Rice Bran, an Alternative Nitrogen Source for *Acetobacter xylinum* Bacterial Cellulose Synthesis

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Rice bran was introduced as an alternative nitrogen source to Hestrin and Schramm (HS) medium in HS *Acetobacter xylinum* culture media in bacterial cellulose (B.C) synthesis. The results indicated an unchanged composition of crude protein per increase in incubation days while diluted protein increased with incubation period. Fourier transform infrared spectroscopy (FTIR) results showed the absorbance of OH groups. It was confirmed that the BC yield was directly proportional to the rice bran content. A general observation indicated a gradual transformation from a highly crystalline structure to a very amorphous structure as rice bran content increased with the endothermic decomposition of samples being recorded between 113.5 °C and 125.6 °C.

Keywords: Bacterial cellulose; Rice bran extract; Nitrogen source

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INTRODUCTION

Cellulose, the most abundant polymer on earth, is a homopolymer of d-glucopyranose residues linked by β-(1 → 4) glycosidic linkages (Liu and Sun 2010). It is obtained from various sources such as plants, microbes, naturally occurring chemical reactions, and enzymatic synthesis (Bomble *et al.* 2017). However, the most explored and utilized cellulose among the various sources has been plant and enzymatic cellulose, partly because of the consistency in obtaining them and also the characteristics that they demonstrate (Coseri 2017). Plant-derived cellulose is harvested from the cell walls of the plant (Lima *et al.* 2017). However, this type of cellulose comes with several impurities, including lignin, pectin, and hemicelluloses, *etc.* (Wu *et al.* 2016), thereby requiring further purification to obtain the pure cellulose, hence affecting the cost of production. This has led to a shift in focus to bacterial or enzymatic cellulose production (Islam *et al.* 2017)

Gluconacetobacter, *Acetobacter*, and *Komagataeibacter* genera have been found to be the best cellulose producers within the bacteria kingdom (Illeghems *et al.* 2016; Pacheco *et al.* 2017) with *Acetobacter* giving the purest and highest fibril yield (Franz and Blaschek 1990). The cellulose produced by these microorganisms, known as bacterial cellulose (BC) is obtained as a gel-like three-dimensional mat formed by entangled nanofibrils of cellulose (Pacheco *et al.* 2017). Structurally, BC is composed of a dense and well-organized three-dimensional network of fibrils that forms porous sheets with higher crystallinity, thermal stability, and mechanical strength far superior to plant cellulose (Stumpf *et al.* 2018). The BC also possesses high water-absorbing capabilities in the form of a hydrogel, moderate biocompatibility, and partial degradability (Islam *et al.* 2017). Nevertheless, in terms of BC chemical interaction with other function groups,

due to the abundance of OH groups, there is a higher tendency of reactivity. However, its higher crystalline nature makes hydroxyl group accessibility difficult, therefore hindering proper deposition of functional modifiers (Troev 2018). Furthermore, BC application remains limited due to the high costs of production arising from culture media preparations (Patel *et al.* 2017); as such, strategies including the genetic improvement of the cellulose-producing microorganisms and the formulation of low-cost culture media are being investigated (Jang *et al.* 2017).

Hestrin and Schramm (HS) proposed a culture medium that has become the backbone of BC production for the past decade (Fan *et al.* 2016). This proposed culture media was composed of glucose, yeast extract, peptone, and mineral salts (Schramm and Hestrin 1954). Throughout the years, many modifications have been made to the proposed composition of HS medium, such as the use of mono and disaccharides such as fructose, sucrose, maltose, cellobiose, xylose, galactose, and alditols, which have been successfully used as carbon sources (Esa *et al.* 2014; Pacheco *et al.* 2017). Other methods that have also been proposed were the use of industrial wastes or by-products from agro-forest and food industry BC production (Carreira *et al.* 2011). Typical examples of industrial waste and agro-forest used in BC production included Konjac powder (Lin *et al.* 2014), beet molasses (Pacheco *et al.* 2017), sugar-cane molasses (Costa *et al.* 2017), and corn steep liquor (Li *et al.* 2016), as a source of carbon, nitrogen, and other nutrients.

Among all the modifications to the culture media for bacterial growth, many have focused on the carbon source provision for the bacteria with respect to which carbon source affords a better yield of cellulose. In contrast, only a fraction of the research focused on the nitrogen source, making use of ammonium sulfate derivatives (Santos *et al.* 2013), and even that was abandoned because it was concluded that the BC yield was significantly low (Santos *et al.* 2013). Nature has demonstrated multiplicity in nitrogen sources, but the question remains as to whether these nitrogen sources are life-supportive enough for *Acetobacter* and apparent BC yield (Cragg and Newman 2010). Among these organic sources of nitrogen, rice bran has been demonstrated to be more cost-effective and a better replacement for yeast extract (Sutanto *et al.* 2017).

Rice bran is a major co-product of the rice milling process, accounting for 5% to 8% of milled rice (Patil *et al.* 2016). It is a natural source of protein (14% to 16%), fat (12% to 23%), crude fiber (8% to 10%), carbohydrates, vitamins, minerals, essential unsaturated fatty acids, and phenolics (Patil *et al.* 2016). In China and other countries, approximately one million tons of bran are produced annually and used predominantly for animal feed (Rosentrater and Evers 2018). The use of rice bran has gained increased attention over the past few decades due to the fact that during the processing of whole rice, large amounts of the grain's outer layers are removed, raising the concentration of nutrients in the bran, hence making it a very important source of nutrients for the food industry and human consumption. Nevertheless, a drawback to the use of rice bran in bacterial synthesis is enzyme rancification (Braghini *et al.* 2009; Faccin *et al.* 2009), which occurs as a result of the presence of endogenous lipoxygenase (Brandicourt *et al.* 2015; Umeno *et al.* 2017). In view of this, a proposed enzymatic inactivation was prescribed to cater for its instability through heat (Gul *et al.* 2015; Cervantes-Elizarrarás *et al.* 2017)

It was confirmed that enzymatic inactivation did not only stabilize the rice bran content but also there was a significant increase in the nutritional values (Rafe and Sadeghian 2017). The values ranged from 11.6% to 13.6% dry basis (d.b.) (Patil *et al.* 2016). It was realized that the protein content of unstabilized rice bran was 13.0%,

whereas the highest protein content (13.6%) was observed for the rice bran sample exposed to longer heat treatment (Patil *et al.* 2016). With the increase in the time of exposure at any given available power, protein content was increased (Malik and Saini 2017; Wang *et al.* 2017a)

Despite all of the functional potential of rice bran, there have been relatively few attempts to use this material in bacterial culture and basically no use in *Acetobacter xylinum* species synthesis (Zhu *et al.* 2013; Matano *et al.* 2014). Therefore, this research focuses on the use of rice bran as an alternative nitrogen source to yeast extract in *Acetobacter xylinum* bacterial cellulose synthesis.

EXPERIMENTAL

Materials

Peptone, D-mannitol, and yeast extract were purchased from Sinopharm Chemical Reagent Co., Ltd. (Ningbo, China), while the bacterial strain was obtained from Shanghai Xiejiu Bio-Tech Co., Ltd. (Shanghai, China). In addition, 1,4-diazabicyclo[2.2.2]octane (DABCO) was obtained from Sigma-Aldrich (Shanghai, China).

Bacterial strain

The bacterial strain used was *Acetobacter xylinum* supplied by Shanghai Xiejiu China (Shanghai, China). The strain was cultured in mannitol yeast extract (MYE) agar containing 100 g D-mannitol, 10 g yeast extract, and 5 g peptone, at 28 °C for 3 days. Working cultures were routinely prepared in MYE and stored at 4 °C until further use.

Methods

Differential scanning calorimetry (DSC)

The DSC curves were obtained using a DSC-Q200 calorimeter (TA Instruments Co., Pennsylvania, USA) that was calibrated according to the manufacturer's recommendation: standard indium 99.99% purity, m.p. 156.6 °C, and ΔH 28.56 J g^{-1}. A mass sample was approximately 6 mg and the samples were heated from 0 °C to 300 °C using an aluminum crucible with a perforated lid with a 1.0-mm orifice, under N_2 atmosphere, with a flow rate of 50 mL min^{-1} and a heating rate of 1 °C min^{-1}.

Fourier transform infrared spectroscopy (FTIR)

A Nicolet FTIR coupled spectrophotometer (Thermo Fisher Scientific Inc. Massachusetts, USA) with gas cell and a DTG S KBr detector (Dexter Research Center, Inc., Michigan USA) was used. For the coupling, a stainless-steel transfer line of 120 cm in length and 3 mm in diameter was employed, which was heated to 250 °C. The gas cell was maintained at 250 °C and the spectra were obtained with a resolution of 4 cm^{-1} and Omnic software (Thermo Fisher Scientific Inc Massachusetts, USA), fitted with spectra from 46 different libraries, was used for data analysis.

X-ray diffraction (XRD)

The XRD measurements were performed using a Bruker D8 Advance X-ray diffractometer (Bruker, Shanghai, China) with copper-radiation (wavelength λ = 1.54 Å). The scanning speed was set at 4° min^{-1} XRD. The XRD data were retrieved over a 2θ range between 0° and 100° to be represented with the peaks indexed. XRD analysis

was strongly dependent on how well the crystallographic data inputs were selected. The analysis consisted of the identification of the phases present in the samples, associated with choosing the crystallographic information files with the best fit results (evaluated on the basis of the agreement indices - R_{wp}, χi^2).

Preparation of BC

Acetobacter xylinum (*G. xylinus*) BC pellicles were synthesized in a Hestrin and Schramm (HS) medium with minor modification (2.5 % D-mannitol, 0.3% bacto-peptone, 2.5% yeast) (Santos *et al.* 2013) and dissolved in 100 mL distilled water at neutral pH. The flasks were incubated statically at 30 °C for 7 days.

Table 1. Culture Modification Chart of Rice Bran Against Different Carbon Sources and Calculated Nitrogen Content of the Media

Culture Modification	Coded Name
Peptone(0.3g) + D-Mannitol (2.5g) + Rice Bran Extract(0.5g)	S = 1
Peptone (0.3g) + Glucose(2.5g) + Rice Bran Extract(0.5g)	S = 2
Peptone (0.3g) + Glycerol(2.5g)+ Rice Bran Extract(0.5g)	S = 3

SAMPLE	C: N RATIO FOR D-M/P	NITROGEN INDEX OF YEAST EXTRACT (%) (Mean± SD)	NITROGEN INDEX OF RICE BRAN (%) (Mean± SD)
Control	8.3:1	1.380±0.011	-
S=1=1	8.3:1	1.380±0.011	0.238±0.035
S=1=2	8.3:1	1.380±0.011	0.476±0.069
S=1=3	8.3:1	1.380±0.011	0.714±0.024

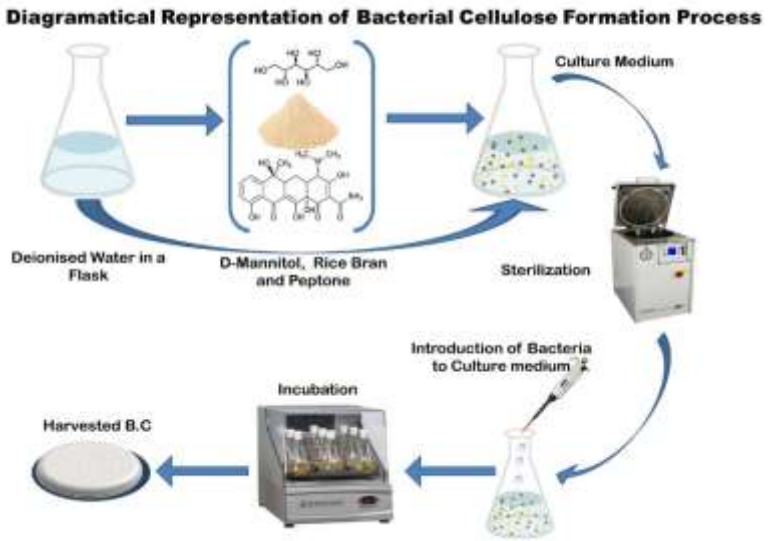

Fig. 1. Schematic representation of BC synthesis process

The synthesized cellulose was dipped into 0.1 M NaOH for 4 h at 80 °C to clear the cells and culture liquid (Long *et al.* 2014). Afterwards, the pretreated BC was rinsed three times in pH 7 distilled water. Modifications were therefore made to the carbon and nitrogen sources with the measuring parameters remaining the same. To obtain the best combination of carbon-nitrogen sources for the bacterial growth, three main carbon sources were selected against the focal nitrogen source in rice bran extract. The mode of modification of the culture media and the process of bacterial cellulose synthesis are summarized in Table 1 and Fig. 1, respectively. In order to achieve the goal of substituting yeast extract with rice bran, the carbon to nitrogen ratio(C:N) for the best carbon source in D-mannitol and one part of the nitrogen source in peptone was kept constant whiles the second nitrogen source in rice bran was added at varying amounts.

Colony count

To ascertain the bacterial growth support of the carbon and nitrogen source substituents, a plate count was performed using the serial dilution protocol, with the results shown in Fig. 2. Plate count agar was first prepared using 0.6% glucose, 0.8% bacto-peptone, 2.5% yeast (Chao *et al.* 1997), and 2.5 g of agar, which was dissolved in distilled water (neutral pH). The same recipe was used as the diluting solvent but without agar. The broth was then sterilized for 9 h, spread on six plates, and allowed to settle for 12 h (Chao *et al.* 1997). The number of plates corresponded to the amount of medium to be used in calculating the number of replications and dilutions (Carvalhal *et al.* 1991). In this protocol, 9.0 mL of the diluent was pipetted into each of the 6 test tubes and tightly closed to dilute it 10 fold (dilution = 10^{-1}). For the bacterial sample of each culture media, 1 mL of the culture medium was pipetted aseptically into a 9-mL tube to dilute it 10 fold (in other words, dilution = 10^{-1}). Next, 1 mL of the previous 10^{-1} dilution was transferred to another 9-mL test tube. This process was repeated until all 6 tubes were exhausted to complete the dilution. For each dilution, a separately sterilized pipette was used to provide consistency in the quantity of bacterial present (Carvalhal *et al.* 1991). This was followed by a drop of 0.1 mL of each dilution onto an agar plate that was tilted for the drop to run down the plate. The plates were then incubated for 24 h to determine the colony, shown in Fig. 2**.**

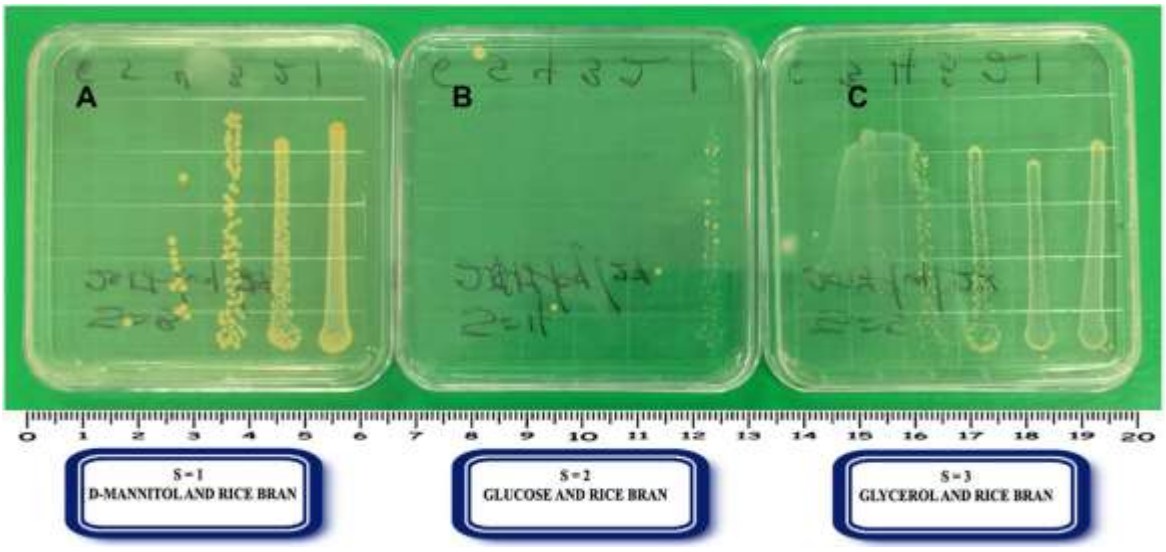

Fig. 2. Plate count confirmation of bacterial growth support of the nutrient sources

The results shown in Fig. 2 indicated that the alternative nitrogen base in rice bran extract with D-mannitol was more life supportive to the bacterial growth than glucose and glycerol even though glycerol demonstrated an easy multiplication of the bacteria. This was the first confirmation that yeast extract could not be the only very reliable nitrogen source for *A. xylinum* growth and cellulose synthesis. To further confirm the growth support of rice bran to the bacteria, a plate count analysis was performed which showed 1.26×10^5, 1.56×10^5, and 1.95×10^5 for nitrogen sources increase 0.238±0.035, 0.476±0.069 and 0.714±0.024, respectively

RESULTS AND DISCUSSION

Nutrient Content of Rice Bran in *A. xylinum* Culture Media Cellulose Synthesis

To substantiate the nitrogen source sufficiency during the incubation period of cellulose synthesis, a fermentation analysis was performed to analyze the dry matter, crude protein, and diluted protein content of the rice bran with the results summarized in Table 2. It was realized that the dry matter of fermented rice bran increased ($p < 0.01$) among different times of incubation treatments as a result of water evaporation during the fermentation process. It was stated that the increase of substrate temperature due to carbohydrate breakdown released water from rice bran. These results were also confirmed in a previous study (Purwadaria *et al.* 2003). Further analysis revealed higher cell growth in aerobic fermentation than anaerobic fermentation, leading to higher metabolic activity caused by substrate temperature rise.

Moreover, increasing the substrate also affects the growth of bacteria, leading to the restoration of organic matter, such as carbohydrates, fat, and protein, which were used during the metabolic process by the microbes and further biosynthesis (Purwadaria *et al.* 2003). It was revealed that a decrease in the substrate at 72 h incubation showed an increase in substrate water content. The results of rice bran content determination during incubation revealed a decrease in rice bran content with a longer time of incubation, showing significant differences among treatment times ($p < 0.01$). These results were in line with the result published by Supriyati *et al.* (2015). The results hence confirmed the usage of rice bran as an energy source by the bacteria. According to the references, rice bran is used by the bacteria for cell biosynthesis and activation of energy during molecule transport, maintaining cell structure, and cell mobility. This was confirmed by the 48 h of incubation, where there was a significant decrease in the rice bran content. This phenomenon showed that after 48 h, there was exponential growth and cleavage, providing an optimum yield according to the nutrient substrate availability.

Analysis of the crude protein content of fermented rice bran, as shown in Table 2, revealed no significant changes in protein content among treatments times ($p > 0.05$), as was also reported by Zhu *et al.* (2013). The crude protein content of the fermented substrate had been affected by the protein content of the raw material (Kim *et al.* 2016). In view of this, the crude content could not be supportive enough for the multiplication of the bacteria, hence slowing the bacterial growth and cellulose synthesis due to low adaptation time (Supriyati *et al.* 2015). Nevertheless, diluted protein of fermented rice bran increased with increasing incubation time ($p < 0.01$) (Todhanakasem *et al.* 2014). During the fermentation process, the organic molecule complex was broken into simple

molecules, and non-diluted molecules became diluted, leading to increasing digestibility (Rosales *et al.* 2018).

Because a longer incubation time demands a higher nitrogen content to support the bacterial growth and cellulose synthesis, it was suggested that the bacteria break down the protein substrate into an amino acid and deaminate to ammonia as nitrogen sources. The increases in this metabolism apparently promoted a higher nitrogen substrate (Fontana *et al.* 2017). However, it was confirmed that during fermentation, the increase in protein content was low, but the diluted protein was 50% and only a simple dilute and a low molecular weight could be used during transportation through the cytoplasm membrane.

A further observation was that nitrogen retention of fermented rice bran increased significantly with longer incubation time ($p < 0.01$) (Schramm and Hestrin 1954; Supriyati *et al.* 2015). The increment was ascribed to a higher diluted protein content, thus ascertaining the fact that fermentation increased the nutritive value of the substrate. It was stated that fermentation causes protein, fat, and carbohydrate of the fermented product to hydrolyze easily and have a higher digestibility (Annor *et al.* 2017).

Table 2. Dry Matter, Organic Matter, Crude Protein, Diluted Protein, and N Retention of Fermented Rice Bran in Culture Medium per Times of Incubation

Treatments	Dry Matter (% as fed)	Organic Matter (% DM)	Crude Protein (% DM)	Diluted Protein (% CP)	N Retention (%)
0 h	62.45±0.29^{a}	90.67±0.61^{b}	10.57±0.28	38.84±3.58^{a}	31.42±4.60^{a}
24 h	65.96±0.21^{b}	88.90±0.36^{b}	10.15±0.21	56.47±3.49^{b}	31.44±0.24^{a}
48 h	71.35±0.07^{d}	87.69±0.30^{a}	10.12±0.73	75.20±7.35^{c}	36.17±3.15^{a}
72 h	69.70±0.24^{c}	87.73±0.19^{a}	10.60±0.40	74.19±8.89^{c}	47.36±0.75^{b}
144 h	68.50±0.22^{c}	86.65±0.18^{a}	10.58±0.36	71.18±8.76^{c}	45.28±0.65^{b}
288 h	64.72±0.20^{c}	84.74±0.19^{a}	10.43±0.61	69.28±.7.69^{c}	41.46±0.52^{b}

SAMPLE	INITIAL NITROGEN (N) N (g/L)	FINAL NITROGEN(N) N (g/L)	APROXIMATE NITROGEN USED N (g/L)
CONTROL	3.8±0.02	1.37±0.014	2.43±0.011
S=1=1	2.7 ±0.012	1.49±0.006	1.21±0.016
S=1=2	3.1±0.08	1.27±0.017	1.83±0.023
S=1=3	3.2±0.03	1.12±0.024	2.08±0.010

Harvesting and Purification

After 9 days of cultivation, BC was harvested and purified by soaking in a solution of 0.5 N NaOH at 90 °C for 2 h to remove the bacterial cells and other medium components. The culture medium was centrifuged, and the nitrogen content was calculated, with the results shown in Table 2. The membranes were freeze-dried in the presence of liquid nitrogen. Figure 3 shows the bacterial cellulose of yeast extract synthesis (A) and rice bran synthesis (B). It could be clearly seen that the rice bran that synthesized bacterial cellulose was much cleaner and more transparent than that of the yeast extract synthesized cellulose. This was evident in the purification, because the yeast extract synthesis cellulose took a total of 24 h to clean, while the rice bran synthesized cellulose only took a total of 4 h. This was partly attributed to the coloring nature of yeast extract that gets trapped during the fibril formation. The factors that affected the

transparency of the cellulose were confirmed in the XRD and DSC test results in Figs. 7 and 8, respectively.

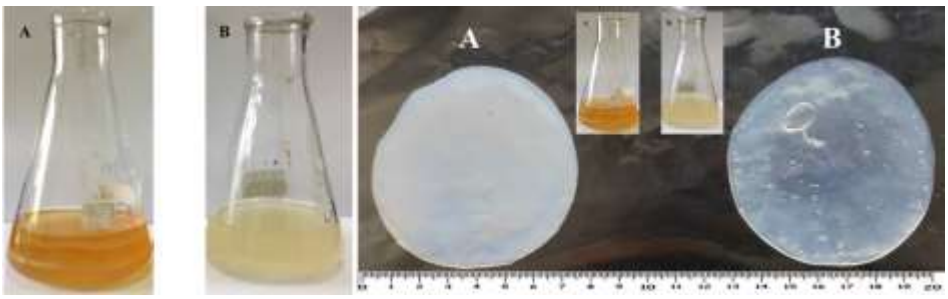

Fig. 3. Culture medium of yeast extract (A), and rice bran (B), and the respective harvested cellulose

Optimization of G. xylinum BC synthesis with rice bran

To confirm the bacterial yield of rice bran and D-mannitol using the same culture medium, all other parameters were held constant and the rice bran content was increased in a ratio of 1:1(0%), 1:2(100%), and 1:3(150). The cellulose for all three samples was harvested after 9 days of incubation, and the thickness and weight of the harvested cellulose of all samples were measured. The results are shown in Fig. 4. It was confirmed by the results that BC yield was in direct proportion to the rice bran content increment. There were no significant BC yield differences between the ratio of 1:1 and 1:2, but there was a 100% increase in BC yield when the ratio was increased to 1:3 with corresponding weights demonstrating the same pattern.

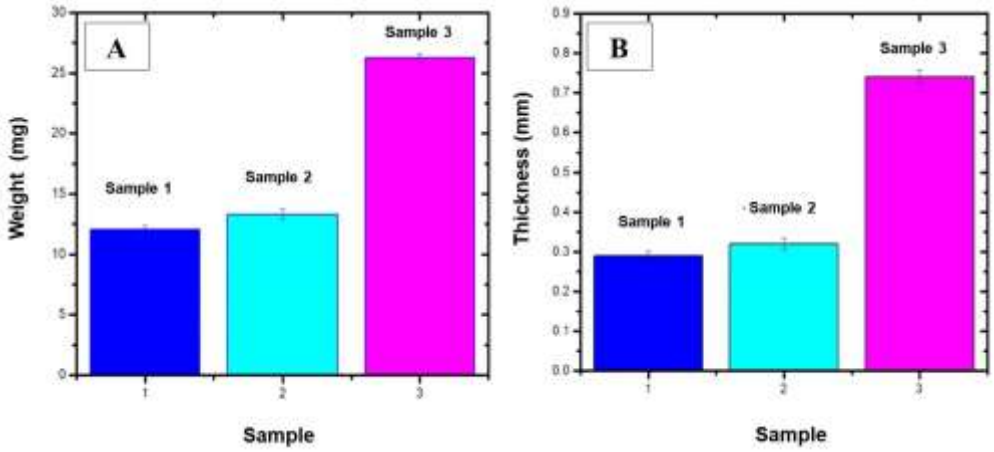

Fig. 4. Bacterial cellulose weight (A) and thickness (B) corresponding to the rice bran content

FTIR spectra

Figure 5 shows the FTIR spectra of all four samples. Cellulose allomorphs showed minor differences in the infrared bands except for the vibration peak at 3423 cm^{-1} corresponding to intramolecular OH stretching (Nielsen *et al.* 2008). It was observed that BC samples with the ratio 1:1 (B) and 1:2 (C) demonstrated broader bands in OH stretching that were associated with the partial adsorption of the OH group, leading to the transparency of the cellulose compared with yeast extract BC (A) and ratio 1:3 (D). There was also a slight variation in the hydrogen bonds at 2893 cm^{-1} due to CH and CH_2 stretching (McKean 1975). However, it was reasonable that these two bands were

displaced due to the rearrangement of the cellulose chains and hydrogen bonding pattern. Other main vibrational peaks exhibited virtually no change due to the polymorphic transformation: 1642 cm^{-1} corresponded to OH from absorbed water, 1427 cm^{-1} due to CH_2 symmetric bending, 1375 cm^{-1} due to CH bending, 1330 cm^{-1} due to OH in-plane bending, 1255 cm^{-1} corresponded to C-O-H bending, 1161 cm^{-1} due to C-O-C asymmetric stretching (β-glucosidic linkage), 1063 cm^{-1} due to C-O/C-C stretching, and 895 cm^{-1} due with asymmetric (rocking) C-1 (β-glucosidic linkage) out-of-plane stretching vibrations. This band was then associated with the cellulose II lattice (Smalley and Wakefield 1970).

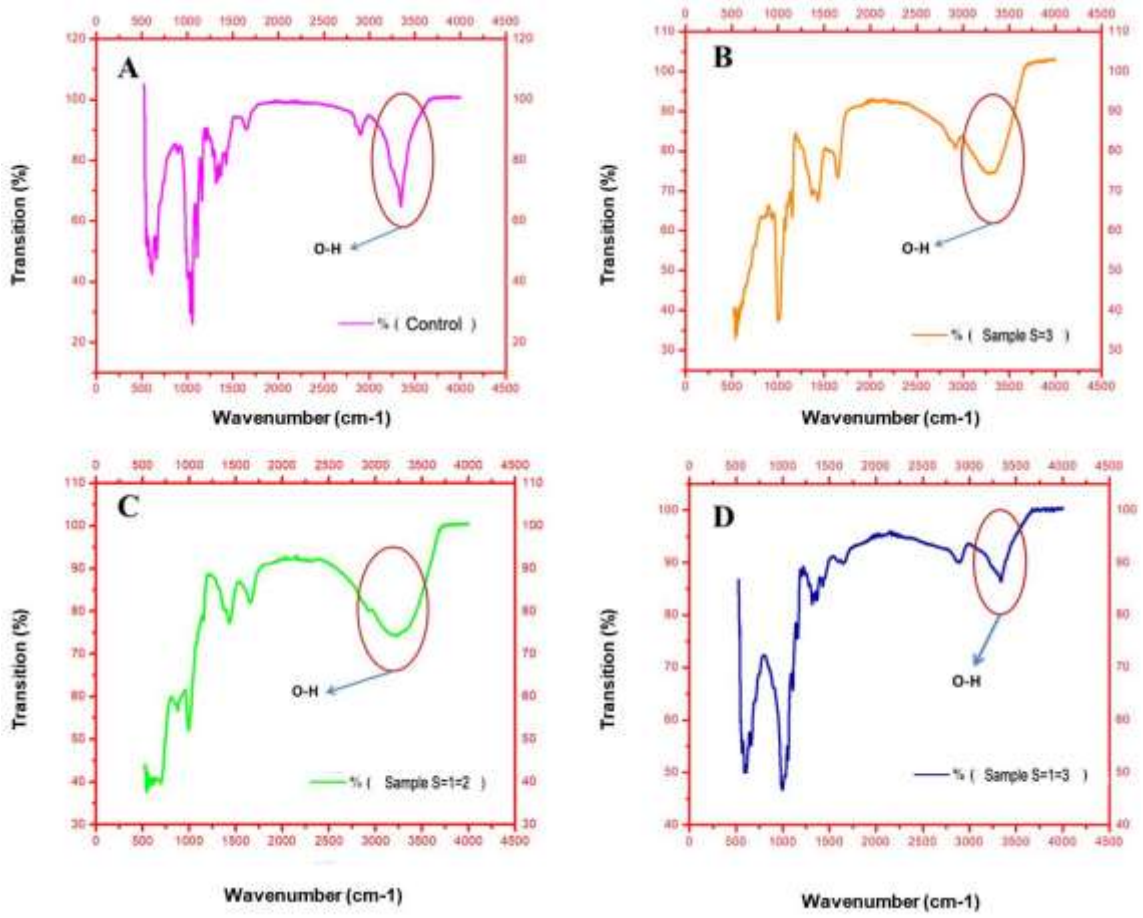

Fig. 5. FTIR spectra of D-mannitol and yeast extract (A); D-mannitol and rice bran of ratio 1:1 (B); D-mannitol and rice bran of ratio 1:2 (C); and D-mannitol and rice bran of the ratio 1:3 (D)

SEM analysis

Figure 6 shows the morphological characteristics as depicted in SEM images of BC produced from yeast extract and D-mannitol (A), 1:1 ratio of D-mannitol and rice bran (B), 1:2 ratio of D-mannitol and rice bran (C), and 1:3 ratio of D-mannitol and rice bran (D). The images were further analyzed to generate a histogram of the mean area morphology of the individual cellulose. From Fig. 7, it could be clearly seen that there was a gradual transformation in the morphological structure of the cellulose from the highly crystalline structure A to the highly amorphous structure of D. It was realized that the higher crystalline structure was first broken down to the amorphous structure using 1:1 (B), which revealed a sheet of cellulose films against fibrils. Meanwhile, when the

ratio of D-mannitol to rice bran increased to 1:2 (C), the structure of the cellulose began to take a form of crystallinity that was slightly different from A. This phenomenon was confirmed by the XRD results.

Image analysis using ImageJ software confirmed a gradual change in the morphology by calculating the mean area of the cellulose and the diameter. It was revealed that sample A had fiber diameter that ranged from 40 nm to 90 nm with a mean area of 113.4 nm, while sample B had a fiber diameter that ranged from 300 nm to 700 nm with a mean area of 112.1 nm. However, there was a drastic increase in the mean area of the BC sample C to 116.7 nm leading to a much finer fiber diameter that ranged from 6 nm to 15 nm, while BC sample D even demonstrated a much larger mean area of 130.1 nm with fiber diameter that ranged from 40 nm to 85 nm.

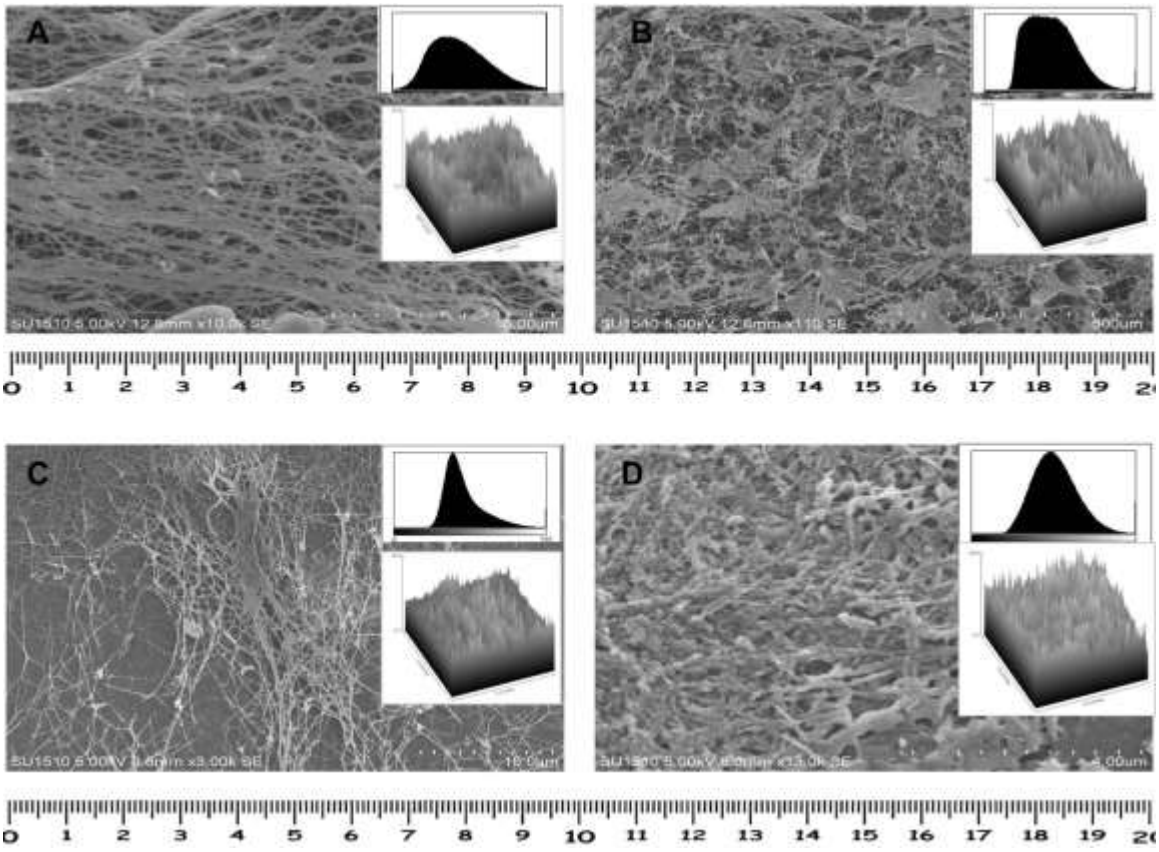

Fig. 6. SEM images of the cellulose sample synthesized using D-mannitol and yeast extract (A); D-mannitol and rice bran with ratio 1:1 (B); D-mannitol and rice bran of ratio 1:2 (C); and D-mannitol and rice bran of ratio 1:3 (D)

XRD analysis

The crystal structures of all four BC samples were analyzed by XRD, and the results are presented in Fig. 7. Per the results, apart from sample B, all other samples demonstrated three major crystalline peaks at 2θ = 14.7°, 16.8°, and 22.7° corresponding to the (−110), (110), and (200) crystal planes indicating the typical cellulose I (Yan *et al.* 2017). However, a general observation indicated a gradual transformation from a highly crystalline structure to a very amorphous structure from sample A to sample D (Wang *et*

al. 2017b). It was also seen that the intensity for samples B, C, and D steadily decreased from highly crystalline to highly amorphous.

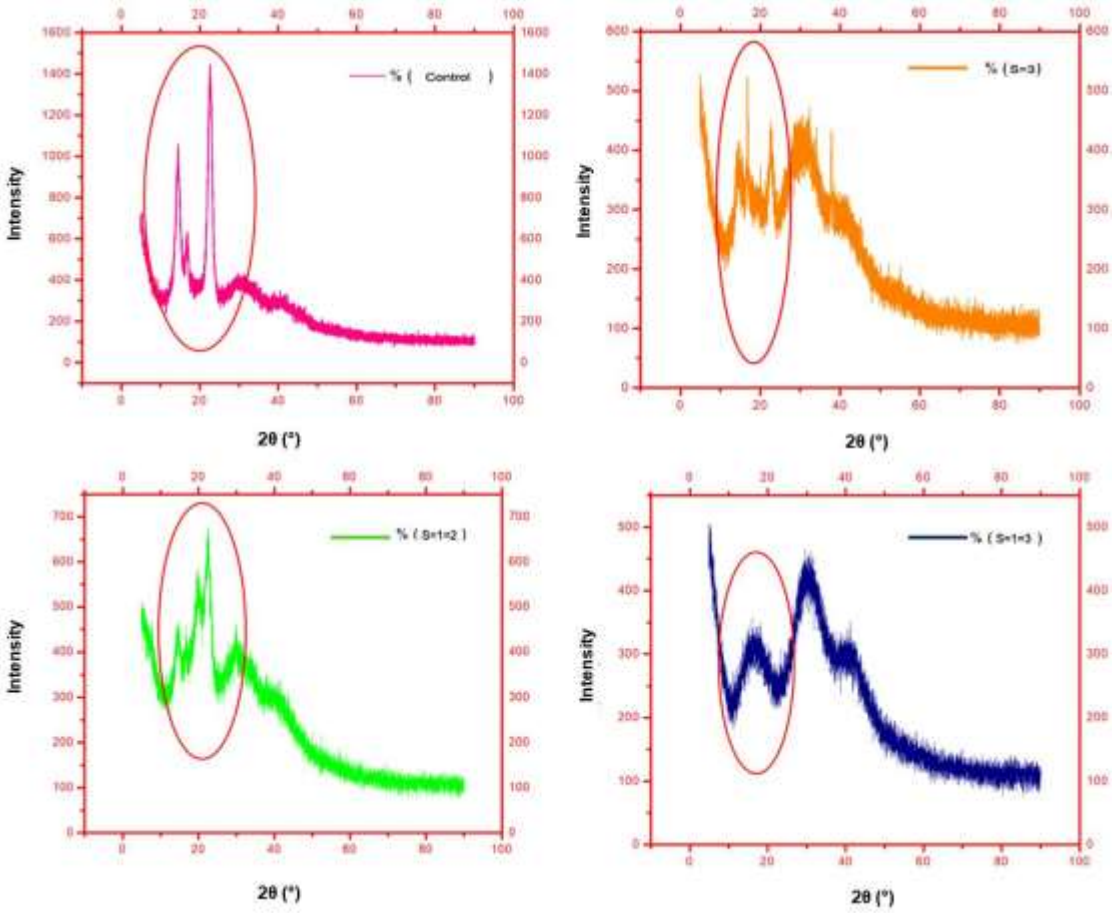

Fig. 7. XRD results of the cellulose sample synthesized using D-mannitol and yeast extract (A); D-mannitol and rice bran with ratio 1:1 (B); D-mannitol and rice bran of ratio 1:2 (C); and D-mannitol and rice bran of ratio 1:3 (D), revealing the crystalline and amorphous regions of the celluloses

DSC analysis

The differential scanning calorimetric traces of the four samples are shown in Fig. 8 with the enthalpy change records displayed in Table 3. It was revealed that all four samples underwent endothermic decomposition at around the same temperature. However, sample (A) had basically no recrystallization, while the other samples of D-mannitol and rice bran had an increasing recrystallization peak that corresponded to the increase in rice bran extract content. The endothermic decomposition for samples A, B, C, and D began with evaporation of water recorded at 91.08 °C, 94.70 °C, 96.78 °C, and 86.29 °C, respectively, which corresponded to the enthalpy 186.2 J/g, 394.7 J/g, 239.0 J/g, and 336.8 J/g. The degradation temperatures of all four samples were recorded at 116.05 °C, 113.53 °C, 125.59 °C, and 119.94 °C for sample A, B, C, and D, respectively. Surprisingly, sample A demonstrated no recrystallization at all, while the remaining samples of B, C, and D demonstrated recrystallization beginning at 212.28 °C, 196.28 °C, and 193.54 °C, respectively, which corresponded to the respective enthalpy 19.78 J/g, 41.08 J/g, and 135.3 J/g, and their respective completed recrystallization temperature at

224.88 °C, 210.73 °C, and 214.72 °C. The results suggested three things with respect to the characteristics of the bacterial cellulose of B, C, and D. The findings first suggest the direct relation of the amorphous state of the cellulose to the nature of nitrogen source present. Secondly, a higher flexibility of the OH bonds in the bacterial cellulose is suggested when rice bran extract is used as the nitrogen source for BC synthesis. Lastly, the results suggest the possibility of a better molecular interaction of BC with other functional groups.

Table 3. Enthalpy Changes of All Four Samples and Their Respective Temperatures

Samples (Wt%)	T_{vap} (°C)	Enthalpy (J·g^{-1})	T_d (°C)	T (°C)	Enthalpy (J·g^{-1})	T_{rec} (°C)
A (CONTROL)	91.08	186.2	116.05	0	0	0
B (S = 3)	94.70	394.7	113.53	212.28	19.78	224.88
C (S = 1 = 2)	96.78	239.0	125.59	196.28	41.08	210.73
D (S = 1 = 3)	86.29	336.8	119.94	193.54	135.3	214.72

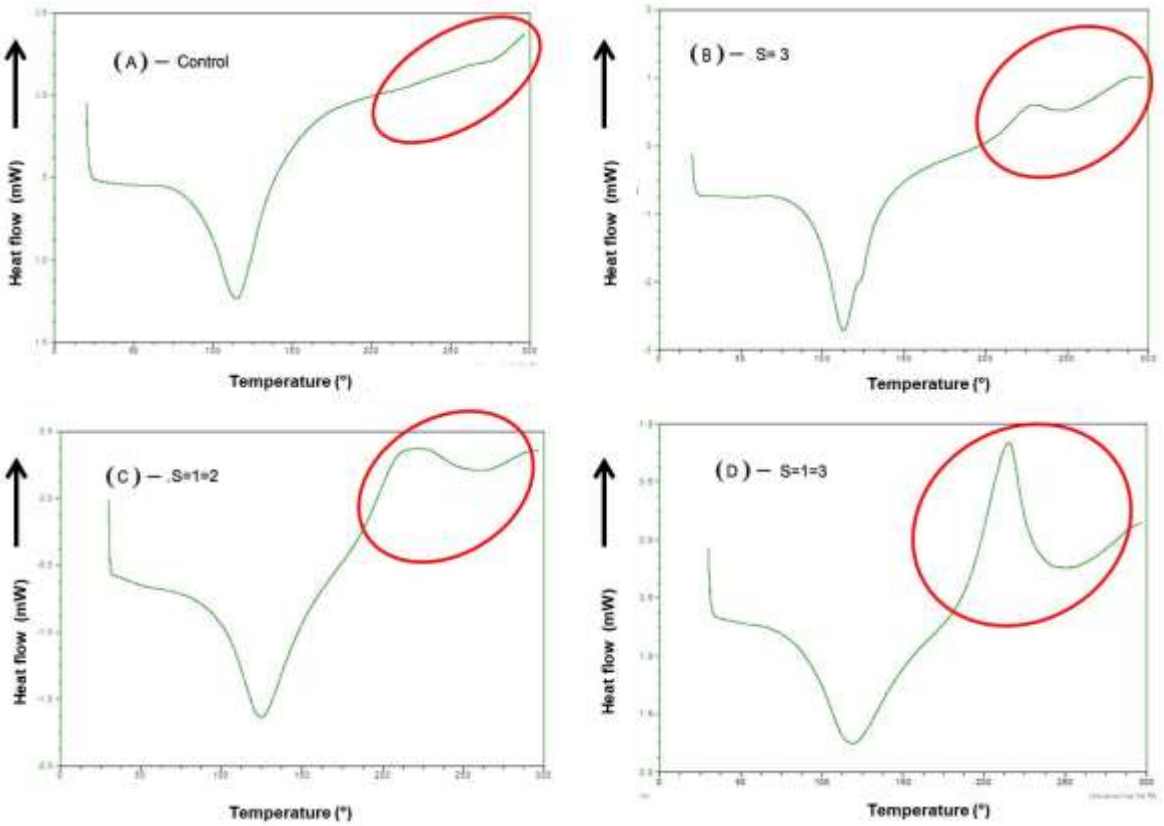

Fig. 8. DSC traces of the cellulose sample synthesized using D-mannitol and yeast extract (A); D-mannitol and rice bran with ratio 1:1 (B); D-mannitol and rice bran of ratio 1:2 (C); and D-mannitol and rice bran of ratio 1:3 (D), which shows the various stages of decomposition and recrystallizations

Mechanical properties of all rice bran generated BC samples were tested to confirm the respective strength against yeast extract generated BC, with the results shown in Fig. 9. It was observed that the strength of rice bran generated BC increased with increasing rice bran content and only about 10% lower than the strength of yeast extract generated cellulose.

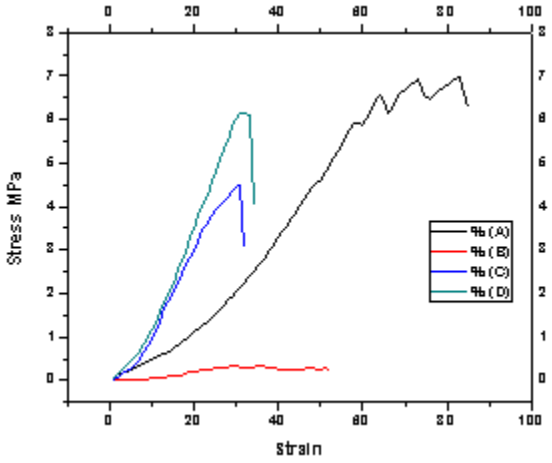

Fig. 9. Mechanical properties of D-mannitol and yeast extract cellulose (A); D-mannitol and rice bran cellulose of ratio 1:1 (B); D-mannitol and rice bran cellulose of ratio 1:2 (C); and D-mannitol and rice bran cellulose of the ratio 1:3 (D)

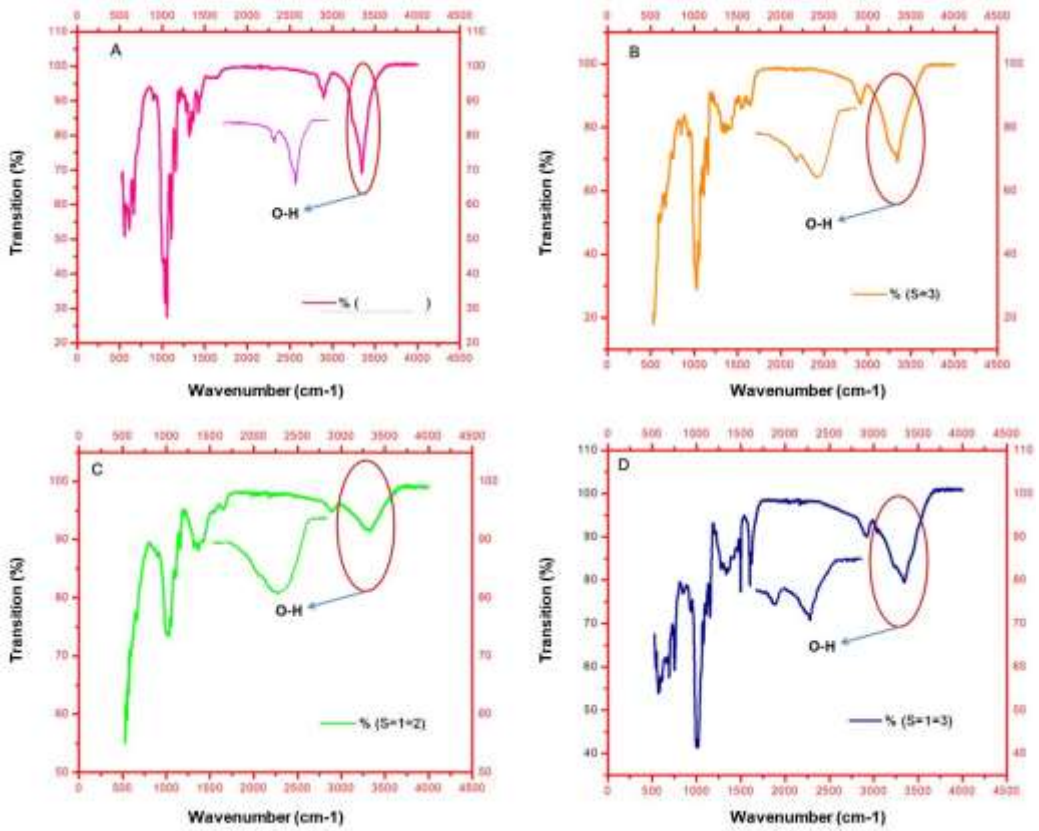

Fig. 10. Infra-red spectra of D-mannitol and yeast extract (A); D-mannitol and rice bran of ratio 1:1 (B); D-mannitol and rice bran of ratio 1:2 (C); and D-mannitol and rice bran of the ratio 1:3 (D) after they were exposed to primary amine of aniline to confirm the molecular interaction of all samples

BC Interaction with other functional groups

To confirm the free OH group presence in the cellulose and an apparent reactivity with other functional groups, all four samples were exposed to the primary amine of aniline for 1 h, after which the samples were washed, dried, and tested using FTIR. To the authors' surprise, sample A did not show any sign of reactivity with the primary amine. However, all three samples of B, C, and D demonstrated stronger affinity and reactivity to the primary amine of aniline. The results hence confirmed the third suggestion from the XRD test that stated there was partial hydrolysis of the OH groups during the formation of the cellulose using D-mannitol and rice bran, which provided a more reactive site than the cellulose formed from D-mannitol and yeast extract. Figure 10 shows the FTIR test of all four samples after exposure to the primary amine of aniline.

CONCLUSIONS

1. *A. xylinum* bacterial cellulose was successfully synthesized using an alternative nitrogen source in rice bran rather than yeast extract. The results indicate the use of diluted protein, and obvious increase in this kind of protein, in the culture media as incubation time also increases, thereby providing sufficient protein needed by the bacteria for cellulose synthesis.
2. It was also noticed that there was a direct correlation between the quantity of the rice bran content present in the culture media and the cellulose yield. This means that a higher amount of rice bran present in the culture media resulted in a higher cellulose yield during the same incubation time frame.
3. Lastly, it was also confirmed that the cellulose synthesis using rice bran as a nitrogen source had a much better recrystallization than the cellulose synthesis using yeast extract because of the partial usage of the OH group during the synthesis process. This made the inter OH bonds more flexible, which led to the cellulose synthesized from rice bran to be more reactive to other functional groups than the cellulose synthesis from yeast extract.

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