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Potential of Biocellulose Nanofibers Production from Agricultural Renewable Resources: Preliminary Study

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Abstract In the present preliminary study, we report results for the biocellulose nanofibres production by *Gluconacetobacter xylinus*. Production was examined by utilizing different feedstocks of single sugars and sugar mixtures with compositions similar to the acid hydrolyzates of different agriculture residues. Profiles for cell proliferation, sugar consumption, and the subsequent pH changes were thoroughly analyzed. Highest biocellulose production of 5.65 g/L was achieved in fructose medium with total sugar consumption of 95.57%. Moreover, the highest production using sugar mixtures was 5.2 g/L, which was achieved in feedstock with composition identical to the acid hydrolyzate of wheat straws. This represented the highest biocellulose yield of 17.72 g/g sugars compared with 14.77 g/g fructose. The lowest production of 1.1 and 1.75 g/L were obtained in xylose and glucose media, respectively, while sucrose and arabinose media achieved relatively higher production of 4.7 and 4.1 g/L, respectively. Deviation in pH of the fermentation broths from the optimum value of 4–5 generally had marked effect on biocellulose production with single sugars in feedstock. However, the final pH values recorded in the different sugar mixtures were ~3.3–3.4, which had lower effect on production hindrance. Analyzing profiles for sugars' concentrations and cell growth showed that large amount of the metabolized sugars were mainly utilized for bacterial cell growth and maintenance, rather than biocellulose production. This was clearly observed with single sugars of low production, while sugar consumption was rather utilized for biocellulose production with sugar mixtures. Results reported in this study demonstrate that agriculture residues might be used as potential feedstocks for the biocellulose nanofibres production. Not only this represents a renewable source of feedstock, but also might lead to major improvements in production if proper supplements and control were utilized in the fermentation process.

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Introduction

Cellulose is one of the most abundant natural raw materials that has been widely studied during the past decades. Traditionally extracted from plant tissues, cellulose can be produced using certain bacterial strain of *Gluconacetobacter xylinus*. Bacterial cellulose (BC) is highly superior over plant-derived cellulose due to its higher crystalinity and purity, nanostructure, higher degree of polymerization, in addition to its higher water holding capacity and permeability to oxygen [1–7]. The high surface to volume ratio along with the unique poly-functionality, hydrophilicity and biocompatibility all make the biocellulose nanofibers potential material for a wide range of biomedical applications [4]. Furthermore, the high elastic modulus and breaking strength of the nanofibers can lead to introducing several advanced functional nanocomposites [3–5, 8]. BC has also many important applications in industries such as electronics, paper, medical devices, and food industry [4].

In general, conventional static culture method has been utilized for several years to produce BC, since the low shear environment in static culture promotes higher production. BC can also be produced under agitated culture conditions [9, 10]. In addition to the culture methods, feedstock has also been a component of investigation and interest as well with the objective of maximizing BC production. Previous investigations have mainly focused on examining several feedstocks. Fructose has been widely reported and utilized as the most suitable carbon source for the production of biocellulose [9]. The widely used medium of fructose and corn steep liquor was used in shake cultures achieving production of 5–8 g/L [11]. Furthermore, a production of 7–9.2 g/L was achieved in conventional stirred tank bioreactors, while lower yields of 5.6–6.4 g/L were obtained in airlift bioreactors [9, 11–13]. D-xylose medium has also been used to produce BC, which achieved lower production rates than fructose [14]. From the early stages of research, glucose was used as a standard medium [15] and recently, Czaja et al. [16] and Son et al. [17] achieved BC production of 3.33 and 4.16 g/L, respectively, using feedstock containing glucose. Furthermore, molasses has also been investigated as a feedstock to produce BC effectively [18].

Although, enough studies concerning utilizing single sugars have been carried out to improve the production of BC, the possibility of using sugar mixtures as feedstock has yet to be investigated. Investigating optimum compositions of sugar mixtures can introduce new routes to produce BC more economically, compared with the conventional feedstock examined to date. For instance, cheaper sugar mixtures can be obtained from agricultural residues such as wheat straw (WS), corn fibers (CF) and Distiller's Dried Grains with Solubles (DDGS) and can widely be utilized in fermentations. Recent developed technologies for agricultural biomass conversion have proven that agricultural wastes have potentially enough carbon sources to produce value-added bio-based products. Several studies within the same stream of interest have shown that their hydrolizate solutions contain sugar mixtures of glucose, xylose, arabinose, galactose and mannose [19–21]. Interestingly, the individual sugars present in WS, CF and DDGS have previously been examined for biocellulose production, but never been examined as sugar mixtures. Against this background, we postulated that it would be essential to examine the possibility of producing BC using sugar mixtures instead of the single sugars widely examined. This will eventually become a vital biomaterial and will be used in the creation of a wide variety of consumer products. To reach this objective, we examined in the present study productions

of BC using sugar compositions identical to those of the acid hydrolyzates of some agriculture residues as a preliminary study to investigate the utilization of real hydrolyzates-based feedstocks in future work.

The results obtained in this study have special importance for the future production of BC. When large-scale BC fermentation using renewable resources becomes a reality, this might lead to a major new industry. Moreover, the inexpensive production of BC will open new avenues for less specialized applications and will be able to deliver new products to the market at a competitive price.

Materials and Methods

Materials

G. xylinus (ATCC 700178) was purchased from the American Type Culture Collection (ATCC), Manassas, VA 20108, USA. Chemicals; KH_2PO_4, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$, $FeSO_4.7H_2O$, $CaCl_2.2H_2O$, $NaMoO_4.2H_20$, $ZnSO_4.7H_2O$, $MnSO_4.5H_2O$, $CuSO_4.5H_2O$, Inositol, Nicotinic acid, Pyridoxine hydrochloride, D-pantothenic acid calcium, Riboflavin, Folic acid, D-biotin, thiamine hydrochloride, fructose, glucose, sucrose, L(+) arabinose, D-(+)-xylose, mannose, and D-(+) galactose; were purchased from Sigma-Aldrich and were used as received. Corn steep liquor (CSL) was supplied by Casco, London, Ontario-Canada, upon request, and was used as received.

Methods

Bacterial Strain and Culture Conditions

G. xylinus (ATCC 700178) was reactivated according to the ATCC guidelines using sterile YGC 459 liquid medium (50 g/L of glucose, 5 g/L of yeast extract, and 12.5 g/L of $CaCO_3$, pH5.0). The bacterium was then cultivated on Petri plates having sterile YGC 459 agar medium (15 g/L agar, pH5.0) and incubated (Symphony 8.5A, VWR) at 29°C for 7 days.

Production of BC Nanofibers

The total initial concentration of sugars in the feedstock was always kept at 40 g/L with pH 5.0. The compositions of the sugar mixtures that were examined as feedstock were identical to the sugar compositions of acidic hydrolyzate extracts of WS, CF and DDGS as previously reported in the literatures [19–21]. Table 1 summarizes the composition of the different feedstock examined in the present study.

Table 1 Compositions of the different feedstocks of sugar mixtures that were used in BC production by *G. xylinus* (ATCC 700178) (each has a composition identical to that of the agriculture residue hydrolyzate).

Feedstock	Glucose g/L	Xylose	Arabinose	Galactose	Mannose	Reference
Mix_1-WS	19.2	13.3	3.3	2.3	1.75	Qureshi et al. [19]
Mix_2-DDGS	17.95	12.7	7.8	0.9	0.6	Ezeji and Blaschek [21]
Mix_3-CF	21.35	10.1	6.45	2.05	0	Grohmann and Bothast [20]

Media composition was as follows: 20 mL/L of CSL (nitrogen source), total sugars (carbon source: single or mixtures; Table 1) 40 g/L, 1 g/L of KH_2PO_4, 0.25 g/L of $MgSO_4.7H_2O$, 3.3 g/L of $(NH_4)_2SO_4$, 3.6 mg/L of $FeSO_4.7H_2O$, 14.7 mg/L of $CaCl_2.2H_2O$, 2.42 mg/L of $NaMoO_4.2H_20$, 1.73 mg/L of $ZnSO_4.7H_2O$, 1.39 mg/L of $MnSO_4.5H_2O$, 0.05 mg/L of $CuSO_4.5H_2O$, 2 mg/L of Inositol, 0.4 mg/L of nicotinic acid, 0.4 mg/L of pyridoxine hydrochloride, 0.2 mg/L of D-pantothenic acid calcium, 0.2 mg/L of riboflavin, 0.2 g/L of folic acid, 0.2 μg/L of D-biotin and 0.4 g/L of thiamine hydrochloride [22].

To prepare the inoculum solution, 7-day-old *G. xylinus* (ATCC 700178) culture plates were aseptically flooded with 20 mL of sterile distilled water and the bacterium was gently suspended using a sterile cell spreader. The resultant bacterial cell suspension was vortexed (VWR Analogue Vortex Mixer) in sterile sample bottles and used as the inoculum solution.

Fermentation experiments were carried out in 500 mL conical flasks containing 200 mL of medium in each. Prior to use, all flasks were sterilized at 121 °C for 15 min in an autoclave (Sanyo MLS 3780). Each flask was inoculated using 2 mL of the inoculum solution containing 1.5×10^7 cells/mL. After collecting the initial sample from each, flasks were incubated at 29 °C for 7 days with shaking speed of 175 rpm (MaxQ 2000). Samples of 3 mL were aseptically taken from each flask over a period of 7 days and stored at −81 °C (ultra-freezer-REVCO Elite Plus-ThermoFisher Scientific) until analyzed. At the end, flasks containing BC were treated with excess 2 N NaOH at 100 °C for 15 min in the autoclave. Biocellulose nanofibres were extracted and repeatedly washed with distilled water. Production of BC was quantified gravimetrically based on the dry weight of the insoluble BC obtained at the end.

Analytical Methods Used

For the analysis of cell concentrations of samples collected during the BC production, Flowcytometer (Guava EasyCyte Mini System, Guava Technologies, Inc., USA) was used according to the manufacturer's guidelines. The Guava EasyCyte Mini System contained a blue laser (Excitation 488 nm) which emits 20 mW visible laser radiations, three fluorescent detectors, an automated analyzer unit with an internal hard drive connected to a computer, single sample tube format and CytoSoft Software with expandable software modules. Samples were diluted 20-fold using deionized water. The diluted samples were repeatedly vortexed at high speed for 3 min and thereafter, filtered through 0.8 μm membrane filters (Millipore, USA). A volume of 1 mL of each filtered sample was loaded into the sample loader and circulated through the capillary feeder attached to the system. Guava VaiCount Assay through the built-in Guava® ExpressPluss software Module displayed the direct absolute cell counts as cells/mL in conjunction with subpopulation percentages.

Sugar concentrations were analyzed using pre-calibrated high performance liquid chromatography (HPLC; Perkin Elmer). This instrument was equipped with an Ion Exchange column (Aminex HPX-87H, Biorad, Hercules, USA), a pump Series 200 (Perkin Elmer), Auto sampler Series 200 (Perkin Elmer) and a Refractive Index Detector (HP1047A, Hewlett Packard). Samples of 50 μL were diluted 20-fold with deionized water and filtered (0.45 μm Gelman Acrodisc CR PTEF, Millipore). Total of 50 μL from each diluted sample was injected into the column and circulated for 15 min at a flow rate of 0.6 mL/min using filtered (0.2 μm nylon Millipore) and degassed mobile phase of 5 mM H_2SO_4 [19]. The column temperature was maintained at 60 °C using the column heater CH-30 controlled by an Eppendorff TC 50. Sugar concentrations were quantified from calibration curves that were constructed from standard sugar solutions of known concentrations (10–100 μg/mL).

Results and Discussions

Figure 1 shows the total biocellulose productions and the final pH of the fermentation broths as obtained using different feedstocks composed of single sugars (Fig. 1a) and sugar mixtures (Fig. 1b). Examining Fig. 1a reveals that highest BC production of 5.65 g/L was obtained in the fructose medium, while lowest production of 1.1 g/L was obtained in xylose medium. Sucrose and arabinose media achieved high production of 4.7 and 4.1 g/L of BC respectively, while glucose medium produced only 1.75 g/L. The pH value of the glucose medium that was recorded at the end of the fermentation was 3.0, while pH of 4.59 and 4.13 were recorded in the fructose and sucrose media, respectively (Fig. 1a).

The deviation in fermentation media pH away from the optimum pH of 4–5 inhibits BC production, which might prevent developments in large-scale fermentation unless a precise control over pH is implemented [23, 24]. Surprisingly, production of BC was 4.1 g/L in the arabinose medium although the final pH recorded was 3.78. These results demonstrate lower production inhibition in the arabinose medium compared with the glucose medium where the effect of lower pH on production was clearly marked. In general, the high BC production in fructose medium obtained in this study is in good agreement with several published results, which confirmed the importance of fructose for BC production by *G. xylinus* (ATCC 700178) [11, 14]. Furthermore, the high production obtained in the sucrose medium can be related to the formation of UDP-glucose in sucrose metabolism, resulting in high BC production [25]. The low production obtained in glucose medium can be related to that some glucose oxidizes to gluconates producing acids, which lowers the pH of the medium.

Total production obtained in the sugar mixtures media with different compositions (Table 1) are shown in Fig. 1b. According to this figure, the highest BC production using sugar mixture was 5.2 g/L, which was obtained in the Mix_1-WS medium. This production is lower than the production in fructose medium and relatively higher than productions obtained among the different media tested in this study. Production obtained using the other two sugar mixtures in Table 1 (Mix_2-DDGS and Mix_3-CF) were 3.75 and 2.4 g/L, respectively. This is still considered as relatively high production compared with production

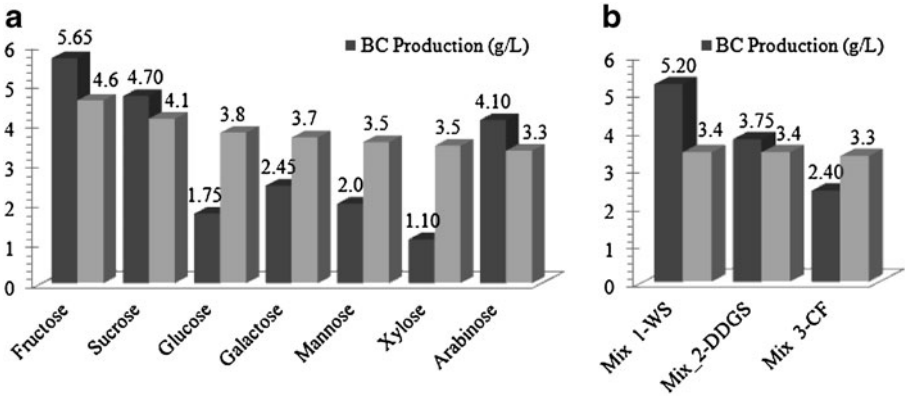

Fig. 1 Final BC production and final media pH for the different feedstocks utilized by *G. xylinus* (ATCC 700178) that contain: **a** single sugars and **b** sugar mixtures (total initial sugar concentration was 40 g/L)

obtained in different single sugar media in Fig. 1a. The final pH values recorded in the different sugar mixtures were almost similar (pH ~3.3–3.4). Apparently, the deviation in pH from the optimum value reported for BC production using *G. xylinus* had lower effect on production hindrance when sugar mixtures are used in feedstock compared with that of the single sugars with similar final pH in Fig. 1a.

Figure 2 (a and b) show respectively the changes in sugar concentrations that were recorded in different feedstock composed of single sugars and sugar mixtures. According to Fig. 2, sugar concentration profiles recorded in all media were almost similar with sharp drop during the first 24 hours of fermentation. However, changes in sugars concentrations observed in the remaining days of fermentation were much smaller. The sugar consumption rates of the different sugars in the media used in the present study were calculated based on the total hourly sugar consumption during the BC production over the 7 days of fermentation. Figure 3 shows the changes observed in these consumption rates for single sugars (Fig. 3a) and sugar mixtures (Fig. 3b). Examining Fig. 3a shows that the different single sugars had close consumption rates of 1.30–1.65 g/L h during the first day of fermentation. Obviously, sucrose and fructose had the highest consumption rates, while xylose had the lowest. In the second day and onward, consumption rates dropped drastically for all single sugars to a similar consumption rate of ~0.1 g/L h. Similarly, the overall sugar consumption rates were comparably high in the first day of fermentation in feedstocks of sugar mixtures as shown in Fig. 3b (i.e. 1.25–1.37 g/L h). Moreover, these rates dropped to the similar rates observed with single sugars in the remaining days of fermentation (Fig. 3b).

The lowest single sugar concentration observed during fermentation was for sucrose followed by fructose, as shown in Fig. 2a. This simply explains the high BC production obtained with both sugars although fructose achieved higher BC production (Fig. 1a). The higher concentration of xylose over the fermentation period also reflects lower consumption of the sugar and explains the lower production of BC obtained in Fig. 1. Apparently, this

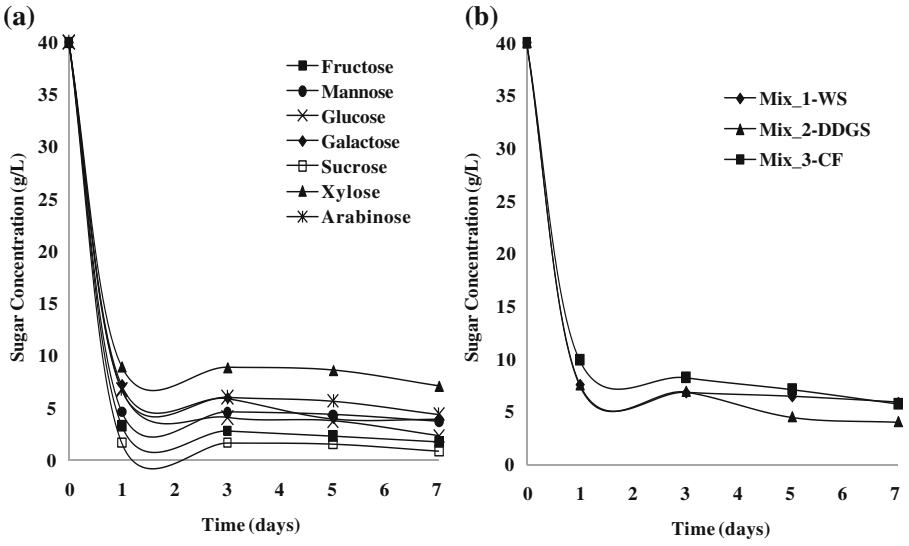

Fig. 2 Changes in sugars concentrations during BC production by *G. xylinus* (ATCC 700178) using different feedstocks containing: **a** single sugars and **b** sugar mixtures (total initial sugar concentration was 40 g/L)

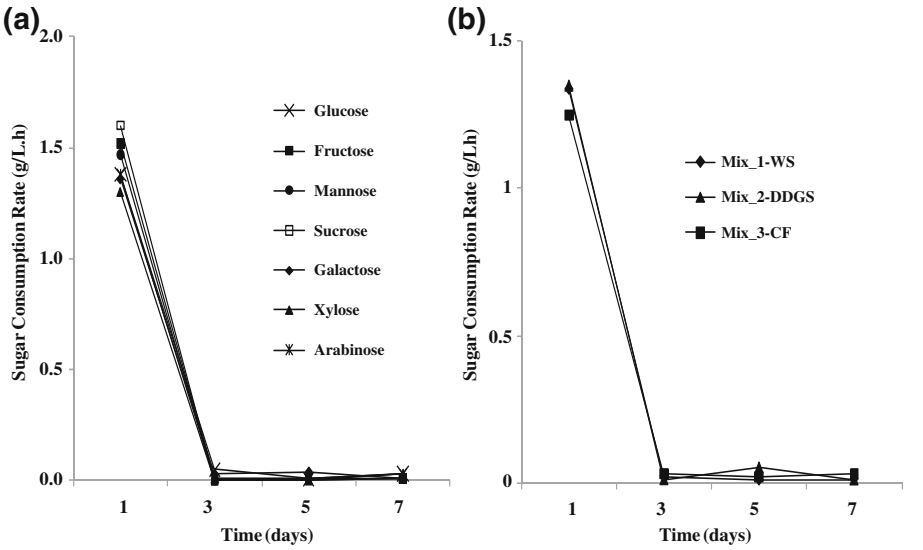

Fig. 3 Total sugars consumption rates calculated based on the total hourly sugar consumption during the BC production by *G. xylinus* (ATCC 700178) in different feedstocks containing: **a** single sugars and **b** sugar mixtures (total initial sugar concentration was 40 g/L)

lower consumption is due to the complexity in metabolizing xylose [11, 14]. The lowest overall sugar concentration among sugar mixtures media was observed for Mix_2-DDGS as shown in Fig. 2b, although Mix_1-WS achieved higher production. In general, higher sugar concentrations remained unconsumed by the bacteria at the end of the fermentation for the sugar mixtures than that for single sugars, although similar amounts of sugars were utilized initially in all fermentations. In general, approximately 85.22% of the total sugars were consumed in Mix_1-WS and Mix_3-CF media as shown in Table 2. This amount is the lowest compared with all other media tested except for the low BC producing xylose medium.

The 89.95% of sugars that were consumed in Mix_2-DDGS medium during BC production is relatively lower than the other media, except for the arabinose medium with 88.97% sugar consumption. The maximum production that was obtained in fructose medium was based on 95.57% total fructose consumption. This achieved an overall yield of 14.77 g BC/ g sugar, which is lower than the yield obtained using Mix_1-WS (i.e. 17.72 g/g sugars; Table 2). BC yields that were obtained with Mix_2-DDGS and arabinose media were almost similar (11.67% and 11.52%, respectively). In general, sucrose consumption was 97.77%, which was the highest among that of all media containing single sugars, with BC yield of 12.02% achieved. Again, xylose was the least consumed with 83.3% consumption and lowest yield of 3.34 g/g sugar. Total consumption of glucose was 94.22%, which is close to that of fructose, although this did not result in comparable BC production. Presumably, this may be related to the fact that the majority of glucose is usually metabolized for energy production [22]. Apparently, the high fructose consumption may have stabilized pH over 4.5 favoring cell proliferation and BC production [24]. According to literatures, *G. xylinus* produces acetates, pyruvates or succinates in fructose media that promote BC synthesis [26]. Furthermore, this bacterium also produces ethanol, which has been proven to enhance BC production [7, 26]. The low consumption of glucose may have resulted in low average cell concentration in the glucose medium. *G. xylinus* also

Table 2 Results calculated for the yield, sugar consumption, and cell proliferation during BC production by *G. xylinus* (ATCC 700178) using different feedstocks containing single sugars and sugar mixtures.

Feedstock	BC yield, g BC/g sugar	Sugar consumption		Average cell concentration (10^7 cells/mL)	Average cell proliferation rate (10^7 cells/mL.h)[b]
		Total, g/L	Fraction[a], %		
Fructose	14.77	38.23	95.57	4.0	0.29
Sucrose	12.02	39.11	97.77	10.6	0.53
Glucose	4.64	37.69	94.22	6.4	0.36
Galactose	6.79	36.08	90.20	5.5	0.41
Mannose	5.50	36.31	90.77	6.2	0.30
Xylose	3.34	32.92	83.30	7.2	0.48
Arabinose	11.52	35.59	88.97	4.7	0.26
Mix_1-WS	17.72	34.09	85.22	4.8	0.29
Mix_2-DDGS	11.67	35.98	89.95	7.2	0.45
Mix_3-CF	6.96	34.23	85.57	4.1	0.29

[a] Calculated based on the consumption out of 40 g/L

[b] Calculated based on the hourly increase in cell count

metabolizes glucose to produce gluconic acid or acetic acid, which generally reduces the pH [27–31]. This might explain the low final pH 3.0 that was obtained in this study (Fig. 1). In addition, *G. xylinus* produces an exo-1,4-*P*-glucosidase using glucose instead of ethanol as in the fructose medium [32]. This decreases the degree of polymerization of the biopolymer and reduces its production [6, 33, 34]. These facts apparently have attributed to hinder the BC production in the glucose medium. In addition, *G. xylinus* is known to share glucose in acetan biosynthesis [11]. The increase of acidity in this glucose medium may also be due to acetan, although information on levels of acetan production in glucose media is inadequate.

Changes in bacterial cell concentration (i.e. *G. xylinus* (ATCC 700178)) that was measured during fermentation in the media of single sugar and sugar mixtures are shown in Fig. 4a, b, respectively.

Bacterial cell concentration increased reaching high concentration during the first day of fermentation in the xylose and arabinose media as shown in Fig. 4a. This demonstrates that larger amount of the metabolized sugars were mainly utilized for the cell growth rather than BC production, especially for xylose with the lowest production obtained. The growth in cell concentration was delayed until after the first day of fermentation in the sucrose, mannose and fructose media. This indicates favoring BC production over the cell growth as proven by the high BC production obtained (Fig. 1). Highest average cell concentration of 10.6×10^7 cell/mL (calculated based on the hourly increase in cell count; see Table 2) was observed in the sucrose medium. This high cell proliferation correlates with the high total sugar consumption of 39.11 g/L (97.77%; Table 2) that was prevailed at 1.6 g/L.h (Fig. 3b). However, BC production in this case was not consistently the highest (4.7 g/L; Fig. 1a). Apparently, sucrose was utilized intensively for cell proliferation and for their maintenance, which recorded the highest rate of $\sim0.53\times10^7$ cells/mL.h (Table 2). This can be related to that a portion of the bacterial cells are non-cellulose producing cells (i.e. *Cel*$^-$ mutants) [35]. According to Park et al. [35], *Gluconacetobacter hansenii* produced up to 26% of non-cellulose producing cells in a jar fermentor, which affected the final BC production. Surprisingly, the average cell concentration in fructose medium was the lowest (i.e. 4.0×10^7 cell/mL; Table 2) although has

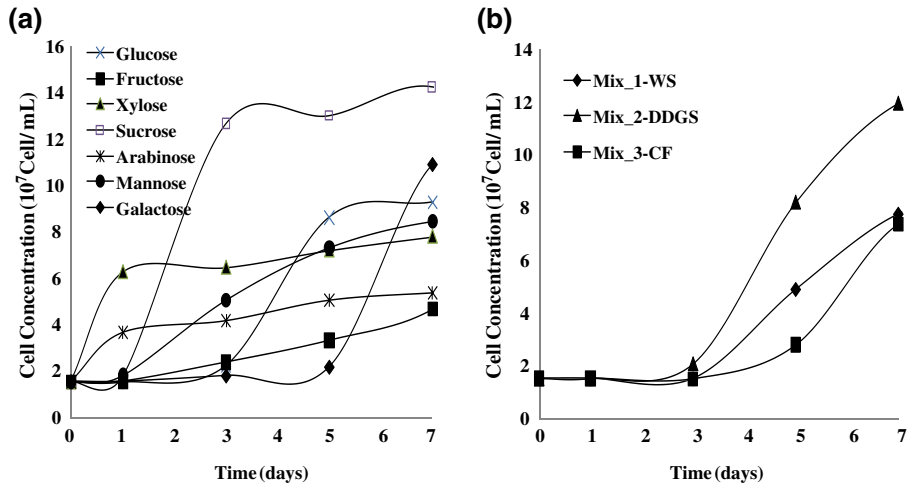

Fig. 4 Changes in bacterial cell concentrations during BC production by *G. xylinus* (ATCC 700178) in feedstocks containing: **a** single sugars and **b** sugar mixtures (total initial sugar concentration was 40 g/L)

achieved highest production of BC. This demonstrates favoring BC production over cell growth in fructose medium. Cell growth in the glucose medium was observed after the third day, while it was observed later after the fourth day for galactose. Again, this represents favoring energy production and cell maintenance for both sugars respectively as discussed above.

Figure 4b shows that bacterial cell concentrations started to increase after the third day of fermentation in the media composed of sugar mixtures. This explains the high BC production, which was favored over the cell growth. The highest average cell concentration was observed in Mix_2-DDGS medium (7.2×10^7 cell/mL; Table 2), which achieved BC production of 3.75 g/L (Fig. 1). The average cell concentrations in Mix_1-WS and Mix_3-CF were almost identical (4.8×10^7 and 4.1×10^7 cell/mL, respectively; Table 2), although highest BC production was obtained in Mix_1-WS (5.2 g/L; Fig. 1b). This all confirms the limited effect of cell concentration on BC production. It is worth mentioning that the average cell concentrations in the two media of fructose and Mix_1-WS that achieved the highest BC productions were very close to each other (4.0×10^7 and 4.8×10^7 cell/mL, respectively). Table 2 further indicates that the cell proliferation rate in Mix_2-DDGS was high (~0.45×10^7 cells/mL h) although BC production was relatively low (3.75 g/L; Fig. 1b). More details on the consumption of each individual sugar in the sugar mixtures are summarized in Table 3.

Examining results in Table 3 shows that over 15 g/L of glucose has been consumed in all mixtures, which represents almost 90% of its initial content. Overall consumption of xylose, galactose and mannose in all media containing sugar mixtures was the lowest. This can be explained based on the low xylose consumption in the feedstock containing single xylose in Fig. 3a. Arabinose consumption was generally lower than that of glucose similar to what was observed in the feedstocks with single sugars (Table 2). In general, maximum sugar consumption was achieved with Mix_1-WS, although arabinose was consumed at a higher rate in Mix_2-DDGS (i.e. 89.49% compared with ~84.6% for the other two mixtures in Table 3). According to Fig. 5, individual sugars were mostly consumed during the first day of fermentation. Surprisingly, sugars consumptions slightly increased after the third day of fermentation for Mix_2-DDGS and Mix_3-CF, but clearly decreased after the first day of fermentation for Mix_1-WS. Consumption of glucose was the highest for all sugar mixtures

Table 3 Results calculated for the individual sugar consumption during BC production by *G. xylinus* (ATCC 700178) using different feedstocks containing sugar mixtures.

Medium	Glucose		Xylose, galactose and mannose[d]		Arabinose	
	Fraction[b], %	Consumption rate[c], g/L.h	Fraction, %	Consumption rate, g/L.h	Fraction, %	Consumption rate, g/L.h
Mix_1-WS	88.49	0.67	81.77	0.57	84.54	0.095
Mix_2-CF	88.80	0.25	80.57	0.27	84.65	0.07
Mix_3-DDGS[a]	92.37	0.62	87.15	0.4	89.49	0.26

[a] Mannose content is negligible in Mix_3-DDGS (Table 1)

[b] Based on the total amount consumed

[c] Based on the hourly rate of sugar consumption

[d] Represented by a single peak with close retention time in the HPLC column

in the first day of fermentation, although consumption of xylose, galactose and mannose was relatively higher for the Mix_3-CF mixture. According to the results from single sugar media, galactose achieved highest yield of BC, followed by manose, glucose, and then xylose (Table 2). Moreover, cell growth was the highest with xylose medium, followed by glucose and mannose, then galactose. Based on that, the high BC production obtain in Mix_1-WS can be explained based on promoting production through utilizing galactose and mannose, while glucose was mainly utilized for cell growth. Moreover, according to Son et al. [17], high concentrations of glucose hinder the BC production, although they observed that it might enhance production at latter periods of fermentation. The higher consumption of xylose, galactose and mannose in Mix_3-CF after the first day and in the presence of high glucose content did not lead to higher BC production or cell concentration. In general, the relatively higher production and higher yield of BC achieved with feedstock of sugar mixtures can be attributed to the possibility that higher sugar concentration in single sugar feedstocks may have caused catabolite repression. This can inhibit enzymatic reactions involved in sensing endogenous levels of sugars, which clearly can be avoided by utilizing sugar mixtures [36].

It is important to mention that none of the sugar mixtures examined contain fructose or sucrose, which achieved the highest production among single sugars tested (Table 2). This demonstrates potential supplements to improve production of BC. Moreover, it may be vital to balance the nutrients by supplying adequate minerals and vitamins such as nicotinamide and methionin as they are essential for the cell growth and high BC production by *G. xylinus* [22, 37]. It may also be important to stabilize the pH and eliminate other inhibitive substances such as acetic acid, furfural, hydroxymethylfurfural, lignin derivatives, phenolic acids and aldehydes that may also contain the acid hydrolyzate of WS [38, 39]. Further investigations are ongoing in order to deliver biocellulose nanofiber to the market at lowest cost possible by improving the efficiency of production and the use of cheap feedstock from renewable resources.

Conclusions

This preliminary study demonstrated potential ability to produce BC using agricultural residues as feedstock, which represents economical and environmental benefits. The effect

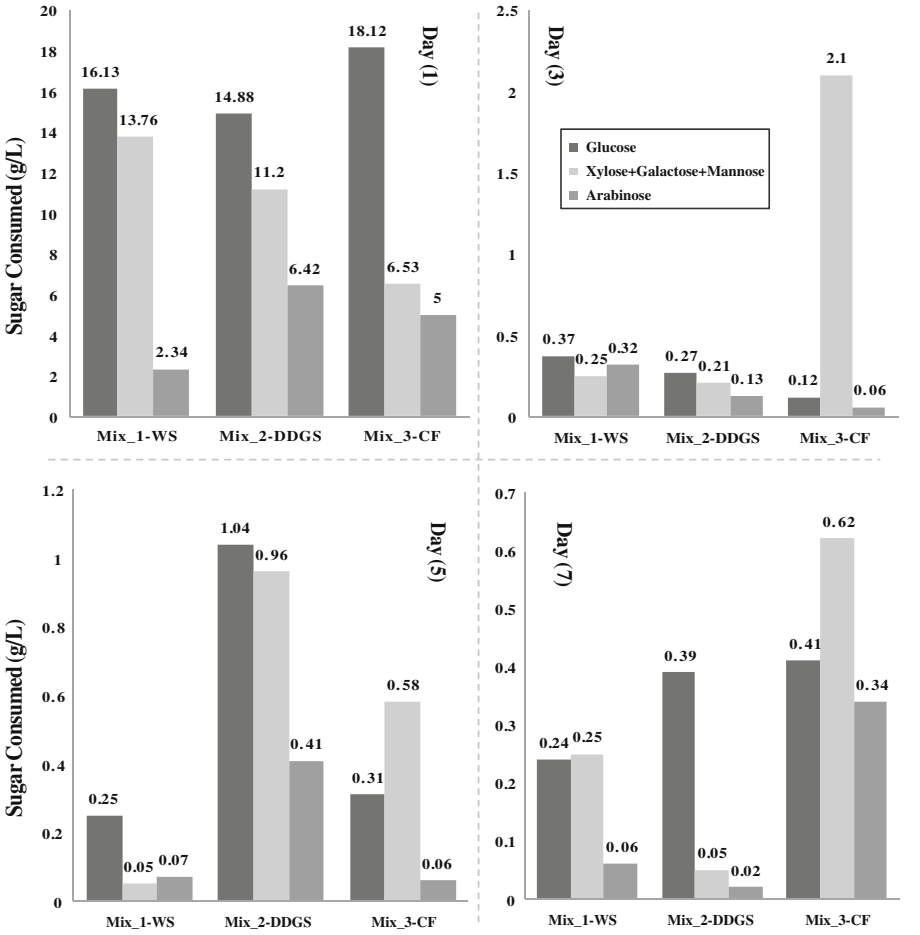

Fig. 5 Changes in individual sugar concentrations obtained for the different sugar mixture based feedstocks during BC production by *G. xylinus* (ATCC 700178) (total initial sugar concentration was 40 g/L)

of sugars in the feedstock on the production of biocellulose nanofibers by *G. xylinus* (ATCC700178) and subsequent changes in each liquid medium were investigated.

Feedstocks examined are either single sugars or mixtures of sugars including fructose, sucrose, glucose, arabinose, galactose, mannose and xylose. Production was first achieved using single sugars in the feedstock to establish the essential background for comparison. Careful literature search showed that although the use of single sugars has widely been investigated in biocellulose production, no work was done that utilizes multiple sugars in the feedstock. Compositions of sugar mixtures examined were similar to the composition of acid hydrolyzates of WS, DDGS and CF. Among the single sugars, fructose gave the highest BC production of 5.65 g/L. Moreover, 95.57% of fructose was consumed, which achieved lowest change in pH and low average cell proliferation rates. Sucrose and arabinose achieved high BC production as well (4.7 and 4.1 g/L, respectively), while xylose achieved the lowest BC production and highest change in final pH of the medium among all other single sugars examined with high average cell proliferation rates. Highest production of 5.2 g/L was obtained from the sugar composition identical to the WS

although it has high initial content of xylose. Production of BC obtained from DDGS and CF sugar mixtures were lower (i.e., 3.75 and 2.4 g/L, respectively). In general, media containing sugar mixtures produced more nanofibers than that of many media containing single sugars. Interestingly, total sugar consumption of 85.22% was observed with this medium although it achieved highest yields among all feedstocks tested. These facts demonstrate the high potential to produce the biocellulose nanofibers based on renewable agricultural residues.

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