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ORIGINAL ARTICLE

Influence of Glucose, Urea and Bacteria Concentration on Yield and Carbon Conversion of Nata De Cassava Prepared Using Liquid Tapioca Waste Medium

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ABSTRACT – Nata de Cassava can be obtained from bacterial cellulose and synthesized by *Acetobacter xylinum* using the liquid tapioca waste as the medium. This research aimed to investigate the influence of concentration of carbon and nitrogen sources and the type of bacteria. The liquid tapioca waste (200 ml) was heated in a beaker glass at 70-80 °C and then added with 5, 7.5, and 10 % (w/v) of sugar and 0.1, 0.2, and 0.5 % (w/v) of urea. The mixture was poured into a container and then cooled. Furthermore, 10, 15, and 20 % (v/v) of *A. xylinum* was added and incubated at room temperature. After ten days, the Nata de Cassava was harvested, sterilized, and immersed in ethanol, then dried in an oven at 60 °C. The results of FTIR, XRD and SEM analysis showed that Nata de Cassava had been successfully synthesized. The highest Nata de Cassava yield of 2.41 % was produced from the composition of 15 % of *A. xylinum*, 10 % of glucose and 0.1 % of urea in the fermentation medium. In addition, the highest carbon conversion ratio of 26.15 % was derived from the composition that used used 10 % of *A. xylinum*, 5 % of glucose and 0.2 % of urea. According to the results, the liquid tapioca waste could be used as an abundant, cheap and suitable culture medium for the production of bacterial cellulose, Nata de Cassava.

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INTRODUCTION

Bacterial cellulose is a gelatinous membrane at the liquid-air interface of a culture medium consisting of cellulose fibers produced by bacteria. These cellulose fibers are described as elongated threads [1]. Bacterial cellulose can be synthesized by static, agitated/shaking culture, and bioreactor culture methods. However, in general, bacterial cellulose synthesis is carried out using the static culture method. The static culture method is a simple and the most frequently used method for the production of bacterial cellulose [2]. Bacterial cellulose can be synthesized by various bacteria, such as *Acetobacter azotobacter, Rhizobium, Agrobacterium, Pseudomonas, Salmonella, Alcaligenes, Sarcina ventriculi* [2], *Komagatacibacter xylinus* [3], [4], *Gluconacetobacter hansenii*, *Komagataeibacter rhaeticus* [5], *Acetobacter xylinum* [5]–[9], *Gluconacetobacter oboediens* [10], *Gluconacetobacter liquefaciens* [11], *Tea fungus* [12], *Gluconacetobacter sucrofermentans* [13], *Acetobacter pasteurianus* [14]. The most effective bacteria to produce bacterial cellulose are *A. xylinum*, *A. Hansenii* dan *A. Pasteurianus*. In addition, *A. xylinum* is reclassified as *Gluconacetobacter xylinus*, and presently, it is known as *Komagataeibacter* [15], [16]. *A. xylinum (G. Xylinum)* bacteria in acidic conditions can grow and produce bacterial cellulose commercially and produced with high productivity [2], [6], [8].

Hestrin-Schramm (HS) is a standard medium and the most appropriate medium used in the synthesis of bacterial cellulose. However, HS is expensive [6], therefore, the research on using a low-cost medium for bacterial cellulose production is necessary. Several mediums have been used to produce bacterial cellulose, such as pineapple peel waste juice [17], vinasse [3], wood hot water extract [6], distillery effluent [10], sugar beet molasses and cheese whey medium [8] and acidic food industry by-product [13].

The yield of bacterial cellulose is influenced by several factors, including carbon and nitrogen sources. Carbon, as the source of bacterial cellulose production has a significant influence on the yield and morphology of bacterial cellulose [1]. In addition, because cellulose is a carbohydrate polymer, the type of carbohydrate source is considered as one of the most important parameters affecting bacterial cellulose yield. Anindya, *et al.*, 2019 indicated that bacterial cellulose yields were strongly influenced by the type and concentration of carbon sources used within the growth medium [5]. Glucose is a carbon source that has been widely used for the production of bacterial cellulose. Glucose as the sole carbon source was completely consumed by bacteria after ten days of fermentation [8]. The higher concentration of glucose in the medium is expected to produce a higher yield of bacterial cellulose in the growth medium, especially at shallow growth conditions. Nitrogen is another essential component in cell growth and bacterial cellulose production [1]. The high production of bacterial cellulose was related to the presence of nitrogen that enhances bacterial growth, thereby increasing bacterial cellulose yield [8].

In our study, we focused on Nata de Cassava as the bacterial cellulose synthesized from liquid tapioca waste medium using *A. xylinum* and sugar as a source of carbon as well as urea as a source of nitrogen. The use of liquid waste from tapioca production because of its cheapness and abundance. Our previous work initiated the successful synthesis of Nata

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de Cassava [18]. The various culture environment that consisted of bacteria strain, nutrition, pH, and oxygen delivery that are important and affect the properties of bacterial cellulose was subjected to the synthesis of Nata de Cassava [1]. Therefore, this research aimed to investigate the influence of glucose, nitrogen and bacteria concentration on the yield and carbon conversion ratio of Nata de Cassava.

EXPERIMENTAL METHOD

Materials and Instruments

Liquid tapioca waste was obtained from the process of tapioca starch production (pH 5). *Acetobacter xylinum InaCC B422* was obtained from the Indonesian Culture Collection (InaCC), National Research and Innovation Agency (BRIN), Indonesia. Microcrystalline cellulose (CAS 9004-34-6) was purchased from Merck. Sugar, urea, and ethanol are obtained as technical grades.

The yield of Nata de Cassava is the amount of Nata de Cassava produced from the fermentation process. It is the result of the amount of the obtained Nata de Cassava (mg) per unit volume of culture medium (ml) as calculated by the following formula [5].

$$Yield = \frac{Weight\ of\ nata\ de\ cassava}{Volume\ of\ culture\ medium} x100\% \quad (1)$$

The Carbon Conversion Ratio (CCR) is the ratio of the amount of the obtained Nata de Cassava to the amount of sugar added into the culture medium. The CCR is calculated based on the formula below [5]:

$$CCR = \frac{Weight\ of\ nata\ de\ cassava}{Amount\ of\ sugar\ added\ in\ culture} x100\% \quad (2)$$

The functional group of Nata de Cassava was analyzed using Fourier transform infrared (FTIR) ATR Shimadzu (Japan) in wavenumber of 400-4000 cm^{-1}. There were a total of 40 scans that were accumulated in transmission mode with a resolution of 4 cm^{-1}.

A Scanning electron microscope (SEM) JEOL JSM IT-200 LA (Japan) at an accelerating 5 kV voltage was utilized to observe the surface and cross-section morphology of the Nata de Cassava. Prior to the analysis, Nata de Cassava samples were coated with gold.

Table 1. Composition, yield and carbon conversion ratio of Nata de Cassava

Nata de Cassava	Glucose (% w/v)	Urea (% w/v)	*A. xylinum* (% v/v)	Yield (%)	CCR (%)
AX10 G5 U0.1	5	0.1	10	0.37	7.34
AX10 G7.5 U0.1	7.5	0.1	10	0.96	12.81
AX10 G10 U0.1	10	0.1	10	1.66	16.62
AX10 G5 U0.2	5	0.2	10	1.31	26.15
AX10 G7.5 U0.2	7.5	0.2	10	1.34	17.84
AX10 G10 U0.2	10	0.2	10	2.26	22.55
AX10 G5 U0.5	5	0.5	10	0.15	3.05
AX10 G7.5 U0.5	7.5	0.5	10	0.22	2.94
AX10 G10 U0.5	10	0.5	10	0.46	4.60
AX15 G5 U0.1	5	0.1	15	0.09	1.83
AX15 G7.5 U0.1	7.5	0.1	15	0.85	11.35
AX15 G10 U0.1	10	0.1	15	2.41	24.07
AX15 G5 U0.2	5	0.2	15	0.81	16.11
AX15 G7.5 U0.2	7.5	0.2	15	0.20	2.66
AX15 G10 U0.2	10	0.2	15	1.10	10.96
AX15 G5 U0.5	5	0.5	15	0.70	13.98
AX15 G7.5 U0.5	7.5	0.5	15	0.55	7.39
AX15 G10 U0.5	10	0.5	15	0.06	0.63
AX20 G5 U0.1	5	0.1	20	0.28	5.58
AX20 G7.5 U0.1	7.5	0.1	20	0.55	7.33
AX20 G10 U0.1	10	0.1	20	1.06	10.55
AX20 G5 U0.2	5	0.2	20	0.53	10.58
AX20 G7.5 U0.2	7.5	0.2	20	0.78	10.42
AX20 G10 U0.2	10	0.2	20	0.96	9.57
AX20 G5 U0.5	5	0.5	20	0.57	11.41
AX20 G7.5 U0.5	7.5	0.5	20	0.63	8.41
AX20 G10 U0.5	10	0.5	20	1.34	13.43

The X-ray diffraction (XRD) pattern was recorded in the wavelength of 1.54 Å, generated in voltage of 40 kV and the filament emission of 15 mA. Prior to analysis, the dried Nata de Cassava films were compressed on a disk. An Aeris Panalytical (Netherlands) was used to analyze them. The radiation reflection of Nata de Cassava samples was measured in the range of 2θ angle of 5°-80° and at room temperature.

The data obtained were analyzed using Analysis of variance (ANOVA) with three factors of Statistical Package for Social Sciences (SPSS version 23.0). Significant means were analyzed at 95% confidence interval ($p<0.05$).

Method and Procedure

Synthesis of Nata de Cassava was conducted with the static culture method [2], [18]. Nata de Cassava synthesis was conducted using liquid tapioca waste as a medium, sugar as a glucose source and urea as a nitrogen source. Liquid tapioca waste (200 ml) was heated in a beaker glass at 70-80 °C and then added with 5,7.5 and 10 % (w/v) of sugar and urea 0.1, 0.2 and 0.5 % (w/v). The composition of variation sugar (glucose), urea and *A. xylinum* is shown in Table 1. The mixture was then poured into a polypropylene container (1000 ml) and cooled at room temperature. Furthermore, 10, 15 and 20 % (v/v) of *A. xylinum* from the inoculum was added and incubated for ten days at room temperature [18]. The volume of bacteria was taken from the bacteria stock solution. After that Nata de Cassava was harvested, sterilized in boiling water, and immersed in ethanol, then oven-dried at 60 °C.

RESULT AND DISCUSSION

Fig. 1 shows the results of the initial condition and after the drying process of Nata de Cassava. In the initial condition (Fig. 1a), Nata de Cassava shows a physical appearance of thick and not transparent due to containing a lot of water and ethanol. However, the after-drying Nata de Cassava (Fig 1b) was thin and transparent due to the evaporation process of water and ethanol in Nata de Cassava during the drying process in the oven.

Figure 1. The Nata de Cassava by *A. xylinum* from the liquid tapioca waste (a) before and (b) after drying

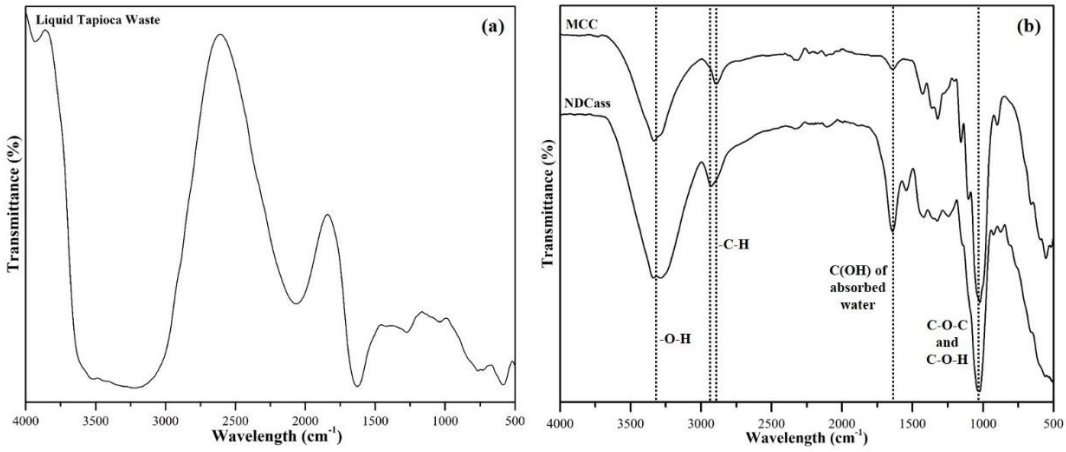

Figure 2. FTIR spectra of (a) liquid tapioca waste and (b) MCC and the optimum yield of Nata de Cassava

Chemical structure

The FTIR analysis of liquid tapioca waste, microcrystalline cellulose (MCC) as a standard and resulting Nata de Cassava was conducted to determine each functional group (Figure 2). The liquid tapioca waste has different FTIR spectra before and after fermentation. The spectra of liquid tapioca waste (Fig. 2a) are dominated by H_2O absorption [19]. The FTIR spectra of Nata de Cassava as the fermentation product of liquid tapioca waste are similar to the microcrystalline cellulose as the standard (Fig. 2b). It indicates that the Nata de Cassava consists of cellulose. There are almost no different FTIR spectra peaks among the resulting Nata de Cassava and MCC (Table 2). The main peak of cellulose in the FTIR

spectra of Nata de Cassava exhibited a broad peak at a wavenumber of 3287 to 3334 cm^{-1} are indicated by the presence of the hydroxyl groups (-OH) associated with the stretch of intra- and inter-chain hydrogen-bonded OH groups, while 2894 to 2927 cm^{-1} are related C-H stretching vibration of CH_2 of the methyl groups [3], [5], [6], [20], [21]. The absorption of 1640 cm^{-1} and 1419-1428 cm^{-1} indicated the the C(O-H of absorbed water) and CH_2 symmetrical bending or the surface of the carboxylate groups [6]. The observed peak at wavenumber 1036 cm^{-1} could be attributed to ether C-O-C and C-O-H stretching vibration of the sugar ring, while the band absorbed of 1324 cm^{-1} was correlated to O-H in-plane bending and 1257 cm^{-1} are related to C-O stretching, which indicated the presence of crystalline region within the structure [3], [6]. Furthermore, the peak at 848 cm^{-1} characterizing β-1,4-glycosidic [22], indicating that cellulosic membranes produced by bacterial cells included cellulose and were not impurities [3]. As shown in Fig. 2b, FTIR spectra peaks of MCC and Nata de Cassava exhibit almost similar positions and intensities and no particular difference. These FTIR spectra of Nata de Cassava showed that the bacterial cellulose production from liquid tapioca waste by *A. xylinum InaCC B422* has been conducted successfully.

Table 2. FTIR peak assignments for liquid tapioca waste, MCC and Nata de Cassava [23]

Liquid tapioca waste (cm^{-1})	MCC (cm^{-1})	Nata de Cassava (cm^{-1})	Assignment
3215	3334	3287	OH stretch vibration
	2894	2927	C-H stretching vibration of CH_2 of the methyl groups
2064	-	-	Several bands from overtone and combinations
1640	1641	1640	C(O-H of absorbed water)
-	-	1544	Protein amide II absorption
-	1428	1419	CH_2 symmetrical bending or surface carboxylate groups
-	1361	1361	C-H bending
-	1321	1324	O-H in-plane bending
-	-	1257	C-O stretching
-	1156	-	Anti-symmetric bridge COC stretching
	1103	-	C-O bond stretching
1038	1021	1036-1023	ether C-O-C and C-O-H stretching vibration of the sugar ring
764	899	848	β-1,4-glycosidic
-	657	658	C-OH out-of-plane bending

Morphology

The surface and cross-section morphology of the Nata de Cassava were analyzed using SEM. The micrographs of the surface morphology of Nata de Cassava are shown in Figure 3. Figure 3 shows that the Nata de Cassava was arranged of rod-shaped nanofiber that forms a fine and dense network, with a nano-sized diameter of approximately 20-120 nm. The nanofiber which forms the Nata de Cassava is arranged randomly. Bacterial cellulose nanostructures formed a highly porous three-dimensional network-like structure. Barshan *et al*., 2019 [3] and Salari *et al*., 2019 [8] obtained similar results. Meanwhile, the cross-sectional morphology shows that the Nata de Cassava comprises fiber layers. These fiber layers indicate that in the static culture method, Nata de Cassava was formed on the liquid-air surface and then grew downwards until all cells were trapped in the gel and became inactive due to a lack of oxygen [24].

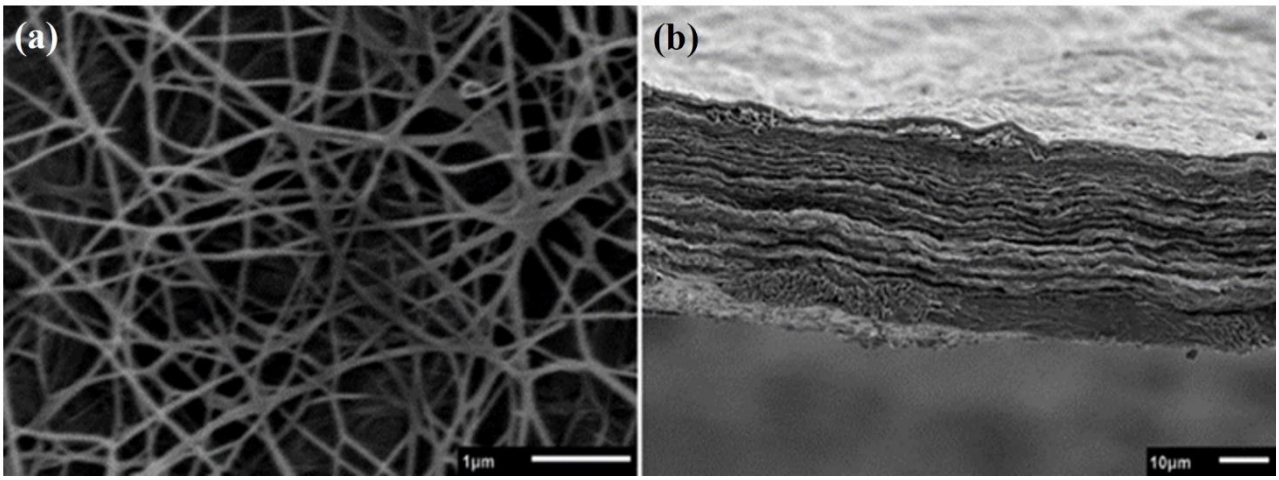

Figure 3. (a) Surface and (b) cross-section morphology of the optimum yield of Nata de Cassava

Crystallinity

Figure 4. shows an X-ray diffraction pattern of Nata de Cassava sample. The Nata de Cassava sample from liquid tapioca waste medium showed peaks around 2θ = 14.5°, 16.6°, and 22.6°, attributed to crystalline plates 101 (amorphous

region), 101 (amorphous region) and 002 (crystalline region), respectively, indicating the typical cellulose I structure [3], [6], [25]. The diffractogram reveals that Nata de Cassava produced by A. xylinum in liquid tapioca waste is similar to plant-based cellulose I structure. There is a slight difference in the Nata de Cassava peaks compared with MCC. The difference peak is only a small change in the intensity, representing differences in the cellulose crystallinity. A similar result was also found in the hot water-extracted medium and sugar cane molasses to produce bacterial cellulose [6].

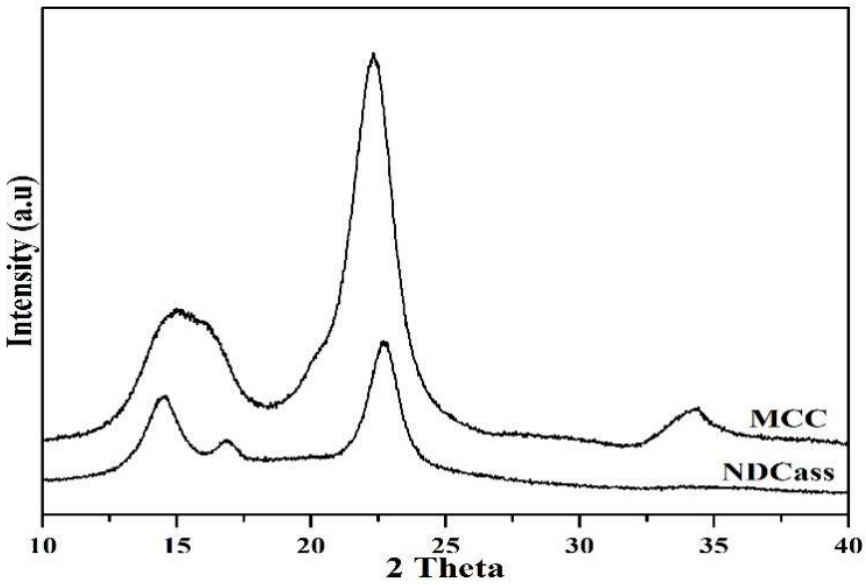

Figure 4. XRD patterns of the optimum yield of Nata de Cassava

Yield: effect of glucose

The effect of glucose concentration on the yield of Nata de Cassava produced is shown in Figure 5. In general, the more glucose added, the higher the yield of Nata de Cassava. The addition of glucose increases glucose concentration, increasing the availability of nutritional sources for the bacteria. Therefore, it can increase the growth and development of bacteria, resulting in higher production of bacterial cellulose. In Fig. 5a, at 10 % composition of *A. xylinum*, an increase in glucose concentration will further increase the resulting bacterial cellulose yield. In Fig. 5b, in the composition of *A. xylinum* with a concentration of 15 % and urea concentration of 0.1%, an increase in glucose concentration also increased the yield of bacterial cellulose produced. However, the composition of *A. xylinum* of 15 % with urea concentrations of 0.2 % and 0.5 % showed a slightly different trend of results. In the composition of *A. xylinum* by 15 % with urea concentrations of 0.2 % and 0.5 %, an increase in glucose concentration from 5 % to 7.5 % decreased bacterial cellulose yield. If the glucose concentration increased to 10 %, it again increased the bacterial cellulose yield in the composition containing 0.2 % urea, but further decreased the bacterial cellulose yield in the 0.5 % urea-containing composition. In Fig. 5c, the 20 % of *A. xylinum* composition also shows the phenomenon that the resulting bacterial cellulose yield increased with increasing glucose concentration that was added to the fermentation medium. This phenomenon of decreasing yield of bacterial cellulose might be due to the excess glucose that is not used by bacteria to produce bacterial cellulose. Salari *et al.*, 2019 reported an increase in glucose concentration in the bacterial growth medium could cause the unutilized of excess carbon sources. At high glucose concentrations, excess glucose that is not used for bacterial cellulose synthesis is oxidized to gluconic acid by *A. xylinum*. In addition, the accumulation of gluconic acid drastically decreases the pH of the culture medium and inhibits bacterial cellulose production [8]. However, if the glucose content in the culture medium is greater than that of the acetic acid, the bacteria can actively and continuously convert glucose into gluconic acid during the fermentation process. Finally, a medium with low pH is suboptimal for bacteria growth, with experimental results indicating that bacterial cellulose biosynthesis terminated when the pH value was outside the suitable pH range for bacterial cellulose formation of $4<pH<7$ [6].

Carbon source has a significant influence on the yield of bacterial cellulose [1]. Anindya *et al.*, 2019 [5] indicated that bacterial cellulose yields were strongly influenced by the type and concentration of carbon sources. Glucose is a carbon source that has been widely used for the production of bacterial cellulose. Glucose showed a more complex relationship with medium height, concentration in the medium and surface area available for growth. The higher concentration of glucose in the medium is expected to produce a higher yield of bacterial cellulose, especially in shallow growth conditions. However, Anindya *et al.*, 2019 also reported that there is always sugar (glucose) that is not used by bacteria to produce bacterial cellulose, so an increase in sugar concentration in the bacterial growth medium can cause excess carbon sources to be useless [5].

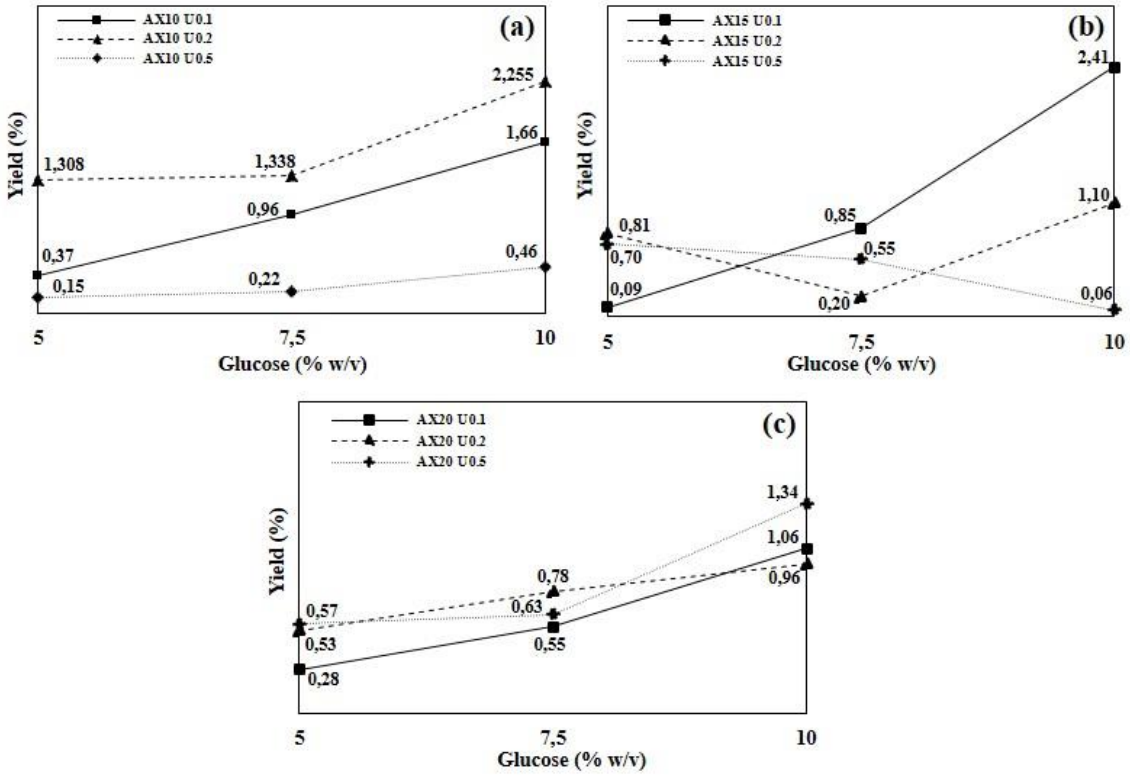

Figure 5. The effect of glucose concentration towards the yield of Nata de Cassava on bacterial concentration of (a) 10 % (v/v), (b) 15 % (v/v) and (c) 20 % (v/v)

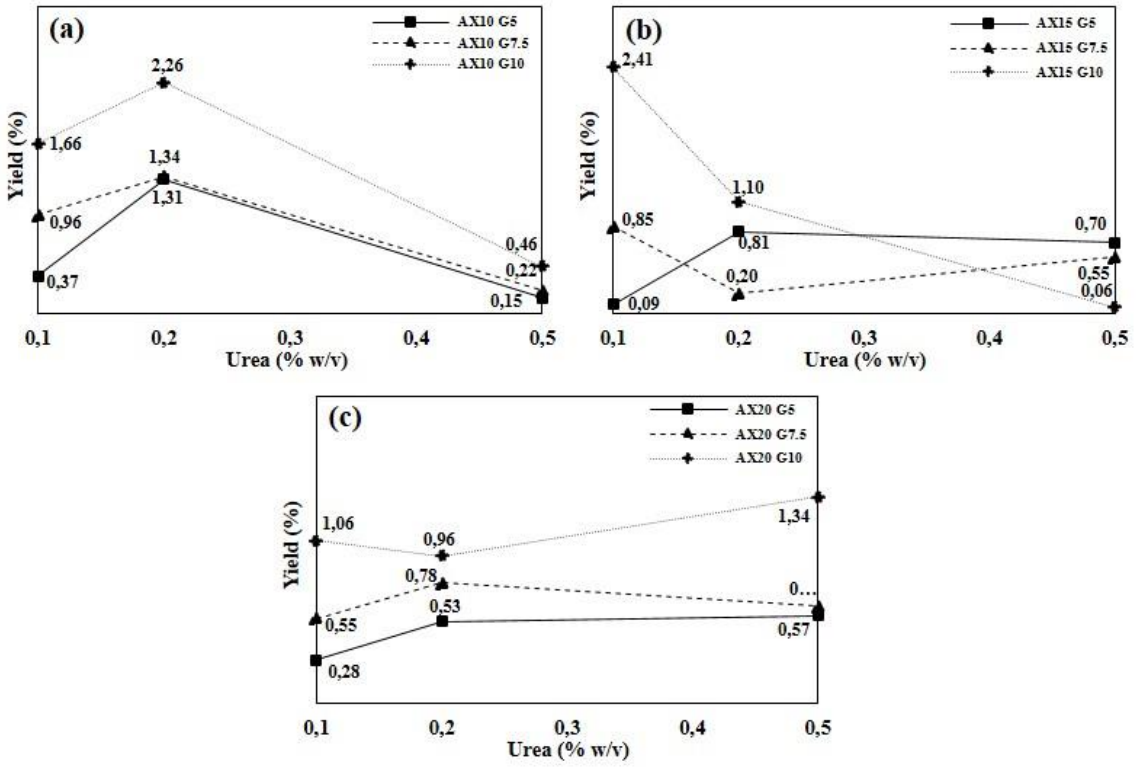

Figure 6. The effect of urea concentration towards the yield of Nata de Cassava on bacterial concentration of (a) 10 % (v/v), (b) 15 % (v/v) and (c) 20 % (v/v)

Yield: Effect of urea

Nitrogen is another essential component in cell growth and bacterial cellulose production [1]. The high production of bacterial cellulose was related to the presence of nitrogen that enhances bacterial growth, thereby increasing bacterial

cellulose yield [8]. Fig. 6a shows that in the use of *A. xylinum* with a concentration of 10 % with various glucose concentrations, the increase in urea concentration from 0.1 % to 0.2 % increased the yield of bacterial cellulose produced. These results are confirmed by Firdaus *et al*., 2018 that increasing the concentration of nitrogen sources can increase the concentration of bacterial cellulose yield. This shows that the bacterium can efficiently utilize the additional nitrogen sources concentration in the medium for bacterial cellulose production [10]. However, when the urea concentration was increased by 0.5 %, it decreased the yield of bacterial cellulose, even though the produced yield was lower when compared to the use of urea by 0.1 %. Fig. 6b shows that with *A. xylinum* concentration of 15 %, the increasing urea concentration from 0.1 % to 0.2 % increased the yield in compositions that contained 5 % glucose but decreased the yield in compositions containing glucose at concentrations of 7.5 % and 10 %, respectively. If the urea concentration was further increased to 0.5 %, it did not significantly affect the produced yield in a composition containing 5 % glucose. The different results were shown in compositions containing 7.5 % and 10 % glucose. The use of a urea concentration of 0.5 % increased the obtained yield in the composition containing glucose 7.5 % but reduced the yield in the composition containing glucose by 10 %. While Fig. 6c shows that with 20 % use of *A. xylinum*, an increase in urea concentration from 0.1 to 0.2 % increased the bacterial cellulose yields in compositions containing glucose by 5 % and 7.5 %, but decreased the yields in compositions containing glucose by 10 %. If the urea concentration increased to 0.5 %, it did not significantly affect the yield in the composition containing 5 % glucose, but decreased the yield in the composition containing 7.5 % glucose and increase the yield in the composition containing 10 % glucose. The phenomenon of decreasing bacterial cellulose yield is probably due to the absence of bacterial cellulose growth and production. This might be due to the presence or the formation of several inhibitors that inhibited the growth and production of bacterial cellulose. This might be related to the lack of balance between carbon and nitrogen in the growth bacterial medium [1].

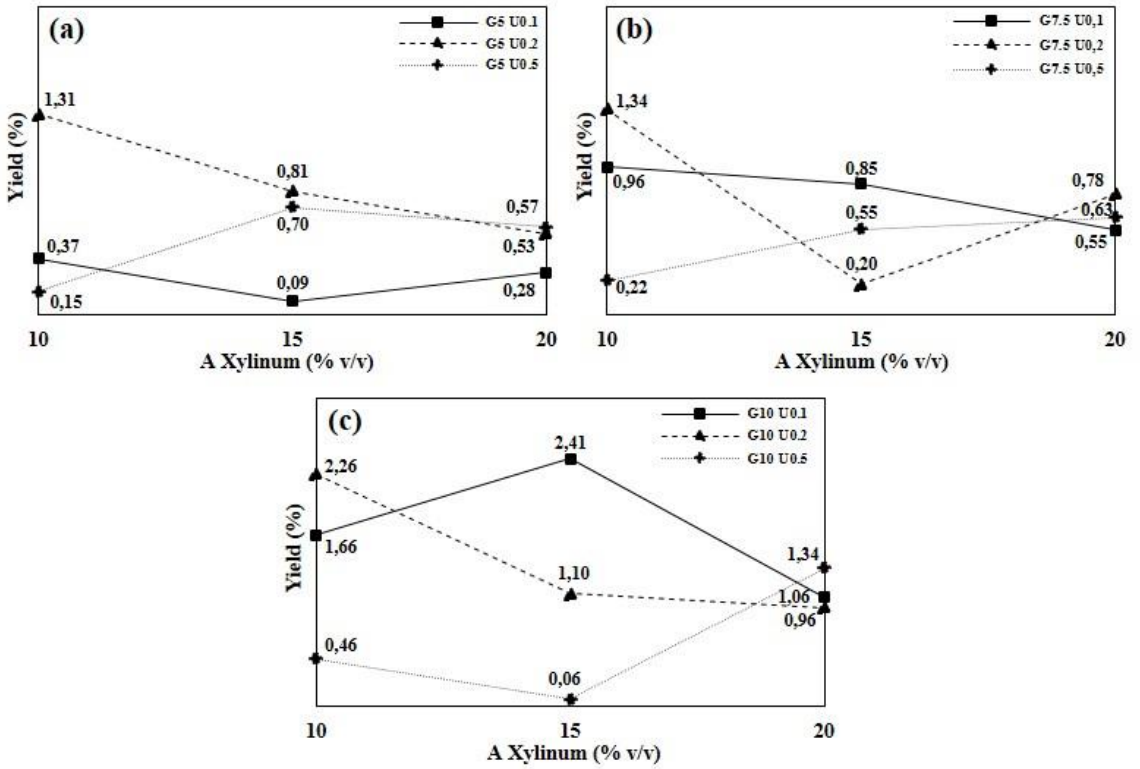

Figure 7. The effect of bacterial concentration towards the yield of Nata de Cassava on glucose concentration of (a) 5 % (w/v), (b) 7.5 % (w/v) and (c) 10 % (w/v)

Yield: Effect of A. xylinum

Fig. 7a shows that at a glucose concentration of 5 %, an increase in the amount of *A. xylinum* from 10 % to 15 % reduced the bacterial cellulose yields in compositions containing urea by 0.1 % and 0.2 %, but increased the bacterial cellulose yields in compositions containing urea with concentrations of 0.5 %. When the amount of *A. xylinum* added to the fermentation medium was increased to 20 %, it would increase the bacterial cellulose yield in the composition containing 0.1 % of urea, but would decrease the bacterial cellulose yield in the composition containing 0.2 % and 0.5 % urea. In Fig. 7b, with a fixed glucose concentration of 7.5 %, an increase in the amount of *A. xylinum* from 10 % to 15 % resulted in a decrease in the bacterial cellulose yield in compositions containing 0.1 % and 0.2 % urea, but increase in bacterial cellulose yield in compositions containing 0.5 % urea. And if the amount of *A. xylinum* was increased to 20 %, it would further reduce the yield of bacterial cellulose in compositions containing 0.1 % urea, but would increase bacterial

cellulose yields in compositions containing urea with concentrations of 0.2 % and 0.5 %. While Fig. 7c shows that at 10 % glucose concentration, an increase in the amount of *A. xylinum* from 10 % to 15 % increased the bacterial cellulose yield in compositions containing 0.1 % urea, but decreased bacterial cellulose yields in compositions containing urea by 0.2 % and 0.5 %, respectively. In addition, when the amount of *A. Xylinum* increased to 20 %, which decreased the bacterial cellulose yield in the composition containing urea with concentrations of 0.1 % and 0.2 %, but increased in the composition containing urea by 20 %. The composition that produced the highest bacterial cellulose yield of 2.41 % was the composition using 15 % *A. xylinum*, 10 % glucose and 0.1 % urea in the fermentation medium. The increase or decrease in the yield of produced bacterial cellulose is highly dependent on the balance of factors that affect bacterial growth, which affects the yield of bacterial cellulose produced [2].

In this study, *A. xylinum* was used. *A. xylinum* is a non-photosynthetic, aerobic bacteria and includes gram-negative bacteria, which can be fed with sugar and converted into bacterial cellulose [3]. *A. xylinum* has been shown to have the highest rate of bacterial cellulose production among all the bacteria types [2]. The effect of the number of bacteria added to the fermentation medium on the bacterial cellulose yield is shown in Fig. 7. In general, the greater the number of bacteria added or used in the culture medium, the lower the yield of bacterial cellulose produced. The addition of the number of bacteria in the medium will change the composition of the balance between the number of bacteria and the nutrients available in the fermentation medium. With the increasing number of bacteria, it requires more nutritional sources and if the available nutritional sources are fixed (not added), the bacteria will likely lack nutrients, thereby inhibiting the growth and development of bacteria and resulting in a decrease in the production of bacterial cellulose produced.

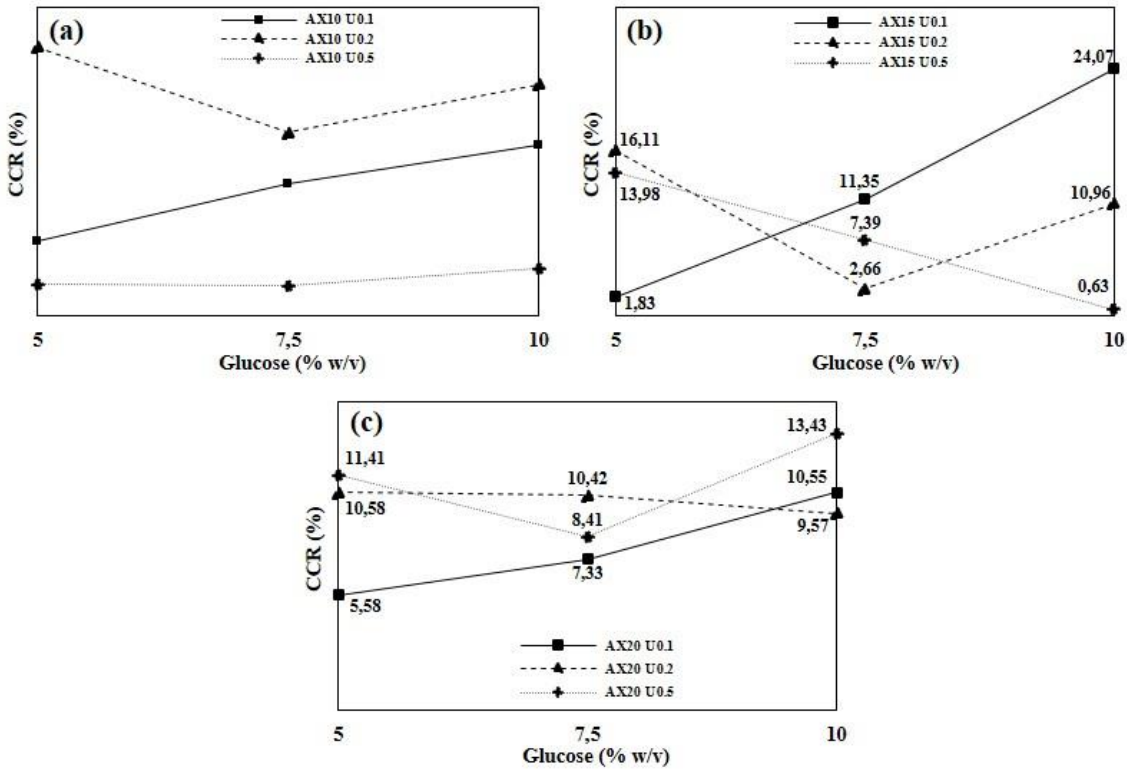

Figure 8. The effect of glucose concentration towards the CCR of Nata de Cassava on bacterial concentration of (a) 10 % (v/v), (b) 15 % (v/v) and (c) 20 % (v/v)

Carbon Conversion Ratio (CCR): Effect of glucose

The carbon conversion ratio was obtained by measuring the weight of produced bacterial cellulose, then divided by the weight of glucose (sugar) added to the culture medium. The interaction between the effect of glucose concentration added to the fermentation medium is shown in Figure 8. In Fig. 8a, at 10 % of *A. xylinum* concentration, the increasing glucose concentration from 5 % to 7.5 % increased CCR of bacterial cellulose in compositions containing 0.1 % of urea, but decreased CCR of bacterial cellulose in compositions containing urea of 0.2 % and did not have a significant effect on the composition containing 0.5 % of urea. When the concentration of glucose in the fermentation medium increased to 10 %, it increased the CCR of bacterial cellulose in the composition containing urea by 0.1 %, 0.2 % or 0.5 %. The increase in CCR of bacterial cellulose might be due to the more glucose added, the more glucose availability in the fermentation medium. The more glucose available, the more glucose can be converted by *A. xylinum* bacteria into cellulose. The increasing number of produced bacterial cellulose increased the CCR of bacterial cellulose. It has been reported that bacteria can convert glucose molecules into bacterial cellulose production [2]. While in Fig. 8b, the

composition of *A. xylinum* with a concentration of 15 % and the increase of the glucose concentration from 5 % to 10 %, resulting in further increasing of the CCR of bacterial cellulose in the composition containing 0.1 % urea concentration, but decreasing the CCR of bacterial cellulose in the composition containing urea concentration of 0.2 % and 5 %. Then if the glucose concentration increased to 10 %, it increased the CCR of bacterial cellulose in the composition containing urea by 0.1 % and 0.2 %, but decreased the CCR of bacterial cellulose in the composition containing urea by 5 %. In Fig. 8c, the composition of *A. xylinum* with a concentration of 20 % with the increase of the glucose concentration from 5 % to 7.5 % resulted in the increasing CCR of bacterial cellulose in the composition containing urea by 0.1 %, did not have much effect on the CCR of bacterial cellulose in the composition containing urea of 0.2 % and decreased the CCR of bacterial cellulose in the composition containing urea by 0.5 %. Then, if the concentration of glucose in the fermentation medium increased to 10 %, it increased the CCR of bacterial cellulose in the compositions containing urea by 0.1 % and 0.5 %, but decreased the CCR of bacterial cellulose in the compositions containing 0.2 % of urea. The decrease in CCR was due to the fact that the more glucose added would potentially cause excess glucose in the fermentation medium, which could not be converted by bacteria into cellulose and inhibited the production of bacterial cellulose. Salari *et al*., 2019 reported, at high glucose concentrations, the excess of glucose that is not used for bacterial cellulose production is oxidized to gluconic acid by *A. xylinum*. The accumulation of gluconic acid inhibits bacterial cellulose production [8], thereby reducing the CCR.

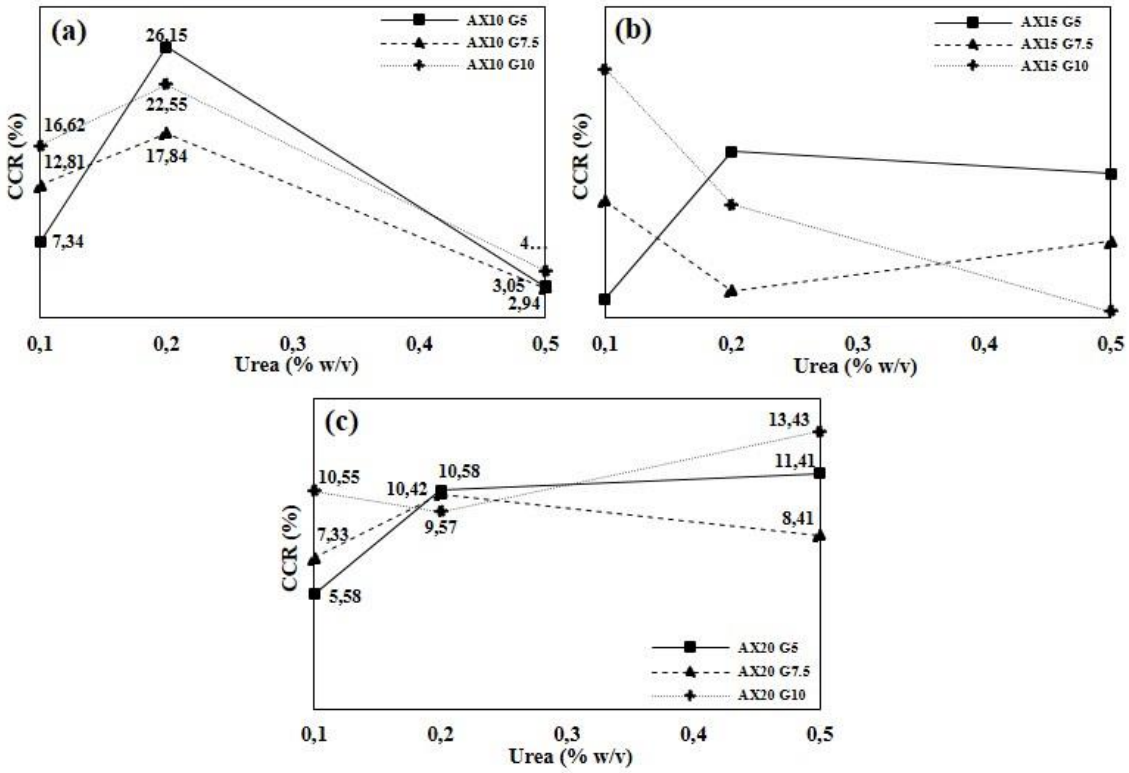

Figure 9. The effect of urea concentration towards the CCR of Nata de Cassava on bacterial concentration of (a) 10 % (v/v), (b) 15 % (v/v) and (c) 20 % (v/v)

Carbon Conversion Ratio (CCR): Effect of urea

The effect of urea as a nitrogen source on the carbon conversion ratio of Nata de Cassava is shown in Figure 9. In Fig. 9a, in a composition containing 10 % of *A. xylinum* concentration with 5 %, 7.5 % and 10 % of glucose concentrations, the increase in urea concentration from 0.1 % to 0.2 % increased the CCR of bacterial cellulose. However, when the urea concentration increased to 0.5 %, it decreased the CCR of bacterial cellulose, even though the resulting CCR of bacterial cellulose was smaller than that of using 0.1 % concentration. This indicated that at the fixed concentration of *A. xylinum* with the various glucose concentration in the fermentation medium, the addition of urea would increase the CCR of bacterial cellulose. However, when the urea concentration is higher, at specific concentrations, it decreases the CCR of bacterial cellulose. The increasing urea concentration can disrupt the balance of conditions that bacteria need to grow and produce bacterial cellulose. Thereby, it inhibits the process of glucose converting into cellulose by the bacteria and lowers the CCR. In Fig. 9b, with an *A. xylinum* concentration of 15 %, the addition of urea from a concentration of 0.1 % to 0.2 % increased the CCR of bacterial cellulose in the composition containing 5 % glucose, but decreased the CCR of bacterial cellulose in the composition containing glucose 7.5 % and 10 %. When the urea concentration was further increased to 0.5 %, it decreased the CCR of bacterial cellulose in the composition containing 5 % and 10 % of glucose, but increased the CCR of bacterial cellulose in the composition containing glucose with a concentration of 7.5 %. While in Fig. 9c,

shows that at 20 % of *A. xylinum* concentration, an increase in urea concentration from 0.1 % to 0.2 % increased the CCR of bacterial cellulose in the composition containing 5% and 7.5 % of glucose, but decreased the CCR of bacterial cellulose in a composition containing 20 % of glucose. Furthermore, if the urea concentration was further increased to 0.5 %, it would increase the CCR of bacterial cellulose in the composition containing 5 % and 10 % glucose, but decrease the CCR of bacterial cellulose in the composition containing glucose 7.5 %. Salari *et al*., 2019 reported that the high production of bacterial cellulose was related to the presence of nitrogen that enhances bacterial growth, thereby increasing the conversion of glucose to bacterial cellulose and increasing bacterial cellulose production [8]. However, if the concentration of nitrogen is excessive, it can disrupt the balance of oxygen and nitrogen bacteria need to grow. The lack of balance between carbon and nitrogen in the growth bacterial medium [1] will inhibit the ability of bacteria to convert sugar into bacterial cellulose and reduce the CCR.

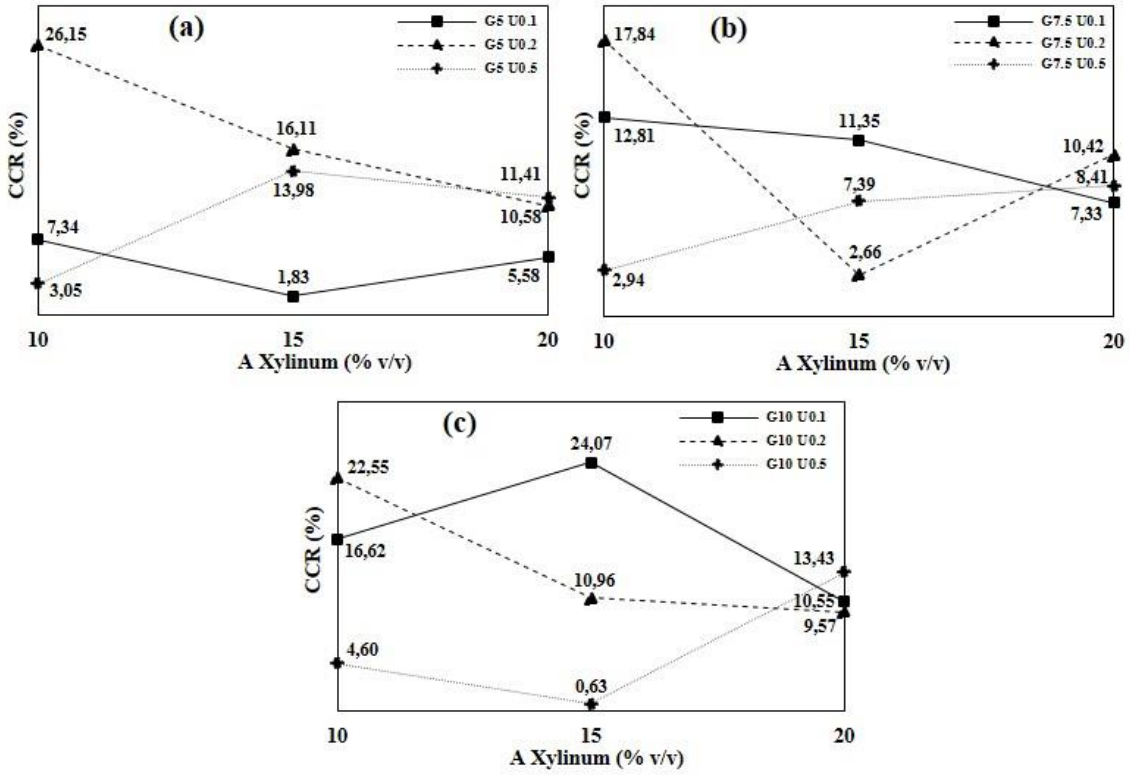

Figure 10. The effect of bacterial concentration towards the CCR of Nata de Cassava on glucose concentration of (a) 5 % (w/v), (b) 7.5 % (w/v) and (c) 10 % (w/v)

Carbon Conversion Ratio (CCR): Effect of A. xylinum

Figure 10 shows the effect of the number of *A. xylinum* bacteria added in the fermentation medium on the CCR of bacterial cellulose. In Fig. 10a, a fixed glucose concentration of 5% in the fermentation medium and an increasing amount of *A. xylinum* from 10 % to 15 % resulted in a reduction of the CCR of bacterial cellulose in the compositions containing 0.1 % and 0.2 % urea. However, this condition parameter increases the CCR of bacterial cellulose on compositions containing 0.5 % urea. When the amount of *A. xylinum* in the fermentation medium increased to 20 %, it increased the CCR of bacterial cellulose in the composition containing 0.1 % urea, but decreased the CCR of bacterial cellulose in the composition containing 0.2 % and 0.5 % urea. In Fig. 10b, with a fixed glucose concentration of 7.5 % and an increasing amount of *A. xylinum* used in the fermentation medium from 10 % to 15 % decreased the CCR of bacterial cellulose in the compositions containing 0.1 % and 0.2 % urea, but will increase the CCR of bacterial cellulose in the composition containing urea by 0.5 %. Then when *A. xylinum* increased to 20 %, it decreased the CCR of bacterial cellulose in the composition containing 0.1 % urea, but increased the CCR of bacterial cellulose in the composition containing 0.2 % and 0.5 % urea. While Fig. 10c shows that when the glucose concentration remains at 10 % and an increase in the number of *A. xylinum* bacteria from 10 % to 15 % resulted in an increase in the CCR of bacterial cellulose in the composition containing urea by 0.1 % but decrease the CCR of bacterial cellulose in the composition containing 0.2 % and 0.5 % urea. Furthermore, if A. *xylinum* was further propagated to 20 %, it decreased the CCR of bacterial cellulose in the compositions containing 0.1 % and 0.2 % urea, but increased the CCR of bacterial cellulose in the compositions containing urea by 0.5 %. The composition that produced the highest CCR of 26.15 % was the composition that used 10 % *A. xylinum*, 5 % glucose and 0.2 % urea in the fermentation medium. The increase in CCR was due to an increase in the concentration of bacteria. The higher concentration of bacteria, the more glucose can be converted by bacteria into cellulose, thereby increasing CCR. However, at the fixed glucose composition, the addition of the number of bacteria in the fermentation medium will change the balance composition of the number of bacteria and the nutrients available in the fermentation

medium. The limited availability of glucose will limit the glucose that can be converted by bacteria to cellulose and decrease the CCR. The increase or decrease in the CCR and yield of produced bacterial cellulose is highly dependent on the balance of factors that affect bacterial growth, which affects the CCR and yield of bacterial cellulose produced [2].

Statistical Analysis

The statistical analysis showed that in the mean of yield of Nata de Cassava, the glucose concentration have significant changes (p=0.018). While both urea and bacterial concentration gave no significant different to the yield of Nata de Cassava with p-value of 0.085 and 0.491, respectively. In the mean of carbon conversion ratio (CCR), all of the parameters did not provide significant different due to their p-value more than 0.05. The p-value for the effect of glucose concentration was 0.376 then 0.061 and 0.420 for urea and bacteria concentration, respectively.

According to the results, the liquid tapioca waste could be utilized as an abundant, cheap and suitable culture medium for the production of bacterial cellulose, Nata de Cassava. This result can provide more value for liquid tapioca waste, so it is expected to reduce environmental problems related to liquid tapioca waste. However, the application of liquid tapioca waste as a medium for the production of bacterial cellulose must be carried out as soon as possible. This is because if it is stored, it will cause an unpleasant odor. Besides, the pH of liquid tapioca waste will also decrease, so it cannot be used directly as a culture medium.

CONCLUSION

In this research, the synthesis of Nata de Cassava as the obtained bacterial cellulose by *Acetobacter xylinum* using the liquid tapioca waste as the medium has been successfully carried out. Based on the cross-sectional morphology from SEM, the FTIR spectra and XRD pattern, it proved that the bacterial cellulose production from liquid tapioca waste by *A. xylinum InaCC B422* has been conducted successfully. The highest production yield of 2.41 % was obtained when the used compositions were 15 % *A. xylinum*, 10 % glucose and 0.1 % urea in the fermentation medium. In terms of carbon conversion ratio, the highest ratio, 26 %, was achieved with the composition that used 10 % *A. xylinum*, 5 % glucose and 0.2 % urea in the fermentation medium. According to the results, the liquid tapioca waste could be used as an abundant, cheap and suitable culture medium for the production of bacterial cellulose, Nata de Cassava.

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