

Determination of BC yield

At the end of the fermentation period, the BC gels formed on the surface of the fermentation media were carefully recovered, washed, and immersed overnight in 1N NaOH solution at room temperature in order to remove bacterial cells and media components. The BC gels were then thoroughly washed with distilled water until the pH of the washing water reached normal ranges (Toda et al. 1997). The BC gels were then immersed in a 96% alcohol solution followed by a diethyl ether solution for few hours. Finally, the BC gels were carefully dried by gentle heating (40°C) then weighted in order to compare the BC production ability of the producing organism using different fermentation media.

Characterization of BC

FTIR-spectra measurement

IR-spectra (4000-400 cm^{-1}) were recorded with a Nexus 670 FTIR spectro-photometer (Iclet Co., USA), using a KBr disc. The technique of O'Connor et al. (1958) was used to calculate the crystallinity index (Cr.I). The mean strength of hydrogen bonds (MHBS) and the asymmetry index (asym. I0) were calculated according to Levdik et al. (1967).

The crystallinity index is the ratio of the absorption band at 1430 cm^{-1} to the band at ~ 900 cm^{-1}. The mean strength of the hydrogen bond (MHBS) is calculated as the ratio of B_{OH} / B_{CH}, where B is the integral intensity of the stretching vibration bands of the subscript group, while the asymmetry index is defined as the ratio of the band width on the low and on the high frequency side of the maximum at half band absorbency. The asymmetry index is also a criterion for hydrogen bonds.

Thermal analysis

Thermogravimetric analyses (TG and DTG) of the examined cellulose samples were done using a PERKIN ELIMER Thermogravimetric Analyzer (TGA 7). The analyses were performed with a heating rate of 10^{o}C/min and a flow rate of 50 ml per minute, under non-isothermal conditions and in the presence of nitrogen.

TG-curve analysis

Kinetic studies, based on the weight loss data, were obtained by TG curve analysis. The activation energy has been evaluated by applying Coat and Redfern method of analysis (Coat and Redfern 1964). For pseudo homogeneous kinetics, the irreversible rate of conversion of the weight fraction of reactant was expressed by the following equation,

$$\frac{d\alpha}{dt} = k(1-\alpha)^n \quad (2)$$

where α is the fraction of material decomposed at time t, k is the specific rate constant, and n is the order of reaction. The temperature dependence of k is expressed by the Arrhenius equation,

$$k = A\, e^{-Ea / RT} \quad (3)$$

where A is the frequency factor (s^{-1}) and T is the absolute temperature for a linear heating rate, a, ($deg.min.^{-1}$):

$$a = dT / dt \quad (4)$$

For calculating the activation energy, Ea, of thermal decomposition when n = 1, equation 5 was used.

$$\log\left[-\log\frac{1-\alpha}{T^2}\right] = \log\frac{AR}{aE_a}\left[1-\frac{2RT}{E_a}\right] - \frac{E_a}{2.3RT} \quad (5)$$

When $n \neq 1$, equation 6 was used.

$$\log\left[\frac{1-(1-\alpha)^{1-n}}{T^2(1-n)}\right] = \log\frac{AR}{aE_a}\left[1-\frac{2RT}{E_a}\right] - \frac{E_a}{2.3RT} \quad (6)$$

Plotting the left-hand-side value, i.e. $\{\log[1-(1-\alpha)^{1-n} / T^2(1-n)]\}$ against $1/T$ using various values of "n" should give a straight line with the most appropriate value of "n" (Basta 1999). The least-squares method was applied to the equation, using values of "n" ranging from 0.0 to 3.0 in increments of 0.5. The correlation coefficient (r) and the standard error (SE) were calculated for each value of "n". The "n" value, which corresponds to the maximum r and minimum SE, is the order of the degradation process. The activation energies and frequency factors were calculated from the slope and intercept, respectively, of the Coat-Redfern equation with the most appropriate value of "n".

RESULTS AND DISCUSSION

Effect of Different Fermentation Media on BC Formation

The results obtained and represented in Fig. 1 show that the maximum yield of BC was produced in both media No. 1 and 2, (0.792 and 1.045 g/l respectively) when inoculated by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) and cultivated for 72 hours under static conditions.

On the other hand, lower amounts of BC (0.6425, 0.6075, and 0.54 g/l) were obtained when the same organism was grown on media No. 3, 4, and 5, respectively.

It was also noticed that the final pH of all fermentation media inoculated by the tested microorganism shifted toward the acidic range, reaching about pH 4.0 to 5.0 at the end of the incubation period.

Thus, the corn steep liquor (CSL), which is a cheap by-product, was more effectively used by the microorganism being evaluated than yeast extract and peptone for the production of BC. This could be explained by the fact that CSL is a complex organic nitrogen source rich in proteins, sugars, vitamins, inorganic ions, and myo-insitol phosphates (Hull et al. 1996).

The encouraging data obtained using CSL medium persuaded us to study the effects of various parameters, as will be described.

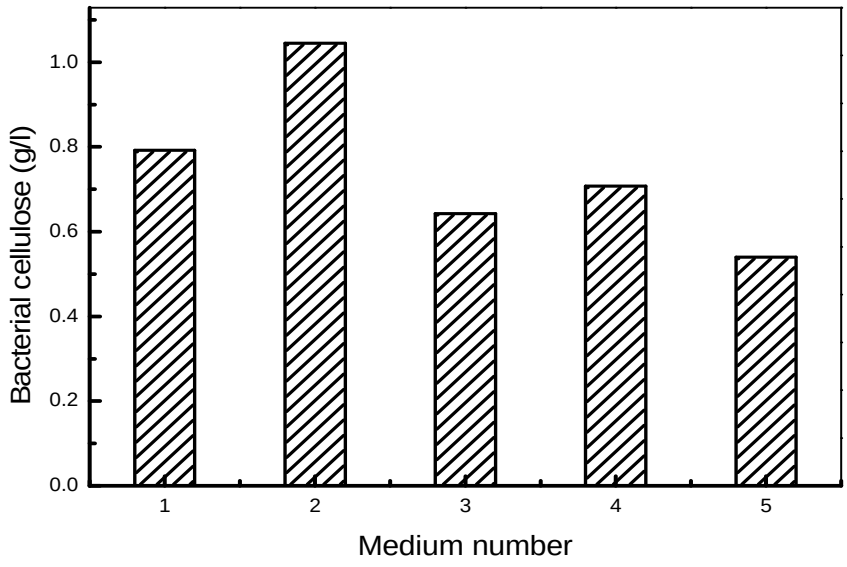


Fig. 1. Effect of different fermentation media on BC production by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) cultivated for 72 hours

Effect of Different Concentrations of Corn Steep Liquor on BC Production

The amount of BC produced by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) was studied when the latter was cultivated for 72 hours on different concentrations of CSL ranging from 4% to 15% (v/v).

The results showed that the BC concentration of 1.16 g/l was obtained upon using 8% (v/v) CSL in CSL medium (Fig. 2). Also, when different concentrations of CSL were

added to the Mannitol medium as a main nitrogen source instead of both the yeast extract and peptone content of the medium, an optimum BC concentration of 0.96 g/l was obtained upon using 8% (v/v) CSL concentration.

Further increase in the concentration of CSL in both media did not result in an obvious increase in the amount of produced BC.

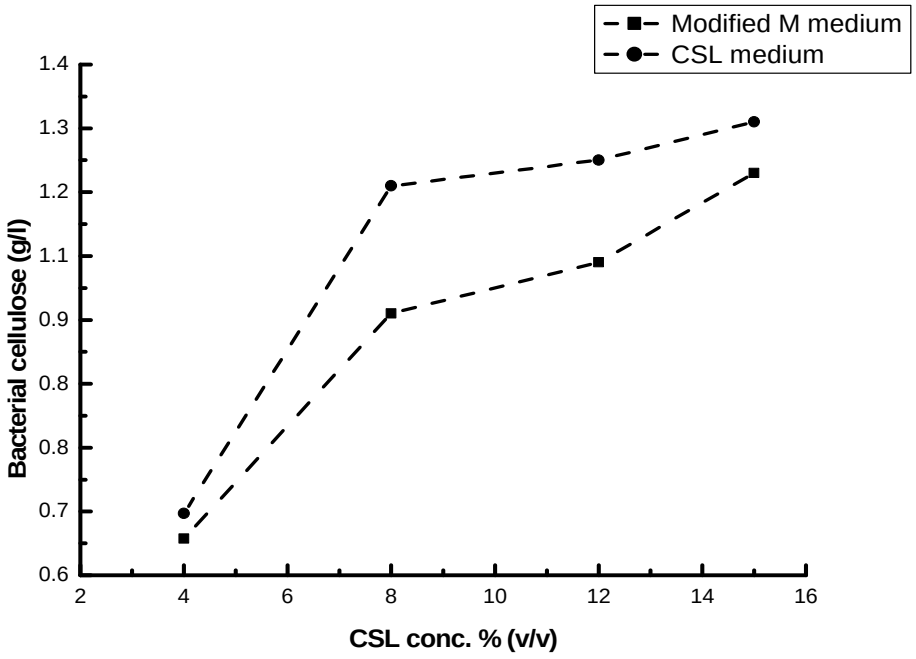


Fig. 2. Effect of different concentrations of CSL in both CSL medium and modified M medium on BC production by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) cultivated for 72 hours

Effect of Different Incubation Periods on BC Production

Results in Fig. 3 show the rapid enhancement of BC production in CSL medium as the incubation period increased up to 144 hours, reaching a maximum BC concentration (C_{max}) of about 3g/l. The BC production rate (Qp) reached 0.026 g/l/h.

Moreover, the glucose consumption curve pattern followed that of BC production very closely, since the amount of residual glucose decreased gradually in the medium and was completely exhausted after 112 hours of the fermentation.

Also, the calculated BC yield coefficient against consumed glucose $Y_{BC/G}$ (gram BC produced/ gram glucose consumed) increased as the incubation time increased and reached a maximum of about 0.2g/g after 144 hours of incubation, which coincided with the C_{max} of BC production.

From these results, we concluded that the optimum yield of BC was reached after 144 hours of cultivation of *Acetobacter xylinum* ATCC 10245 on CSL medium under static conditions.

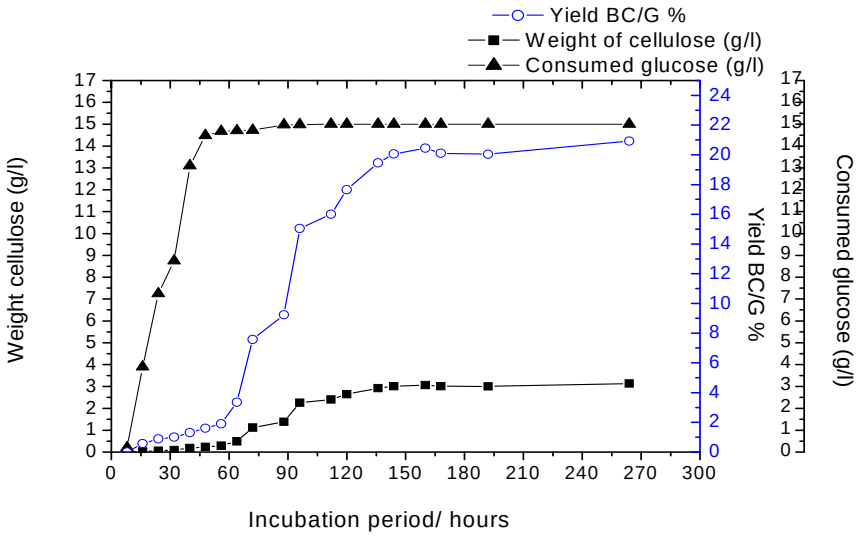


Fig. 3. Effect of different incubation periods on BC production by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) cultivated in CSL medium

Effect of Different Incubation Temperatures on BC Production

Results in Fig. 4 show that the BC production increased as the degree of temperature increased up to 30ºC, where maximum amount of 4.4 g/l was reached after 144 hours of incubation. Further increase in the degree of temperature resulted in an obvious decrease in the amount of BC production. At 37°C, no BC could be detected in the fermentation.

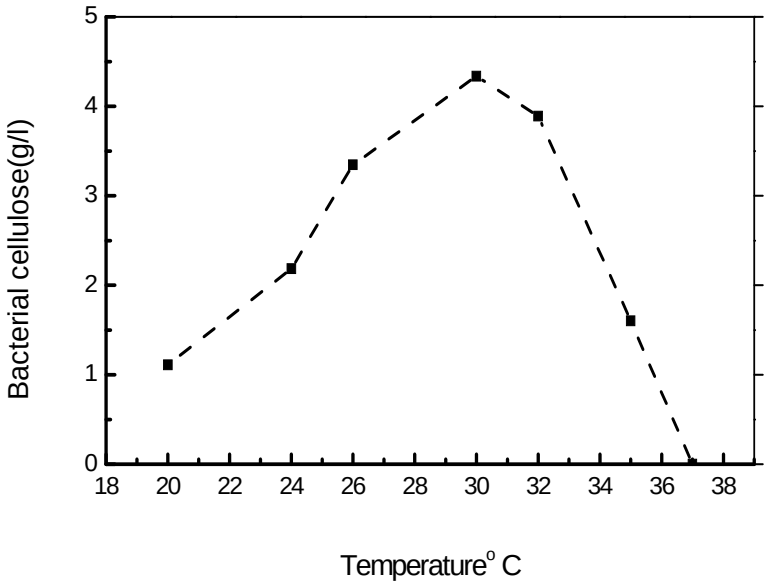


Fig. 4. Effect of different temperatures on BC production by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) cultivated in CSL medium for 144 hours

Effect of Different Concentrations of Molasses Solution on BC Production

This experiment was a trial for the economical production of BC. Therefore, the glucose content of CSL medium as well as the mannitol content of Mannitol medium was substituted by different concentrations of molasses solutions before and after H_2SO_4-heat treatment, since molasses solution is an inexpensive by-product of sugar industries. These media were inoculated and cultivated for 144 hours at 30ºC.

The results illustrated in Fig. 5 (a and b) showed that the BC production increased as the amount of molasses (either treated or untreated) increased in both media until a molasses concentration of 17% (w/v). When untreated molasses solution was used in CSL medium, the BC production increased from 1.425 g/l to 1.97 g/l as the concentration of molasses increased in the medium from 2% to 17% (w/v). On the other hand, when H_2SO_4-heat treated molasses was added to the same medium, the BC production increased from 2.88 g/l to 4.695g/l as the concentration of molasses increased in the same respect (Fig. 5a).

Much lower amounts of BC were obtained when the mannitol content of the medium was substituted by either treated or untreated molasses solutions (Fig. 5b).

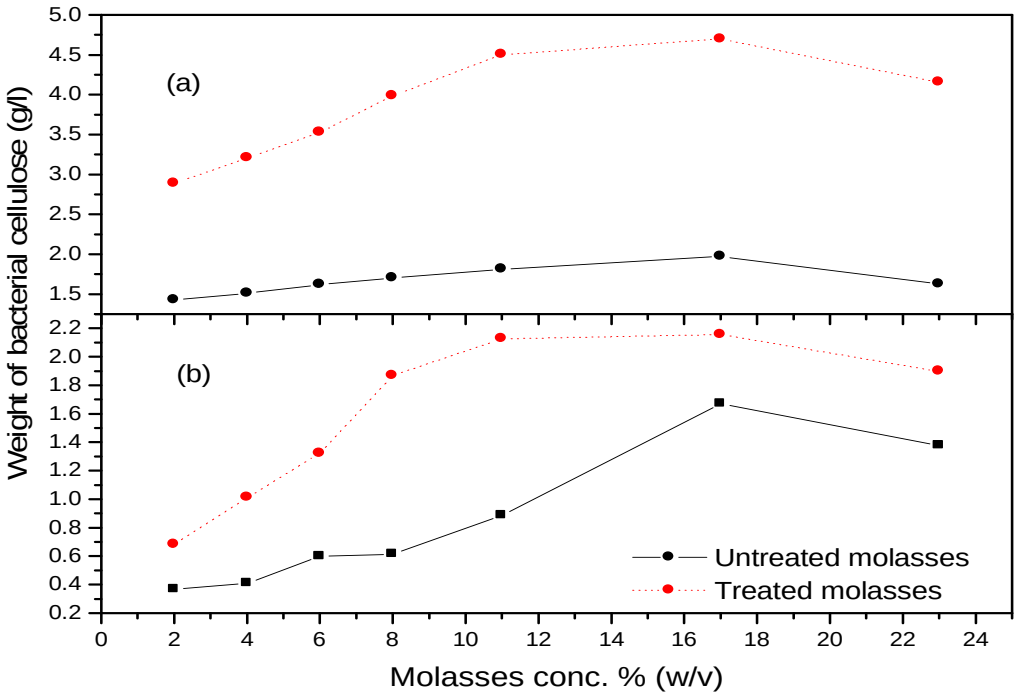


Fig. 5. Effect of different concentrations of molasses solutions (before and after treatment) on BC production by *Gluconacetobacter subsp. Xylinus* (ATCC 10245) cultivated for 144 hours. (a) in CSL medium, and (b) in M medium

It was found that the C_{max} of BC production increased by about 60% upon using H_2SO_4-heat treated molasses instead of untreated molasses in both media. These BC amounts were very close to those obtained by the cultivation of the producing micro-organism in both M (Medium No. 1) and CSL (Medium No. 2) control media (Table 1).

Table1. Maximum BC Concentrations Obtained using Molasses Solutions Before and After Treatment in both CSL and M Media.

Medium	Carbon Source	C_{max}, Maximum BC concentration (g/l)
CSL medium	Glucose	4.467
	Treated molasses	4.695
	Untreated molasses	1.970
M medium	Mannitol	2.400
	Treated molasses	2.115
	Untreated molasses	1.670

The results in Table 1 agree with those of Bae and Shoda (2005), who also obtained an increase in BC production when untreated molasses concentration in the medium was substituted by H_2SO_4-heat treated molasses. The cited authors attributed this result to the fact that the treatment of molasses with dilutes sulfuric results in the hydrolysis of its sucrose content into glucose and fructose in addition to the depolymerization of any present oligosaccharide. These changing in the components of molasses lead to an increase in its nutritional availability to the bacteria. Furthermore, the acid-treatment of molasses causes the removal of many components of the molasses that can be inhibitory to the bacterial growth such as heavy metals, coloring substances, and others.

Comparative Studies of The Properties of BC and Plant-derived Celluloses

In this study the bacterial cellulose samples produced using both conventional and by-product media, as well as some plant derived celluloses (cotton linters, viscose pulp and microcrystalline cellulose), were subjected to thermogravimetric analysis (TGA) technique under non-isothermal conditions. The thermal stabilities of the samples were estimated and compared, taking into account the values of the initial decomposition temperature, maximum weight loss temperature, and the activation energy associated with each degradation stage. The above-mentioned cellulose samples were also examined using FTIR-spectral measurements.

The examined BC pellicles were divided into four groups according to the media in which they were produced, as follows:

- Group 1: BC pellicles produced by the cultivation of the microorganism under test in (medium No. 3) (group 1a) and (medium No.1) (group 1b). These two media were designated as conventional media, as mentioned before.
- Group 2: BC pellicles produced by the cultivation of the microorganism under test in fermentation media containing conventional carbon sources and a by-product nitrogen source: (medium No. 2) (group 2a) and (medium No.6) (group 2b).
- Group 3: BC pellicles produced by the cultivation of the microorganism under test in a fermentation medium containing conventional nitrogen sources and by-product carbon source (medium No. 8).

- Group 4: BC pellicles produced by the cultivation of the microorganism under test in the CSL-heat treated sugar cane molasses medium (medium No. 7). This medium was designated as by-product medium, as mentioned before.

Thermal Stability of the BC Samples Produced Using Conventional Media in Comparison to that of Plant-Derived Celluloses

The non-isothermal TGA and DTGA curves of cellulose samples (either plant-derived or produced by bacteria) are illustrated in Figs. 6-8. Figure 9 represents the plot of correlation coefficient (r), standard error estimation (Se), and activation energy (E_a) as a function of order "n" for the 1st main degradation stage of cotton linter and BC produced in the Schramm Hestrin medium (conventional medium).

Tables 2 and 3 summarize the temperature range, maximum weight loss temperature, r, Se, n, and E_a of the two main degradation stages of BC and natural-based celluloses. The maximum weight loss temperature was the temperature at which the derivative of the TG curve reached a maximum.

The thermograms of the BC, cotton linters (CL), viscose pulp (VP), and microcrystalline cellulose (MC) (Figs. 6 and 7) showed that the thermal degradation of the different types of cellulose took place through three stages. The first stage was due to the evolution of adsorbed moisture and was followed by two main degradation stages (2nd and 3rd stages). The second stage (1st main degradation stage) was due to the decomposition of cellulose, including dehydration, rearrangement, formation of carboxyl and carbonyl groups, evolution of carbon dioxide and carbon monoxide, and formation of carbonaceous char. This stage is called the volatilization stage. The third stage (2nd main degradation stage) was related to the rapid volatilization and oxidation of char, accompanied by the formation of carbonaceous residue. This stage is called the carbonaceous stage.

However, the thermograms of BC samples (Fig. 7) showed that they differed in their thermal degradation property when compared to plant-derived celluloses (CL, VP, and MC; Fig. 6). The weight loss of the 1st main degradation stage of BC samples began at a relatively low temperature. Also, their thermal degradation was rapid and peaked at 284.1°C and 302.8°C in the case of BC produced using Schramm Hestrin and Mannitol media, respectively. On the other hand, cotton linters, viscose pulp, and microcrystalline cellulose showed a slower rate of weight loss and peaked at 342.2°C, 333.3 °C, and 333.02 °C, respectively.

The thermograms of BC samples produced in conventional media (Fig. 7 and Table 2) showed that they differ in their thermal degradation property when compared to plant-derived cellulose (CL, VP & MC; Fig. 6), wherein the weight loss of the 1st main degradation stage of BC samples began at a relatively low temperature. Also, their thermal degradation was rapid and peaked at 284.1°C and 302.8°C in the case of BC produced by pellicles of groups 1a and 1b, respectively. However, CL, VP, and MC showed a slower rate of weight loss and peaked at 342.2°C, 333.3 °C, and 333.02 °C, respectively.

However, the reverse trend was noticed on comparing the carbonization stages, since the temperature determining the beginning of this stage was much higher for the BC pellicles of group 1a compared to that of group 1b or to those of the three natural-based

celluloses tested. Moreover, the E_a of the two main degradation stages of the BC pellicles belonging to group 1a was twice as high as that of the BC pellicles belonging to group 1b or to VP cellulose and was approximately equivalent to that of the CL and MC cellulose.

Based on the values of temperature onset for weight loss, DTG peak temperature, and activation energy of the first main degradation stages, Fig. 7 and Table 2 show that the BC produced using Schramm Hestrin medium had a relatively high thermal resistivity (high onset temp. of the carbonaceous stage & E_a), compared to that of BC produced using Mannitol medium.

Thermal Stability of The BC Samples Produced Using Food Waste Liquors

Figures 7 and 8 and Table 3 show the changes in the shape of the thermograms, onset temperature, as well as the activation energy values of BC samples produced in by-product media.

The thermograms of the BC samples produced in by-product media (Fig. 8 and Table 3) showed that they had more than two main decomposition stages in addition to the stage corresponding to adsorbed moisture. The additional DTGA peak temperatures may be ascribed to the formation of BC with different degrees of decrystallization. In this case the 1st and 2nd degradation stages are regarded as volatilization stages (1st main degradation stage), while the following degradation stage (2nd main degradation stage) was regarded as the carbonaceous stage.

Table 2 represents the characteristics of the group 2 of BC pellicles and showed an increase in the temperature onset as well as the maximum weight loss temperature compared to that of BC pellicles produced using conventional media, i.e., the stability of BC toward thermal energy was improved. But when compared to natural-based cellulose, it was found that the temperature onset and the maximum weight loss of these pellicles was lower to that of the CL but more or less equal to those of both VP and MC.

However, the onset of the temperature determining the carbonization stage of group 2a was much higher than that of any tested BC samples or that of the three plant-derived celluloses under test. Moreover, the E_a of the BC pellicles of group 2a was found to be about twice higher than that of BC pellicles of group 2b or that of VP and was also slightly higher than that of CL and MC.

The results in Table 2 also showed a decrease in the values of the temperature onset as well as those of maximum weight loss of the BC pellicles of group 3 if compared to BC of either group 1 or 2 or to the different types of natural-based cellulose tested. But still the temperature at the onset of the carbonization stage was higher than that obtained upon testing the BC pellicles of group 1 or 2b or that of cellulose of natural origin.

Furthermore, the study the TGA and DTGA curves obtained by the thermal degradation of the BC pellicles belonging to group 4 showed that although the degradation temperature was lower than that of the tested plant-derived cellulose, the maximum weight loss temperature was much higher even than that of BC belonging to group 1, 2, or 3. Moreover, the results showed that the E_a of group 4 was close to 2-fold higher than that of CL, MC, or BC of groups 1a and 2a, 2.5-fold higher than BC of group 3 and about 3-fold higher than that of VP or BC of group 1b or 2b.

The results of thermal analysis proved that the BC pellicles obtained upon using medium No. 7 had the highest thermal resistivity of all the tested cellulose samples,

especially the cotton linters, which were taken as a standard reference for evaluating the cellulose properties. The latter showed an E_a equal to about half that of the BC produced using medium No. 7.

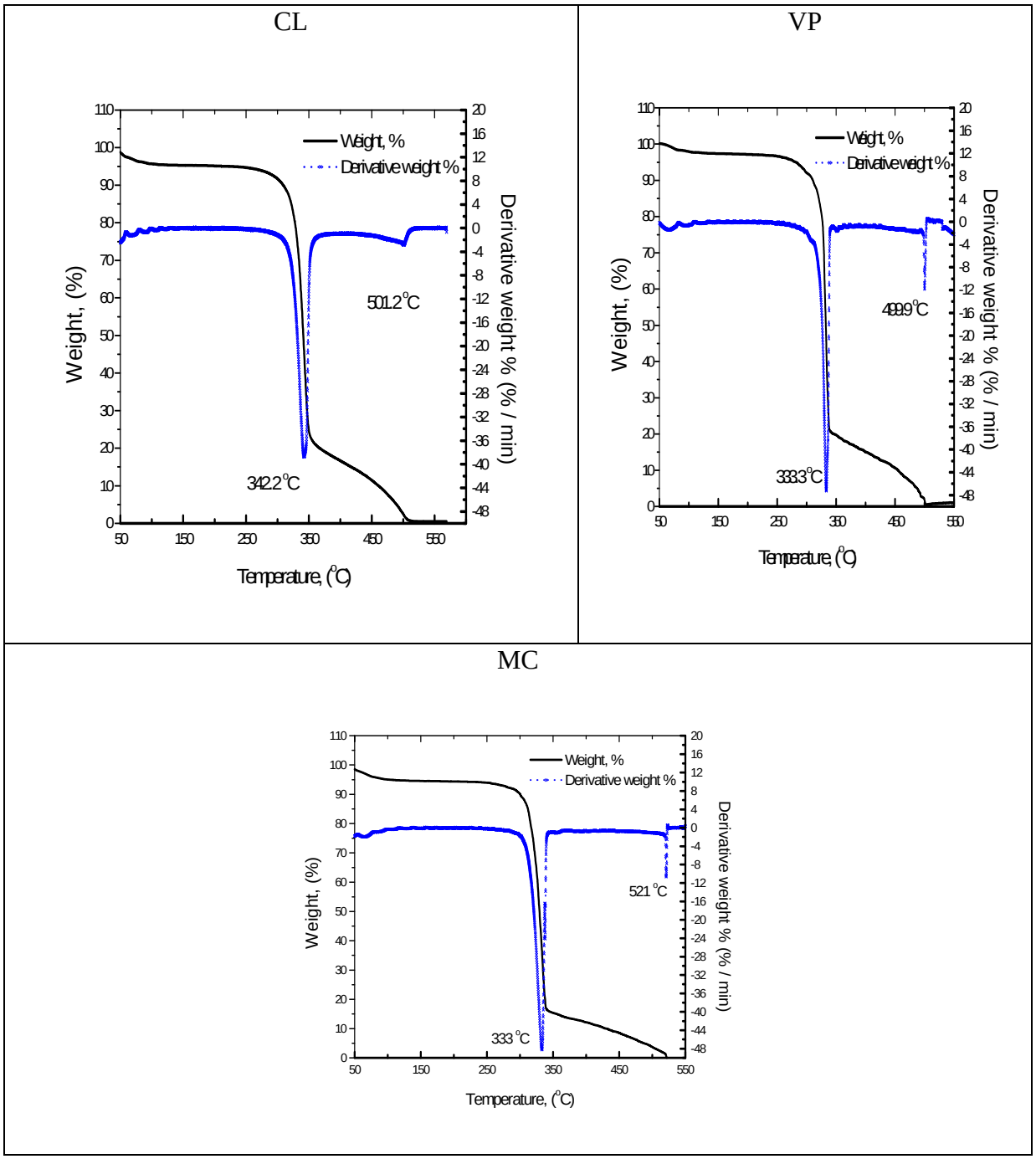


Fig. 6. TGA and DTGA curves of plant derived celluloses

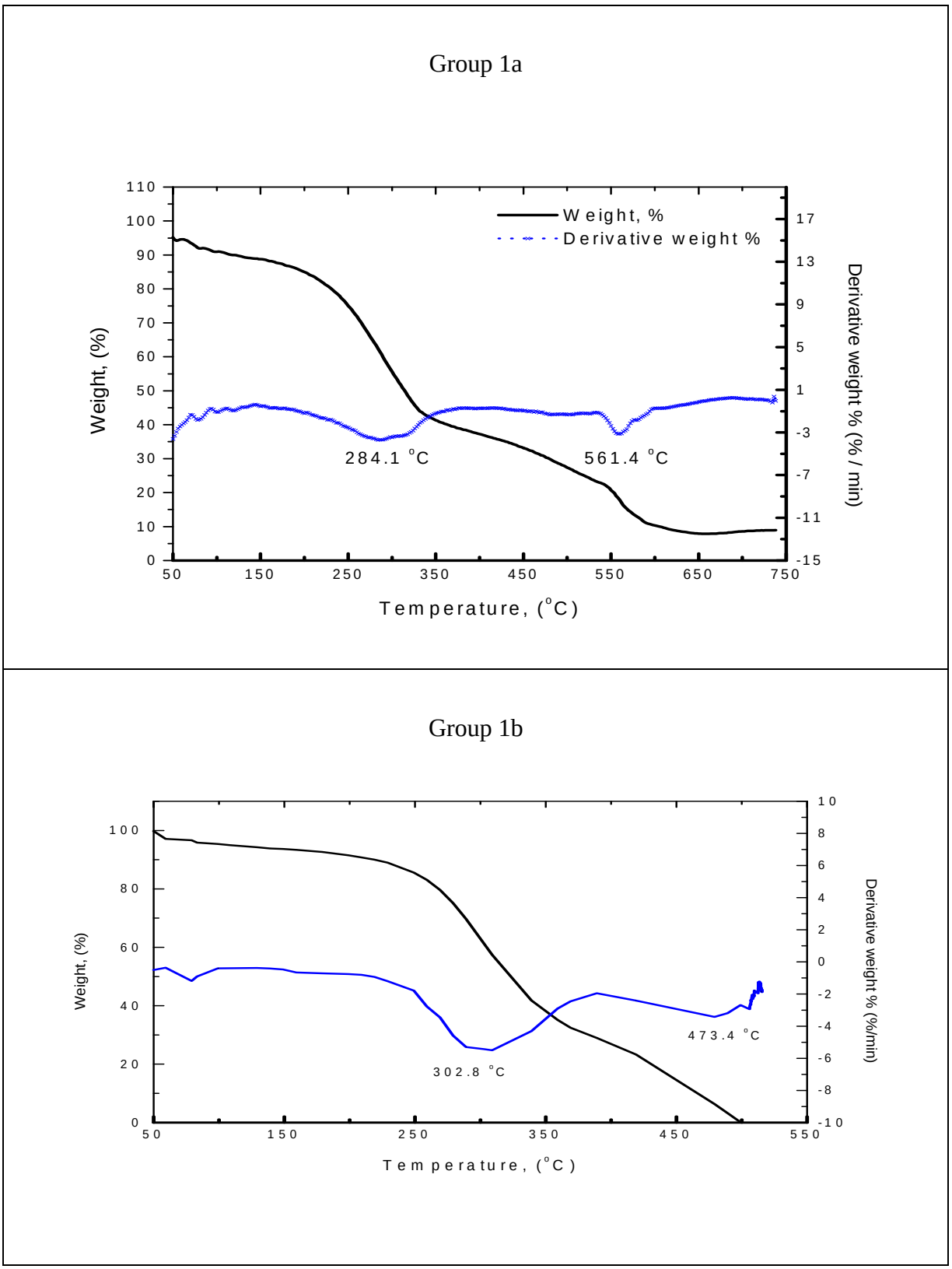


Fig. 7. TGA and DTG curves of BC produced using conventional media

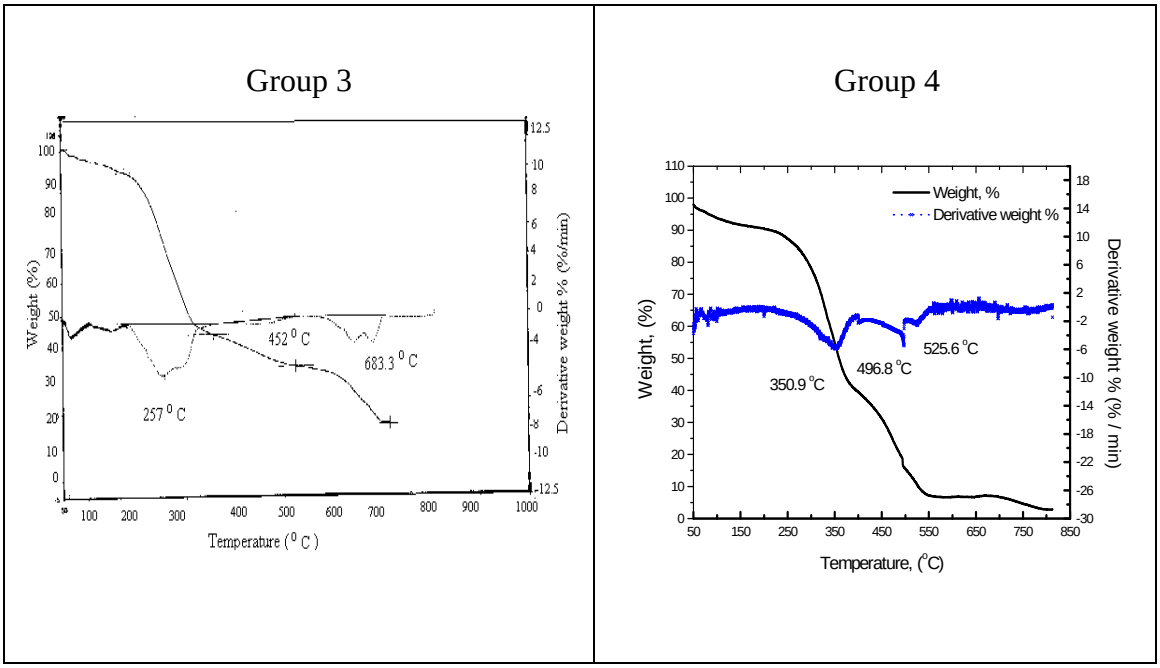


Fig. 8. TGA and DTG curves of BC produce using by-product media

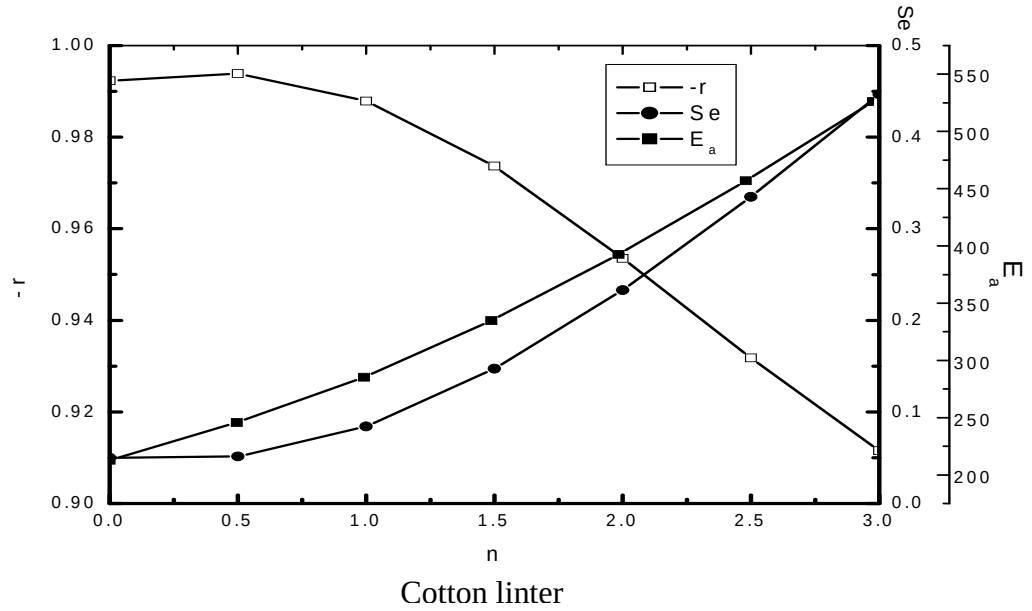
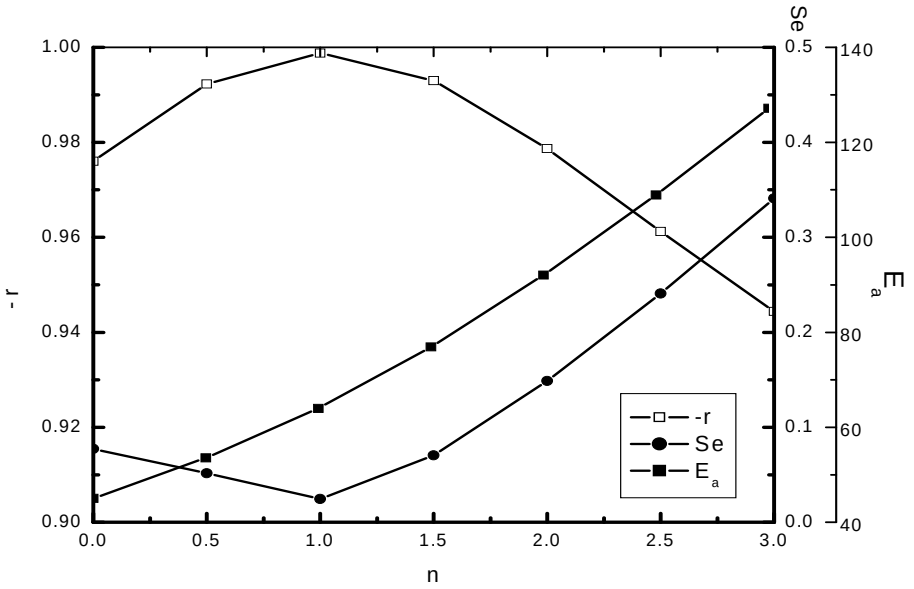


Fig. 9A. Variation of –r, SE, and E_a as a function of order of degradation (n) of the 1st main degradation stage of cotton linter produced using group 1a



Bacterial cellulose

Fig. 9B. Variation of –r, SE, and E_a as a function of order of degradation (n) of the 1st main degradation stage of BC produced using group 1a

IR Spectral Measurements

In addition to the previously mentioned thermal analysis technique, the IR-spectroscopy technique was used to compare the structure of BC with that of plant-derived celluloses, as well as to follow the change in the structure of the produced BC as a result of replacing the conventional carbon and nitrogen sources of the cultivation media by food industrial wastes. This was done by determining the IR measurements, such as the means strength of hydrogen bond (MHBS), the degree of crystallinity (Cr.I), and the band asymmetry (Asym. I). The obtained results are listed in Table 3. Also, the results illustrated in Fig. 10 showed that there was a correlation between IR-measurements and thermal stability of the produced BC.

A comparison of the data obtained from the analysis of the BC samples with that of plant-derived celluloses samples revealed some changes in their structures. The results showed that when the BC pellicles produced using conventional media were studied, they had a lower MHBS and Cr.I. than cotton linters but more or less equal to those of viscose pulp and microcrystalline cellulose. On the other hand, the asymmetry of the bands, corresponding to hydroxyl groups in the region 3000-4000 cm^{-1}, for spectra of these BC was higher than plant-derived celluloses. According to Levdik et al. (1967), this change of asymmetry band indicates that the number of hydroxyl groups entering the strong hydrogen bonds was lower in the BC samples than in plant-derived cellulose, especially cotton linters.

Table 2. Thermal Degradation Measurements of BC Samples Produced Using Conventional and By-Product Media in Comparison to those of Plant-Derived Cellulose

Cellulose origin	Cultivation medium		Main deg. stage	Temp. range (°C)	Maximum wt. loss temp. (°C)	r	SE	"n"	Ea kJ/mole
	Carbon source	Nitrogen source							
CL	-	-	1st	301.1-370.7	342.2	0.9939	0.0513	0.5	245.71
			2nd	447.8-515.2	501.2	0.9946	0.0667	1.5	363.55
									ΣE_a =609.26
VP	-	-	1st	272.1-366.1	333.3	0.9774	0.0859	0.0	238.4
			2nd	371.7-509.6	499.9	0.9847	0.0754	0.5	95.86
									ΣE_a =334.21
MC	-	-	1st	273.8-354.1	333.0	0.9907	0.0874	1.5	245.71
			2nd	447.8-522.4	521.0	0.9795	0.1072	1.0	363.55
									ΣE_a =609.26
BC group 1a	Glucose	Yeast + Peptone	1st	186.3-348.6	284.1	0.9988	0.0246	1.0	63.95
			2nd	536.9-597.4	561.4	0.9960	0.0689	2.0	541.40
									ΣE_a =605.35
BC group 1b	Mannitol	Yeast + Peptone	1st	196.1-399.1	302.8	0.9994	0.0202	1.5	79.71
			2nd	399.1-599.2	473.4	0.9927	0.9759	1.5	212.38
									ΣE_a =292.09
Group 2a	Glucose	Corn steep liquor	1st	201.5- 340	301.8	0.9957	0.0346	0.0	64.867
				449.1- 580.2	538.3	0.9965	0.0525	1.5	196.88
			2nd	580.2- 699.2	632.3	0.9923	0.0940	2.0	349.98
									ΣE_a =611.72
Group 2b	Mannitol	Corn steep liquor	1st	272.9- 415.7	339.3	0.9951	0.0618	1.5	148.09
			2nd	425.0- 586.7	495.1	0.9942	0.0670	1.5	184.57
									ΣE_a =332.59
Group 3	Sugar cane molasses	Peptone + yeast	1st	178.3- 356.7	257.0	0.9972	0.0453	1.5	80.68
				366.6- 525	452.3	0.9919	0.0772	1.5	126.11
			2nd	550.0- 716.7	683.3	0.9992	0.0208	1.0	206.54
									ΣE_a =413.33
Group 4	Sugar cane molasses	Corn steep liquor	1st	249.7- 398.9	350.9	0.9986	0.0267	1.0	96.11
				398.9- 499.8	496.8	0.9897	0.0750	1.0	167.90
			2nd	499.8- 561.2	525.6	0.9895	0.1340	2.5	794.11
									ΣE_a=1058.12

The results also proved that the data of Cr.I of the examined cellulose samples successfully correlated with the values of activation energies of their volatilization stages and that of their total activation energies (ΣE_a). However, there was no conformity between the data of activation energies and that of MHBS. This was probably due to the type and degree of hydrogen bonding formed between the hydroxyl groups upon changing the cultivation media.

On the other hand, the IR-spectral results from the BC pellicles produced using by-product media illustrated that the BC of group 4 had the higher MHBS and Cr.I. if compared to the rest of the produced BC or to the plant-derived cellulose even cotton linters (Fig. 11 and Table 3). According to these results, it was suggested that replacing the conventional carbon and nitrogen sources in the cultivation medium by molasses solutions and corn steep liquor produced BC with a higher degree of crystallization. In contrast, the asymmetry of the bands corresponding to hydroxyl group in the region 4000-3000 cm^{-1} for spectra of BC was lower than plant-derived celluloses. According to Levdik et al. (1967), this change of band asymmetry indicates that the number of hydroxyl groups participating in strong hydrogen bonds was higher in the BC samples than in plant derived celluloses, especially cotton linters.

Table 3: FTIR Measurements ff BC Samples Produced Using Conventional and By-Product Media in Comparison to Those of Plant-Derived Celluloses

Cellulose origin	Cultivation medium		MHBS	Cr. I.	Asym. index
	Carbon source	Nitrogen source			
CL	-	-	3.0280	1.215	1.208
VP	-	-	2.0380	0.911	1.309
MC	-	-	1.6512	0.932	1.380
BC group 1a	Glucose	Yeast +Peptone	1.5140	1.011	1.682
BC group 1b	Mannitol	Yeast +Peptone	2.0080	1.097	1.659
Group 2a	Glucose	Corn steep liquor	2.2100	1.512	1.539
Group 2b	Mannitol	Corn steep liquor	2.6160	1.440	1.526
Group 3	Sugar cane molasses	Peptone + yeast	3.7590	1.506	1.325
Group 4	Sugar cane molasses	Corn steep liquor	3.7800	1.568	1.217

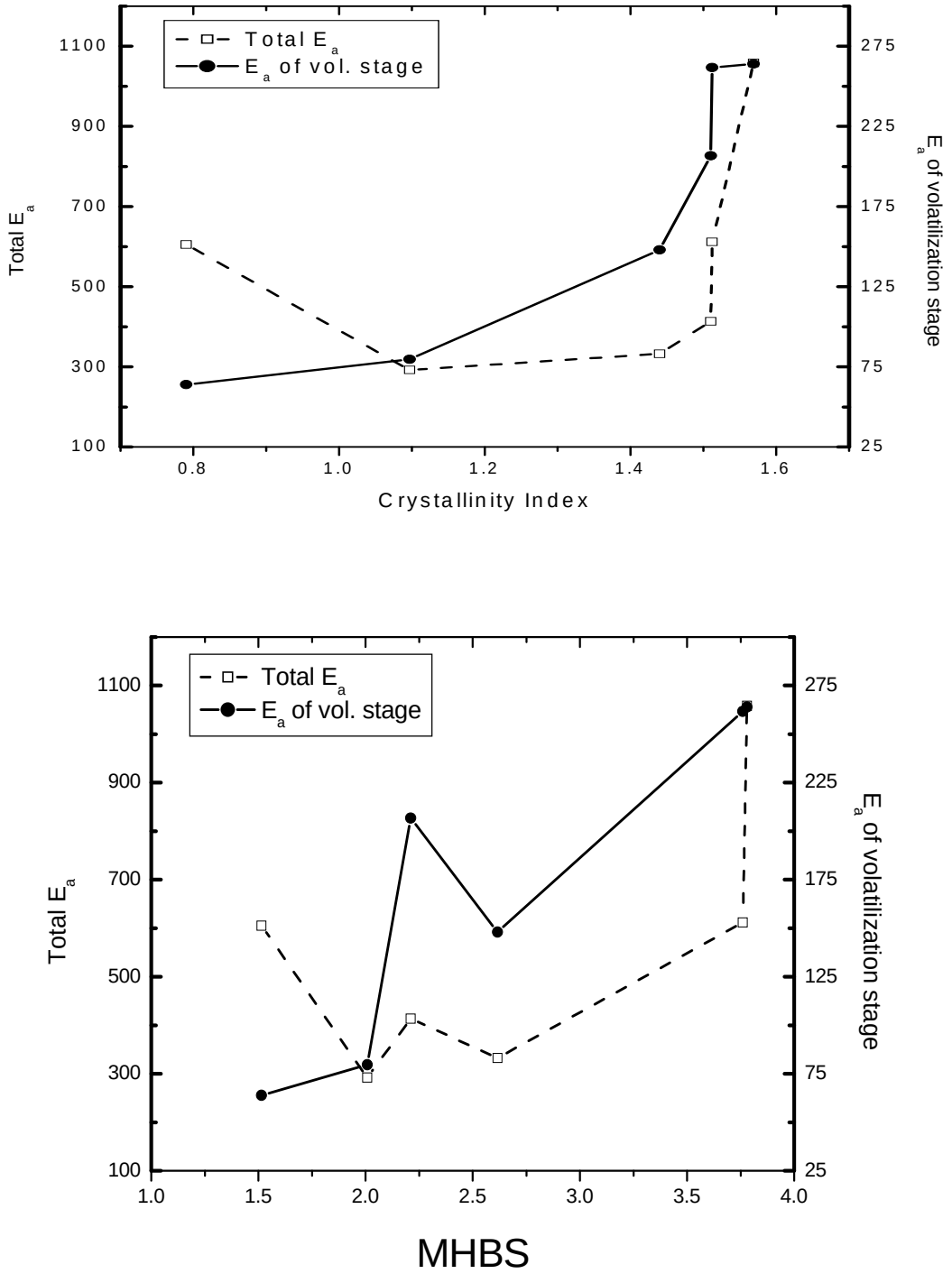


Fig. 10. Correlation between IR-measurements and thermal stabilities of the produced BC

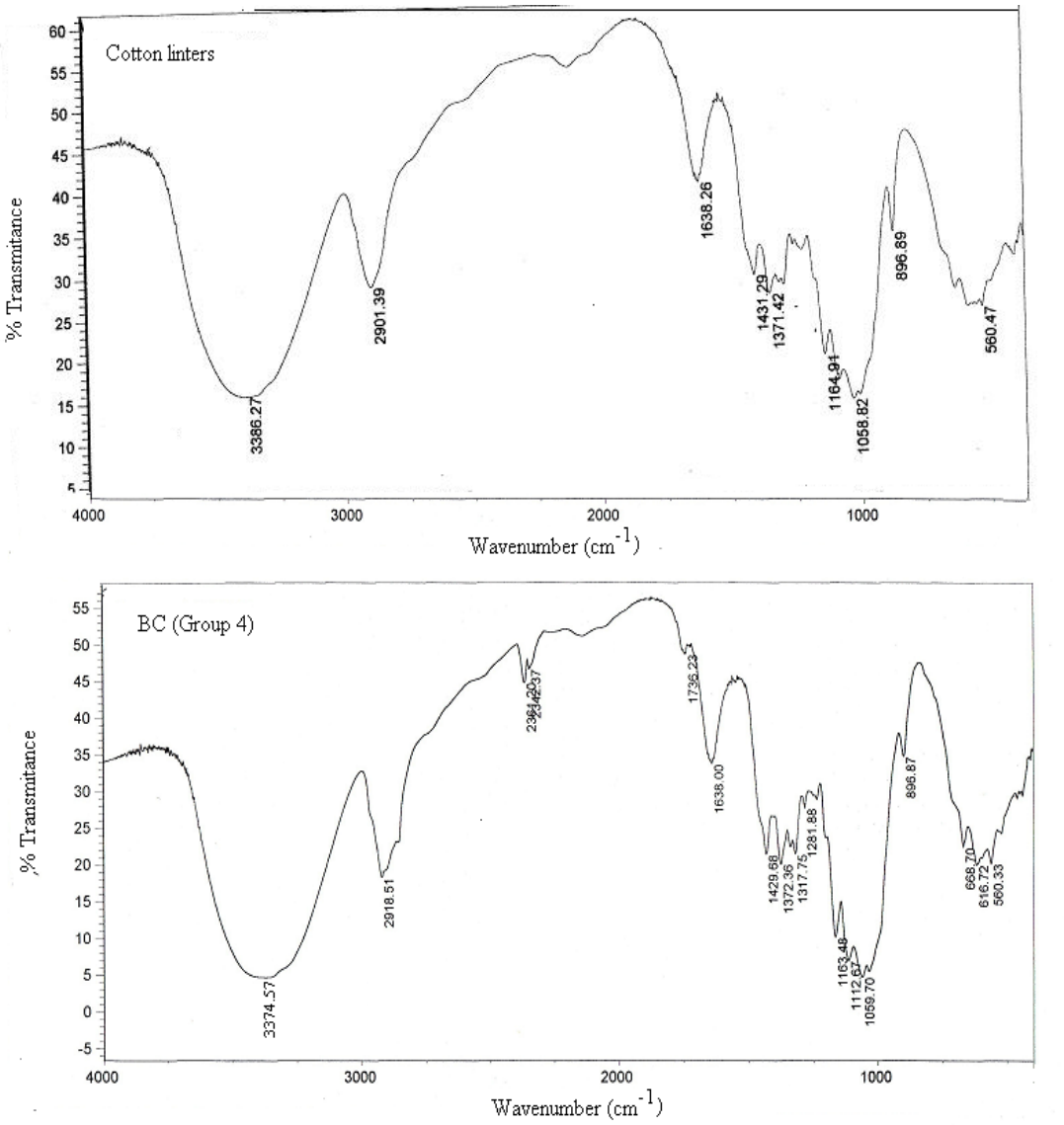


Fig. 11. FTIR-Spectra of cotton linters and BC derived from using treated molasses and CSL medium

CONCLUSION

This study showed that BC, which is a very expensive product having unique physical and chemical properties, can be produced by the static incubation of *Gluconacetobacter subsp. Xylinus* (ATCC 10245) at 30°C for 144 hours in a very cheap medium composed of natural by-products, such as black strap molasses solution and corn steep liquor, as carbon and nitrogen sources, respectively. The produced BC pellicles were characterized by improved thermal properties, as well as higher crystallinity over cotton linters, which allow such celluloses to be use in specific applications (e.g. membranes).

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