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BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media

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Abstract Bacterial cellulose (BC) is used in different fields as a biological material due to its unique properties. Despite there being many BC applications, there still remain many problems associated with bioprocess technology, such as increasing productivity and decreasing production cost. New technologies that use waste from the food industry as raw materials for culture media promote economic advantages because they reduce environmental pollution and stimulate new research for science sustainability. For this reason, BC production requires optimized conditions to increase its application. The main objective of this study was to evaluate BC production by *Gluconacetobacter xylinus* using industry waste, namely, rotten fruits and milk whey, as culture media. Furthermore, the structure of BC produced at different conditions was also determined. The culture media employed in this study were

composed of rotten fruit collected from the disposal of free markets, milk whey from a local industrial disposal, and their combination, and Hestrin and Schramm media was used as standard culture media. Although all culture media studied produced BC, the highest BC yield—60 mg/mL—was achieved with the rotten fruit culture. Thus, the results showed that rotten fruit can be used for BC production. This culture media can be considered as a profitable alternative to generate high-value products. In addition, it combines environmental concern with sustainable processes that can promote also the reduction of production cost.

Keywords Bacterial cellulose · Rotten fruit · *G. xylinus* · Milk whey · Bioprocess

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Introduction

At present, several studies are being conducted worldwide to produce cellulose by using processes that reduce both environmental impact and production cost. Cellulose, which can be produced by several sources, is one of the most abundant macromolecule on Earth (Klemm et al. 2001; Sheykhnazari et al. 2011). Although its production is mostly by vascular plants, an alternative route that not only replaces but also reduces the demand from plants is the production of cellulose from another resource such as a bacterial system (Sheykhnazari et al. 2011; Brown 2003; Castro et al. 2011; Lin et al. 2013).

Bacterial cellulose (BC) is an extracellular polysaccharide secreted mainly by *Gluconacetobacter xylinus*, a rod-shaped, strictly aerobic gram-negative bacterium (Moosavi-Nasab and Yousefi 2011; Li et al. 2012). This microorganism produces a pellicle of BC that has a nanofibrillar structure, with a denser surface on one side and a gelatinous layer on the other side

(Klemm et al. 2001; Kurosumi et al. 2009; Cai and Kim 2010; Nakagaito et al. 2010).

BC does not have lignin and hemicellulose like cellulose from plants, making it a highly pure source of cellulose (Mohite and Patil 2014). BC is also distinguished from its plant equivalent by a high crystallinity index (above 60 %) and different degree of polymerization (DP), usually between 2000 and 6000 (Mohite and Patil 2014). Due to its unique properties, BC has been employed as a new biological material in the food industry, as edible packing material, as wound-dressing materials, artificial skin, vascular grafts, scaffolds for tissue engineering, artificial blood vessels, medical pads and dental implants (SHAH et al. 2013), and industrial products, as sponges to collect leaking oil and materials for absorbing toxins, Optoelectronics materials (liquid crystal displays) (Donini et al. 2010).

The main problems associated with the bioprocess technology are productivity, ease of industrial application, and production cost (Koutinas et al. 2012). Despite all applications, the elevated BC production costs associated with the use of expensive culture media have encouraged studies to optimize the bioprocess aiming to achieve high yields capable of meeting the worldwide demand for cellulose (Donini et al. 2010).

As the substrates, especially the carbon source, can determine the costs of microbial fermentation processes, and particularly for BC production the culture media can be responsible for up to 65 % of the total process cost, several studies have been devoted to find new low-cost carbon sources, as glycerol remaining from biodiesel production and grape bagasse, a residue of wine production, coffee cherry husk extract, and corn steep liquor (Moosavi-Nasab and Yousefi 2011; Vazquez et al. 2013; Castro et al. 2013; Rani et al. 2011; Carreira et al. 2011).

Since acetic acid bacterium such as *G. xylinum* can assimilate several sugars (Verschuren et al. 2000; Ishihara et al. 2002; Bae and Shoda 2004), different sources have been studied by other authors among them: coconut water (Kongruang 2008); pineapple and sugar cane juices (Castro et al. 2011); syrup of a typical fruit of Iran (Moosavi-Nasab and Yousefi 2011); and agroindustry such as grape skins, whey, raw glycerol, and sulfite pulp (Carreira et al. 2011). Recently, Vazquez et al. (2013) modified carbon sources in the Hestrin and Schramm (HS) culture medium by replacing commercial glucose or glycerol with grape bagasse from wine production and remaining glycerol from biodiesel production. Kurosumi et al. (2009) employed different fruit juices such as orange, pineapple, apple, Japanese pear, and grape in the BC production. The present study employed a mix of fruit rotten as an alternative source to reduce the cost of culture media for producing BC.

When fruits cannot be marketed due to poor quality caused by bad weather or other natural disasters, it leads to rotten fruit (Kurosumi et al. 2009). In addition, disposal from dairy

industries has received special attention due to its polluting effect on the environment (Arauz et al. 2009). It is not often recognized that milk can have a significant polluting effect on rivers or streams. It can be as much as 400 times more polluting than untreated domestic sewage. Naturally occurring bacteria break down milk entering a watercourse, consuming the oxygen in the water more quickly than it can be replaced. As a result of decay of oxygen levels, fish and other creatures can suffocate.

Such wastes are rich in sugars, which can be easily assimilated by microorganisms. This makes them suitable for the industrial production of bioproducts by microorganisms (Rosales et al. 2005).

The utilization of the rotten fruits and pomace fruits was evaluated and efficacy system because for fruit processing industries are considerable by-products. They explained that prices for these by-products are lower because they usually have few alternative uses (Kennedy and Krouse 1999). For these reason, applying these materials as culture medium, even treated to reduce fiber and high molecular weight polysaccharides, could be a good alternative for research. The development of culture media to fermentative process is of paramount importance since its composition can impact in the productivity, purification process, and product quality (Kennedy and Krouse 1999).

In the production process, the value of the culture medium represents 30 % of the total cost of production (Rivas et al. 2004). Therefore, development of a process by using culture media from rotten fruits and dairy industry waste would be an important achievement, since it could reduce the cost process and support large-scale development.

Thus, the main objectives of this study were to evaluate BC production by *G. xylinum* by using rotten fruits and milk whey as culture media without supplementation and to verify the effects of this different culture media on BC structure.

Material and methods

Culture media

The BC was produced using the following culture media: Hestrin and Schramm (HS) containing glucose 20 g/L, peptone 5 g/L, yeast extract 5 g/L, sodium phosphate anhydrous 2.7 g/L, and citric acid monohydrate 1.15 g/L; rotten fruits composed of plums, green grapes, pineapples, and apples, which were collected from the disposal of free markets; milk whey from a local industrial disposal; and a mixture of rotten fruits and milk whey. The amount of carbon and nitrogen in each medium was determined by elemental analysis at the Central Analytical of Chemistry Institute, São Paulo University (São Paulo, Brazil). Through the mass of carbon and

nitrogen in each medium, it was possible to calculate the carbon/nitrogen (C/N) rate, according to Eq. 1:

$$C : N = \frac{\text{carbon mass(g)}}{\text{nitrogen mass(g)}} \quad (1)$$

Table 1 presents all the culture media tested and their carbon/nitrogen (C/N) ratio. To obtain the culture media composed of rotten fruits, 250 g of fruits was processed in a blender with 400-mL deionized water. Next, the juice was filtered and centrifuged at 4000g for 20 min. The supernatant was collected and used as culture media. In this culture media, milk whey and HS were sterilized at 121 °C for 30 min and then stored at 4 °C. All media had the pH adjusted to 6.0.

BC production

A preculture of *G. xylinus* ATCC 53582 was prepared using 100 mL of the HS broth (M_0) medium at 30 °C for 48 h under 150-rpm stirring. After this, 10^6 CFU mL⁻¹ was taken from each culture medium. For BC membrane production, 24-well plates were used as the template. Each well was filled with 1 mL of the inoculated culture medium. The plates were kept at 30 °C in a static culture for 0, 24, 48, 72, and 96 h. Analytical methods such as (i) bacterial growth, (ii) cellulose production, (iii) protein and sugar consumption, and (iv) pH were analyzed at each time point.

Analytical methods

The number of viable cells was determined by counting colonies formed on the HS agar surface at 30 °C±0.5 °C at 48 h adapted from methodology (Kouda et al. 1997; Naritomi et al. 1998). The culture medium was centrifuged at 10,000g for 10 min to separate cellulose from the supernatant. The supernatant was collected, and the residual protein levels were determined according to the bicinchoninic acid (BCA) protein assay reagent by using bovine serum albumin as the standard, and sugar levels were determined using a GOD-PAD glucose kit (Laborlab, Brazil), in which the reagent is ready to be used

Table 1 Culture media percentages and carbon/nitrogen ratio (C/N) for bacterial cellulose (BC) production

Culture media	Milk whey (%)	Rotten fruit (%)	HS (%)	C/N
M_0	–	–	80	6.9
M_1	80	–	–	17.7
M_2	–	80	–	57.6
M_3	30	60	–	35.6
M_4	50	40	–	24.2
M_5	30	50	–	27.5

Preculture of *G. xylinus* with 10^6 CFU/mL and initial pH 6.0

and employs enzymatic method for large analytical specificity. The color reaction was measured by UV spectroscopy, using a Molecular Devices Spectramax 384 Plus | UV–Vis Microplate Reader at $\lambda = 505$ nm.

The produced BC was collected, rinsed in distilled water, and immersed in NaOH 1 N at 60 °C for 90 min to remove attached cells. Later, BC was washed in distilled water and dried at 50 °C for 24 h to evaluate the BC yield concentration in mg mL⁻¹ (mass (mg) of BC/volume (mL) of culture medium) (Moosavi-Nasab and Yousefi 2011; Hong et al. 2006).

Kinetic parameters

The BC productivity (P_{BC}) and BC yield coefficient ($Y_{P/S}$), expressed in mg/mL h and g product/g substrate, respectively, were defined as follows:

$$P_{BC} = C_{BC} / t \quad (2)$$

$$Y_{P/S} = (dS/dT) / (dP_{BC}/dt) \quad (3)$$

where C_{BC} is the maximum BC concentration (mg/mL) at time t .

BC production was measured at the end of each period and corresponded to (mg/mL) the dry weight of cellulose per milliliter of culture media, as described by Moosavi-Nasab and Yousefi (2011).

Characterization of BC membranes

All samples were characterized in terms of surface morphology and crystallinity. BC membranes were frozen at –70 °C for 24 h. The samples were freeze-dried (L101 Liotop™) for 3 days. Following, the samples were cross-sectioned, sputter-coated with gold, and analyzed by scanning electron microscopy (SEM).

The X-ray diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions (Adesakin et al. 2011). The patterns of all BC membranes were determined using a PANalytical X'Pert PRO MPD diffractometer with an anode material (copper). The generator settings were (operating current and voltage) 40 mA, 45 kV; scan speed 59.69 s; K-alpha1 [Å] 1,54060; K-alpha2 [Å] 1,54443; step size [°2Th.] 0,0170; scan type was continuous; in a range 0–60 postsynaptic density (PSD) length [°2Th.] 2,12; divergence slit size [°] 0,4785. The diffraction profile was processed by computer-aided fitting analysis and transformed to basic crystallographic features: d-spacings of equatorial lattice planes. The results were attained by computer-

aided fitting analysis. The assays were performed at Instituto de Pesquisas Tecnológicas SP/Brazil (IPT).

Statistical analysis

All the analyses were performed in triplicate and the results expressed as mean values with the error bars calculated representing a confidence limit of 95 % for the measurements. Moreover, the generated data were treated by Tukey test to identify whether changes in the parameters assessed were significant at 95 % significance.

Results

BC production

To verify the effect of culture media on *G. xylinus* growth and BC production, the bacteria preculture was made in a standard culture medium M_0 (HS broth) containing 10^6 CFU mL⁻¹ cells. This procedure was performed to ensure the same number of cells in each well so that BC membranes could undergo identical development.

Figure 1 shows that the experiments performed with culture media containing rotten fruit had a higher BC production. Among these results, after 96 h of bioprocess, the BC production was M_2 , 60.0; M_5 , 52.8; M_4 , 48.1; M_3 , 40.0 mg/mL. The time which promoted the highest BC production varied according the culture media. To the standard media (M_0), the highest production was achieved at 48 h of process while to culture media, M_4 and M_5 96 h promoted the highest production. Regarding the culture media M_2 , the error bars indicate that there was no statistical difference among the BC

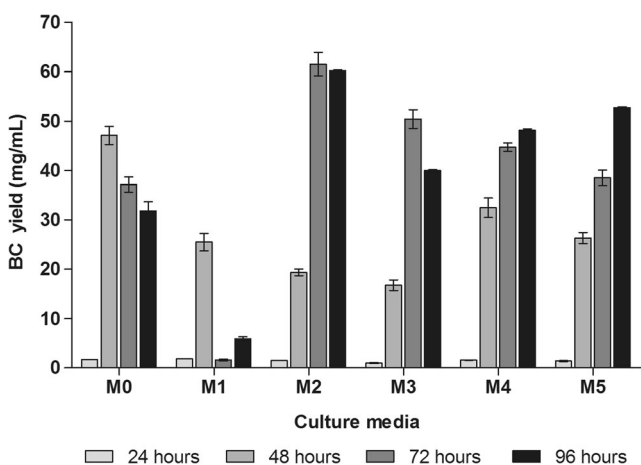


Fig. 1 Bacterial cellulose (BC) yield (mg/mL) in different culture media in static culture for 24, 48, 72, and 96 h. All the experiments were performed in triplicate. The error bars represent a confidence limit of 95 % for the measurements. M_0 HS broth; M_1 milk whey; M_2 rotten fruit; M_3 , M_4 , and M_5 milk whey/rotten fruit

production at 72 and 96 h. The quickest production obtained with culture media M_0 can be a result of the easier assimilation of nutrients present at this media as compared to the ones in the complex media.

Considering the time of 96 h, the use of rotten fruit as culture media promoted an enhance in the BC yield, independently of its concentration. Comparing the results obtained at media M_0 with the others, it achieved an increase in the BC yield around 89, 25, 51, and 66 % for media M_2 , M_3 , M_4 , and M_5 , respectively. Tukey test at 5 % probability was applied to BC yield results, and it was observed that different statistics among all the results are achieved. The “*p*” value obtained to these results was 0.001.

The C/N ratio (Table 1) was different for each media studied, which can have contributed for the results achieved. The media M_2 had the higher C/N ratio, namely, 57.6; this value was 1.6, 2, 2.4, 3.4, and 8 times higher than that in M_3 , M_5 , M_4 , M_1 , and M_0 , respectively. So, the results obtained can be related not only with how easy is the carbon assimilation by the microorganism but also with its amount in the culture media. Although the C/N ratio in M_1 was higher than that in M_0 , the first one was composed by milk whey, in which the main carbon source is lactose, and the microorganism had to develop a different metabolic route promoting a lower BC yield.

To the other hand, culture media containing rotten fruits blended with milk whey contain sufficient nutrients that contribute to the bacterial cell growth and BC production. The results of cell growth and pH were determined at 96 h of bioprocess, which was considered the final time of fermentation. The cell growth was similar to media M_3 , M_4 , and M_5 . The lowest cell growth was achieved with media M_2 while the media M_0 and M_1 provided intermediate results (Table 2).

Table 2 shows the amount of sugar and protein in the media used to produce cellulose before and after the fermentation process compared with BC yield values. At time 0, the total protein content in the medium showed different concentrations, varying from 4 to 12 g/L. The total sugar content varied from 10 to 20 g/L. The results showed a similar pattern of protein consumption; however, the media containing rotten fruit had a higher protein concentration before and after BC production.

The pH was at acidic conditions in the end to all media evaluated. The pH values of media M_0 , M_1 , and M_3 were 3.2, 3.4, and 3.9, respectively, and those of media M_2 , M_4 , and M_5 were 4.5, 5.4, and 4.4, respectively. However, higher production was achieved at pH values around 4–5, as demonstrated in Table 2.

Several previous studies showed that another relevant factor that influences BC production is the volume of the culture medium in a determined type of flask culture. It is important to consider that this parameter is essential for the membrane size and yield of BC production.

Table 2 Sugar and protein concentrations in each culture medium to produce bacterial cellulose (BC) before and after the fermentation process, along with the BC yield values obtained at the final process (96 h)

Culture media	M ₀		M ₁		M ₂		M ₃		M ₄		M ₅	
Time (h)	0	96	0	96	0	96	0	96	0	96	0	96
Proteins (mg/mL)	4.1	0.8	4.0	1.9	10.0	4.1	10.1	2.1	8.1	1.3	9.1	2.2
pH	4.6	3.2	4.9	5.4	3.8	3.43	4.1	3.8	4.2	4.5	4.2	4.4
Log CFU/mL	5.0	6.7	5.0	6.4	5.0	7.3	5.0	7.2	5.0	6.8	5.0	7.0
Sugar (mg/mL)	20.0	2.4	10.0	4.1	16.3	2.9	17.3	4.9	18.2	5.8	17.7	4.2
BC yield (mg/mL)	–	31.8	–	5.9	–	60.2	–	40	–	48.1	–	52.8

M₀ HS broth, M₁ milk whey, M₂ rotten fruit, M₃ M₄ and M₅ milk whey/rotten fruit

Ruka et al. (2012) observed that the surface area/volume ratio of cellulose was dependent on the culture medium volume; for example, 80 mL of culture medium for BC production would give a surface area/volume ratio of approximately 0.71 cm⁻¹ and would most likely provide a suitable depth to microorganism development and to produce high amounts of cellulose. Ratios between 0.57 and 0.71 cm⁻¹ would provide depth of 1.75 and 1.4 cm, respectively, which would most likely give the most cost-effective quantities of media to obtain maximum BC yields. As in this work, the relation between medium consumption/volume was the same for all culture media; this parameter had any influence in the results obtained. The final membranes were approximately 2 mm thin.

However, the BC membrane size is also dependent on the culture media used in BC production. Our results showed that the culture media containing milk whey produced a pellicle that was thinner than that produced by other culture media even if they had started with the same media volume (1 mL). The size of BC is a relevant factor in its production because it is directly associated with the application area.

BC produced after 72 h of fermentation from culture media containing milk whey (M₁) was susceptible to treatment with NaOH, a standard process used to purify BC; therefore, yield calculation, SEM imaging, and DRX analyses could not be continued. However, to show that in a culture media containing milk whey it was possible to produce BC, the sample obtained at 48 h was characterized (data not shown) proving that in this culture media, there was BC production.

Kinetic study

Kinetic parameters are essential not only to estimate the cost of a bioprocess but also to develop control strategies (Baptista-Neto et al. 2000). In this way, some kinetic parameters, specifically BC productivity (P_{BC}) and yield of substrate on product (Y_{P/S}), were investigated, and the results are shown in Fig. 2.

The culture media M₂ showed the highest value for both parameters, namely, 4.45 for yield of substrate on product and 0.63 for productivity. Although the culture media containing

only rotten fruit (M₂) promoted the highest kinetic parameters when combined rotten fruit and milk whey, the conditions carried with a lower percentage of rotten fruit showed better results; as example with the culture media M₃ (60 % rotten fruit), a productivity of 0.42 (h⁻¹) was achieved while that with culture media M₅ (50 % rotten fruit), the value of this parameter was 0.55 (h⁻¹). So, rotten fruit is a suitable media to obtain BC, and its combination with milk whey can prejudice the process.

The productivity achieved in the medium M₂ was approximately (0.63 h⁻¹) 10-fold higher than that obtained in the medium M₁ (0.06 h⁻¹), which contained only milk. The type of sugar present in each medium can be the main reason for the results achieved. Since culture media M₂ showed the best results with respect to kinetic parameters, this media can be used as an alternative to produce BC.

BC characterization

Figure 3 presents the difference in surface morphology by comparing the culture media M₃ and M₄ with other BC

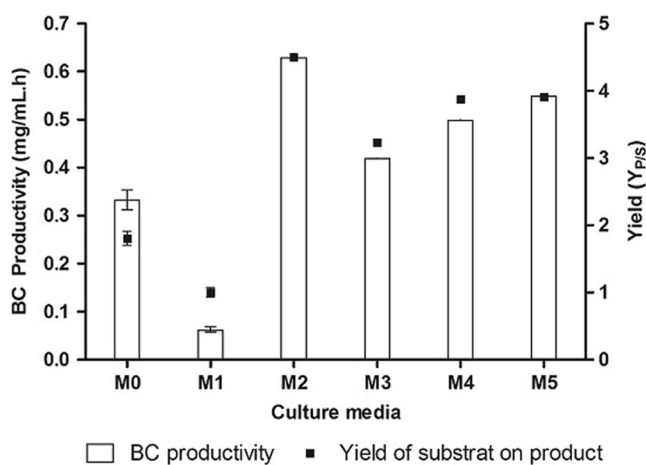
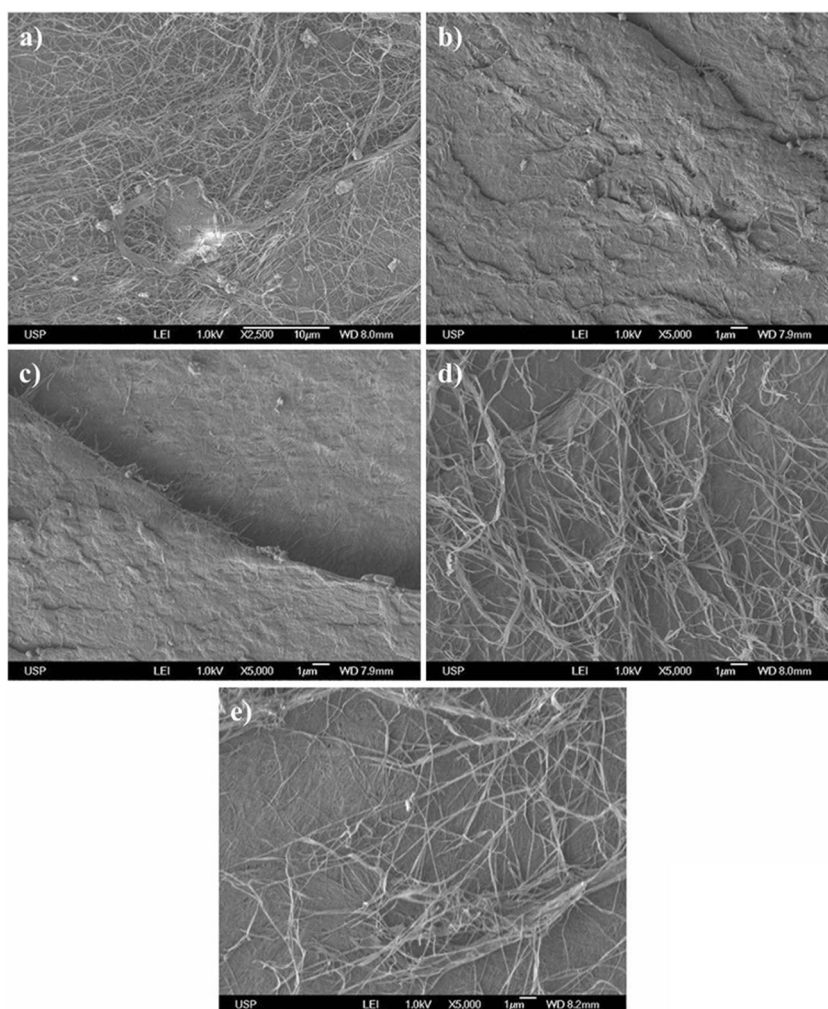


Fig. 2 Bacterial cellulose (BC) productivity (h⁻¹) and yield of substrate in product (Y_{P/S}) at 96 h of fermentation. Gray bars represent the BC productivity and black line represents the yield. The error bars represent a confidence limit of 95 % for the measurements. M₀ HS broth; M₁ milk whey; M₂ rotten fruit; M₃, M₄, and M₅ milk whey/rotten fruit

Fig. 3 SEM images of the top view of the bacterial cellulose (BC) produced in **a** BC standard (M_0 HS), **b** BC from milk whey/rotten fruit (M_3), **c** BC from milk whey/rotten fruit (M_4), **d** BC from milk whey/rotten fruit (M_5), and **e** BC from rotten fruit (M_2)



membranes after 96 h. The SEM images of the BC membranes exhibited their top view (denser side). The loose fibrillar structure of the standard M_0 , M_5 , and M_2 contrasts with the compact and diminished surface porosity of the M_3 and M_4 cellulose. This compact cellulose network structure is composed of a random assembly of fibrils (Sheykhazari et al. 2011). The structure of the BC produced in M_2 , which contained only rotten fruit, was similar to the standard culture media M_0 . These differences can influence the final application of cellulose where more porous or compact structures are necessary to create a certain device or environment (Fig. 3).

BC crystallinity was also investigated using XRD technique, and the X-ray patterns of BC are exhibited in Fig. 4. The obtained X-ray patterns show cellulose with the same chemical structure but with different crystallinity degrees (Table 3). Using the culture media M_0 as the standard sample, 2 main crystalline regions can be observed in the 14 and 22 positions, as previously reported by Yan et al. (2008), Phisalaphong and Jatupaiboon (2008), and Saibuatong and Phisalaphong (2010) who observed crystallinity areas varying from 64.6 to 100 %, respectively. The same peaks were obtained for other samples,

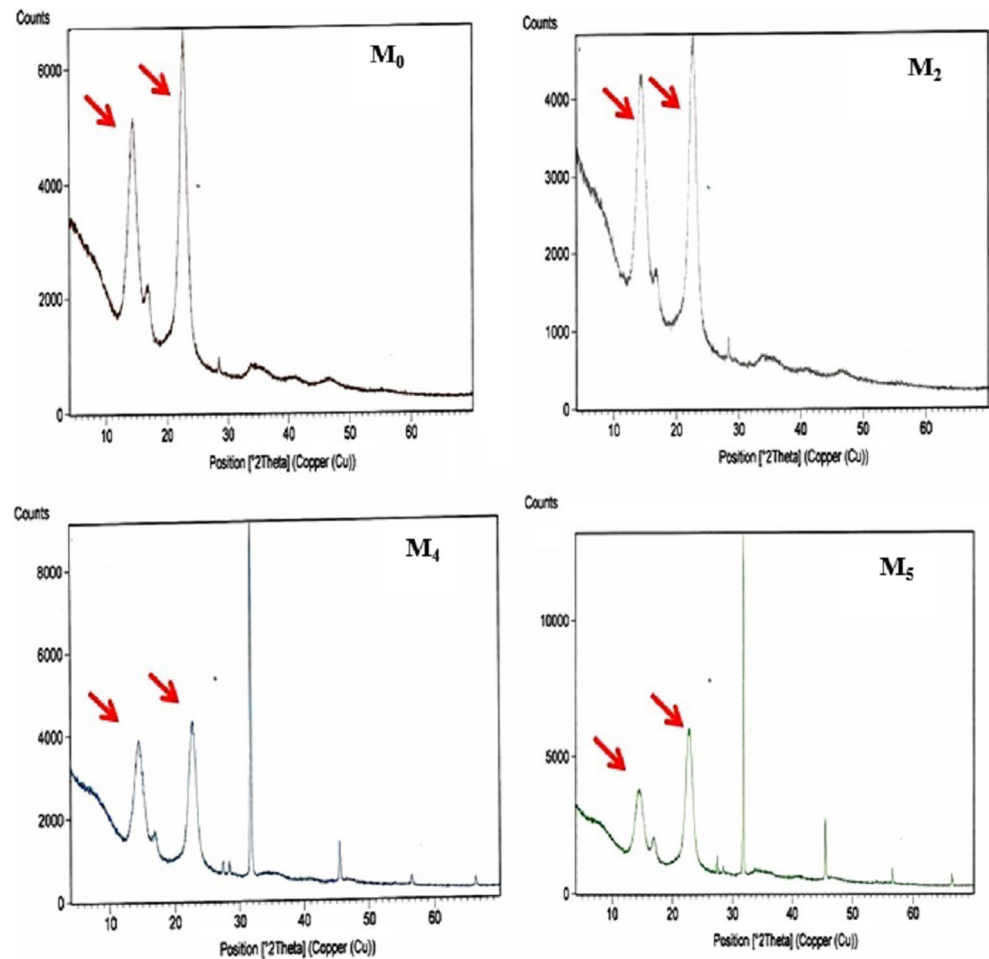
differing only in the crystalline percentage. However, for BC produced in M_2 medium containing rotten fruit, the peak showed a superior crystalline area compared with the standard one (Table 3).

According to the results reported, this bacterial strain is capable of producing quality cellulose at high yields with rotten fruit as the culture medium, with similar characteristics to those reported for BC produced in the standard medium, thus providing favorable conditions for recycling waste.

Discussion

The highest BC yield, determined in mg mL^{-1} (mass [mg] of BC/volume [mL] of culture medium), achieved in this study, was 10-fold higher than the results obtained by Kurosumi et al. (2009). The cited authors studied BC production in different culture media containing fruit juice and showed a higher cellulose yield (6 mg/mL) from culture media containing orange juice after 96 h of cultivation. Zeng et al. (2011)

Fig. 4 Bacterial cellulose (BC) crystallinity by X-ray diffraction and the X-ray patterns. M_0 is standard sample; M_2 rotten fruit; M_4 milk whey/rotten fruit; M_5 milk whey/rotten fruit; *bold red arrows* show the right positions of peaks to BC



demonstrated that BC can be produced after 21 days in a flask culture at a 1.51-mg/mL rate with maple syrup residues as the carbon source. Wu and Liu (2012) studied BC production in HS broth supplemented with thin stillage (TS) and observed that 100 % TS supplement enhanced the production to a concentration of 10.38 mg/mL after 7 days of cultivation.

Table 3 Positions, d-spacing, and crystallinity by X-ray diffraction and the X-ray patterns of bacterial cellulose

Samples	Pos. (°2Th.)	d-spacing (Å)	Crystallinity %
M_0	14.96	5.92	64.66
	22.73	3.91	100
M_2	14.8	5.98	70.69
	22.69	3.91	100
M_3	14.8	5.98	15.70
	22.85	3.89	35.87
M_4	14.74	6.00	59.76
	22.83	3.89	74.52
M_5	14.69	6.03	27.23
	22.77	3.90	37.58

M_0 HS broth, M_2 rotten fruit, M_3 M_4 and M_5 milk whey/rotten fruit

Both productivity and yield of BC were higher in the culture media containing only rotten fruit, i.e., culture media M_2 . This result shows that it was possible to increase BC production by employing rotten fruit as the culture medium. Gomes et al. (2013) employed dry olive mill residues for the production of BC, and the results showed an increase in the production, with lower yields in 96-h BC yield of 0.099 gBC/gS, yield of substrate on product. Jung et al. (2010) employed molasses and corn steep liquor to reduce the cost incurred for culture media and achieved the maximum BC yield of 2.21 mg/mL, which corresponds to dry weight of cellulose per milliliter of the culture media. In our study, the best result was 60 mg/mL from M_2 culture medium, which was 30 times higher than that achieved by Jung et al. (2010).

Vazquez et al. (2013) studied BC low-cost carbon sources such as glycerol from biodiesel production and grape bagasse, a residue of wine. The results showed that maximum BC production was achieved after 14 days of cultivation by employing approximately 10 mg/mL glycerol and 8 mg/mL for grape bagasse.

Some studies describe glucose, unlike other sugars, not only as an energy source but also as an ideal precursor for the assembly of structured cellulose. Consumption during

bacterial incubation is nearly total and in quantitative agreement with the amount of BC produced (Masaoka et al. 1993; Klemm et al. 2001; Castro et al. 2011). Mikkelsen et al. (2009) studied different carbohydrate sources for BC production and observed that *G. xylinus* preferentially metabolizes monosaccharides such as fructose and glucose. In fact, milk whey contains lactose, a disaccharide, in abundance, which is unfavorable for BC production by *G. xylinus* cells. This explains the inferior value in sugar consumption as well as the lower yield value of 5.9 mg/mL observed in culture media containing only milk whey.

BC production involves several enzymes. Their regulation is an essential step for BC production. Enzyme regulation of the branched hexose monophosphate (HMP) involves the Embden-Meyerhof-Parnas (EMP) and tricarboxylic acid (TCA) pathways (Li et al. 2012). As cited before, glucose is the most commonly sugar employed for the biosynthesis of BC, but this substrate generates several by-products such as gluconic acid, which leads to low yields for BC. Alternatively, Li et al. (2012) studied the addition of ethanol and sodium citrate to the medium, which generated fewer by-products. Thus, the use of rotten fruit, which contains fructose as the main carbon source, can both reduce the generation of by-products and increase the yield of BC.

According to literature (Vandamme et al. 1998; Joseph et al. 2003; Park et al. 2006), the pH of the culture medium is a critical factor for BC productivity. Panesar et al. (2012) have observed that lower pH and incubation time result in minimum cellulose production; however, cellulose production increases with an increase in incubation time and pH.

In addition, Pourramezan et al. (2009) indicated that alkaline pH is favorable for cellulose production because of the minimum conversion of glucose into gluconic acid, which increases cellulose production. In this study, pH was not the limiting factor to BC production. The pH values varied from 4.5 to 5.5 and did not exert any effect on the production of BC since a production higher than found in the literature was achieved.

All the samples characterized showed BC with typical characteristics such as nanodimensional network and microfibrils. Furthermore, XRD profiles were in accordance with those presented by other authors (Mikkelsen et al. 2009; Jung et al. 2010; Carreira et al. 2011; Gomes et al. 2013).

With respect to productivity, it is important to consider the C/N ratio in culture media because the media containing rotten fruit have higher carbon concentrations. BC production can be improved by varying not only the carbon concentration but also the type of sugar employed. The results of BC characterization showed that the membrane produced in media M₀ (standard) compared to that produced in media M₂ (compound of rotten fruit) had the same structural characteristics.

In summary, for the success of a fermentation process, operations involved in production and in recovery should be

simple and cost-effective. It should reduce costs and time and maximize revenue, productivity, and quality of the product of interest (Thiry and Cingolani 2002). This study demonstrated an excellent way to produce BC by employing culture media at a low cost, which can be improved by optimizing the process. Furthermore, BC can be produced by employing rotten fruit media as a carbon source to achieve high yields. Indeed, for all the analyzed time points, the ideal period to obtain the BC membranes seems to be 96 h.

Our results showed that use of rotten fruit and its combinations can be used as a substrate for the production of BC by *G. xylinus*. This culture media promotes economic advantages, reduces environmental pollution, and stimulates research on science sustainability. Furthermore, it is essential to science development alternative ways to reuse waste, which bring an ecology awareness and a significant socioeconomic impact. The cost-effectiveness of the media in terms of cellulose yield and productivity is an important factor to optimize the production. Thus, the results of this study provide a profitable alternative to generate high-value products. In addition, they present an excellent viewpoint to produce a bio-material with different possible applications by using waste.

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