

An Observation of Hydrocolloid Effects on Bacterial Cellulose

N.S. Mokthar, N.A.I. Mohd Zackimei, N.F. Abdul Kohar, N.N. Mohamad Kasturi, N. Mohd Zainodin, W.S. Wan Abd Aziz, N.N. Ruzelan and A. Adnan*

Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

Microbial or Bacterial cellulose (BC) is a polysaccharide synthesized by bacteria, with *Komagataeibacter xylinus* being the primary producer. BC is known for its high biodegradability, biocompatibility, and mechanical strength, making it of interest to various industries such as biomedical, pharmaceutical, food, and cosmetics. However, its low production rate has led researchers to explore methods to enhance its production, including the addition of various substances such as food additives, supplementary elements, and hydrocolloids. This study investigates the effects of adding hydrocolloids such as carboxymethylcellulose (CMC) and xanthan gum (XG) at different concentrations on BC features and BC yield produced by *K. xylinus*. The findings indicate that the highest production of bacterial cellulose (BC) occurred at an optimal carboxymethyl cellulose (CMC) concentration of 1.5% (w/v), whereas the most significant BC production was observed at a xanthan gum (XG) concentration of 0.2% (w/v). Moreover, the introduction of CMC and XG had notable effects on BC morphology, crystallinity, and the presence of functional groups.

Keywords: bacterial cellulose; *Komagataeibacter xylinus*, hydrocolloids; BC feature; BC yield

I. INTRODUCTION

Cellulose is a water-insoluble macromolecule that can be obtained from vascular plants, especially in the woods of the plant. Since the vascular plant is the main producer of cellulose, traditionally, to obtain cellulose for industrial purposes, many trees were cut and harmed during the process. To obtain pure cellulose for usage, it is also necessary to remove contaminants such as lignin, hemicellulose, and pectin from the wood stocks obtained (Cheng *et al.*, 2009a). This process has a high manufacturing cost and is not ecologically friendly. Alternative methods, such as using a microbial system to create cellulose, are required due to the wide range of industrial uses for cellulose (Vasconcellos *et al.*, 2018), to avoid harming the environment and reducing plant populations. Cellulose is used in a variety of industrial sectors, including those that produce food, paper, cosmetics, detergents, textiles, pharmaceuticals, and medical sectors

that use it for artificial skin and wound treatment (Cheng *et al.*, 2009b; Rachtanapun *et al.*, 2021).

Microbial cultivation employing fermentation or bioreactors is one option for replacing plant cellulose. Bacterial cellulose (BC) is derived from the microbial system through the oxidative fermentation process. Some of the bacteria strains that can produce it are *Agrobacterium*, *Alcaligenes*, *Aerobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Sarcina*, and *Acetobacter* (reclassified as *Gluconacetobacter* and moved to a new category of *Komagataeibacter* in 2012). Brown discovered *Acetobacter xylinum* in 1886, when he saw cells producing cellulose in the presence of oxygen and glucose (Jacel *et al.*, 2019). Growth of *Komagataeibacter xylinus* in standard fermentation medium will digest the carbon sources from the available saccharides (Rachtanapun *et al.*, 2021). Furthermore, *K. xylinus*' respiratory mechanism permits it to produce gluconic acid from glucose metabolism and acetic

*Corresponding author's e-mail: azila.adnan@umt.edu.my

acid from ethanol oxidation (Campano *et al.*, 2016). The production of these acids results in a reduction in the pH of the culture medium.

Because BC will be released as an extracellular, insoluble gelatinous biofilm is formed, the depth or volume of the culture had no effect on the rate of synthesis (Auta *et al.*, 2017). They are adaptable biopolymers with characteristics that are comparable to those of cellulose found in plants. Unlike plant-derived cellulose, BC is made up of linear polysaccharides of 1,4-glucan chains and is impurity-free (Ciecholewska-Jusko *et al.*, 2021). We required high chemical purity and highly desirable properties like increased flexibility, great crystallinity, excellent tensile strength, great polymerisation, and high water-retention ability from BC because it is economical and environmentally friendly (Ciecholewska-Jusko *et al.*, 2021; Rachtanapun *et al.*, 2021). BC can also be considered biocompatible because it is non-toxic and biodegradable. Non-pathogenic bacteria *K. xylinus* strains are chosen to create cellulose at the industry level because of their high productivity and because they are the most effective producers of BC via oxidative fermentation, claim Ciecholewska-Jusko *et al.* and Cheng *et al.* Nowadays, a lot of improvements have been made in the cultivation method with proper media and growth conditions to increase the production of BC (Cheng *et al.*, 2009b).

When the methods of fermentation system are carried out in a static, agitated, or swirling environment, several forms of BC are synthesised. While fermentation using shaken and stirred procedures results in an unusually shaped and sphere-like pellicle of cellulose, static fermentation results in a three-dimensional connected reticular pellicle. Additionally, when employing a static situation, cellulose production is influenced by the air contribution from the media's top layer, and the end-product is dictated by the amount of the carbon source.

As a result, different cellulose-producing bacteria can be distinguished based on the carbon source they use (Aswini *et al.*, 2020). A prolonged growing period enhances the formation of BC via C-H bonds and hydrogen. As a result, as the pellicle spreads lower and traps the bacteria, rendering them inactive due to a lack of oxygen, BC synthesis is prevented.

Hydrocolloids in BC Synthesis

According to Cheng *et al.* (2009b), CMC is a negatively charged, water-soluble food stabiliser or additive that is produced from cellulose itself. This hydrocolloid is frequently used. As reported by BeMiller (BeMiller, 2019), CMC is frequently used in food manufacturing to absorb and retain water, control crystal formation, serve as an adhesive agent and thickening agent, enhance duration of shelf-life, and create the correct texture or body. Anionic and amphiphilic characteristics make up the CMC backbone, which especially aid in stabilisation (Krempel *et al.*, 2019). It is used to prevent the production of crystallisation of ice in most ice cream products as the primary stabiliser. The product's smoothness and creaminess are preserved by tiny ice crystals (Krempel *et al.*, 2019). The inclusion of CMC will make the fermentation media viscous, reducing the shear stress that the bacterial cell will be under. Furthermore, it will result in BC that has been mixed with CMC (Cheng *et al.*, 2011). This results in the production of thinner nanofibers that are mostly composed of cellulose I β and covered with additives (Zhong, 2020). Compared to pure cellulose, CMC-BC will have slightly different structures and characteristics. The inclusion of CMC during the fermentation process will enhance the quality of BC produced, according to Cheng *et al.* (2009a).

Xanthan gum (XG), another hydrocolloid used in this study, is comparable to CMC in terms of its properties. Xanthan gums (XG) are commonly employed as functional additives to alter the structure, flavour, texture, and shelf life of food products (Dickinson, 2003). A polysaccharide called XG is created through the fermentation of other substances. It comes from the *Xanthomonas campestris* species of Gram-negative bacteria. It is a well-known food additive that is frequently utilised in a variety of industrial fields (Kumar *et al.*, 2018). This significant commercial microbial polysaccharide is composed of a backbone of cellulose-like -D-glucopyranose units and a side group of D-carabinose and D-glucuronic acid (Jian *et al.*, 2012). Due to its exceptional rheological characteristics, such as high biocompatibility, gelling property (Petri, 2015), high viscosity yield, high pseudo-plasticity, and the ability to create an extremely viscous solution at low shear pressures (Rosalam, 2006). XG has also been widely used in a variety of applications. Some

reports show that XG significantly increases the BC production by *K. xylinus* when fermentation conditions are present. Nguyen *et al.* (2022) found that despite being fermented anaerobically, all hydrocolloid-supplemented media, including konjac glucomannan (KGM), a mixture of microcrystalline cellulose (MCC) and carboxymethylcellulose (CMC), and xanthan gum (XG), indicated the presence of BC when compared to the negative control sample. These hydrocolloids were found to improve BC solubility. This increased the efficiency of cellulose manufacturing. Hydrocolloids cling to the membrane of the cellulose fibres during the production of cellulose. This improves the structure and solubility of the cellulose layer (Cheng *et al.*, 2009a). Additionally, Gao *et al.*'s (2020) research found that, within a narrow range, the presence of XG has a significant impact on BC synthesis. They also showed significant changes in BC output as well as enhanced BC structure improvement. Hence, this study elucidates the influences of hydrocolloids on BC yield and properties.

II. MATERIALS AND METHOD

Materials

A strain of lyophilized *Komagataeibacter xylinus* ATCC 53524 was utilised to produce BC, which was obtained from the American Type Culture Collection (Rockville, MD) (Costa *et al.*, 2017).

Media Composition

The standard BC medium was used in the experiment, which was first published by Hestrin and Schramm (1954) and modified by Hungund and Gupta in 2010. The inoculum and fermentation liquid medium composed of 2.0% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.27% (w/v) Na₂HPO₄, and 0.15% (v/v) citrate (Costa *et al.*, 2017).

Fermentation Conditions

Transferring a new culture of *K. xylinus* to liquid HS media, followed by 3 days of stirred cultivation at 30°C, yielded the inoculum culture. The growing culture was agitated to ensure cell dispersion in the inoculum is even (Costa *et al.*, 2017). The resulting cell suspension was transferred into a 250-mL conical flask containing standard medium and each

representative substrate and hydrocolloid at various concentrations, and it was then agitatedly incubated at 150 rpm, 30°C for 5 to 7 days in duplicate trials (Sperotto *et al.*, 2021). The BC pellicles were gathered, quantified, and purified after culture. All studies were conducted in an aseptic environment.

BC Purification and Determination

The harvested BC pellicles underwent a 10-minute centrifugation at 4500 rpm followed by a distilled water rinse. After that, the harvested BC were submerged in 0.1M NaOH for 30 minutes at 80°C to eliminate any leftover bacteria that had attached themselves to them. After numerous rounds of rinsing with deionised water to assure complete alkali elimination, the pellicles had a pH of neutral. To achieve a consistent mass, BC was made and dried at for 24 hours using a (Costa *et al.*, 2017).

Morphology Observation Using Scanning Electron Microscopy (SEM)

To prepare the BC films for SEM, they were chopped into small pieces, applied to copper stubs with adhesive carbon conductive tape, and then covered in gold for 30 seconds. Then, using a scanning electron microscope (Jeol, JSM-6360LA).

Fourier-Transform Infrared (FTIR) Spectroscopy

The spectrum of the samples was recorded, and structural modifications were analysed at room temperature using Thermo Fisher's FTIR Spectra 2000 (Nicolet iS20). The functional group of BC was determined using FTIR.

X-ray Diffraction (XRD)

The percentage of crystallinity and the size of the crystallites were determined. Every freeze-dried BC film's XRD patterns were measured using a diffractometer with CuK radiation, wavelength ($\lambda = 1.54 \text{ \AA}$), generated at a voltage of 40 kV and a filament emission of 30 mA (Rigaku SmartLab®). The scan speed of 2° theta per min, samples were inspected in a 2° range between 5° and 60°. The software SmartLab Studio II was used for data collection.

Statistical Analysis

A one-way analysis of variance (ANOVA) test was used to verify if there are significant differences between the treatments at the significance level of $p < 0.05$ (Costa *et al.*, 2017).

III. RESULT AND DISCUSSION

A. Evaluation of Carboxymethyl Cellulose (CMC) Concentrations on BC Yield

In this study, agitated cultures of *K. xylinus* were grown in HS-medium with various concentrations of CMC, including 0.0%, 1.3%, 1.5%, 1.7%, and 1.9% (w/v). Additionally, a control run without CMC was carried out. Duplicate trials were used in every experiment. Figure 1 illustrates how the effects of CMC at various concentrations on BC yield were compared in this study. The yield of BC increased along with the CMC, which went from 0.0% (w/v) to 1.5% (w/v). When the concentration of BC dropped from 1.7% (w/v) to 1.9% (w/v), the yield of BC began to decline. BC production appears to be negatively impacted by CMC concentrations greater than 1.5% (w/v). At CMC concentration of 1.5% (w/v), the highest amount of BC was produced (0.0743 g), as opposed to the control (0.0% (w/v) CMC), which only produced 0.073 g of BC. The overlapping error bars across treatments show that the differences are not statistically significant, even though a modest increase in BC yield was noted at 1.5% (w/v) CMC. Rather from being a direct result of CMC content, the changes in dry weight could be explained by biological variability. However, the general pattern indicates that a decrease in BC synthesis is associated with increasing CMC concentrations, especially at 1.7% and 1.9%. Since excessive CMC may disrupt the orderly assembly of cellulose fibres during biosynthesis, this drop may be linked to a decline in crystallinity. As seen in the graph, this disruption probably results in a less compact and structurally disorganised matrix, which lowers dry mass recovery.

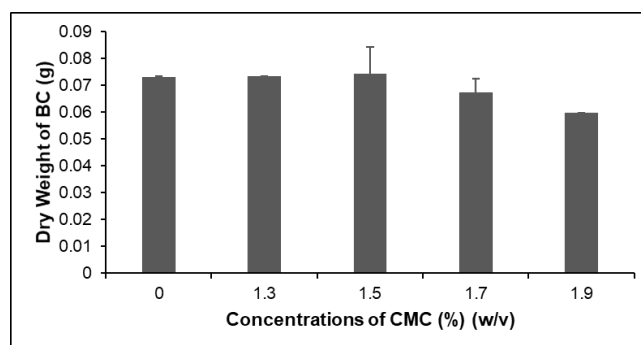


Figure 1. Production of BC cultured with or without CMC at various concentrations (0.0%, 1.3%, 1.5%, 1.7% and 1.9% (w/v)) by *K. xylinus*

Because 1,4-glucans that have been incorporated into microfibrils go through a combined process of polymerisation and crystallisation at an extrusion point on the cell surface, the yield of BC rises when CMC is introduced (Cheng *et al.*, 2011). The crystallisation process is slowed down, which increases the BC yield when natural microfibrils and negatively charged CMC combine to form intramolecular hydrogen bonds (Cheng *et al.*, 2011). Rate-limiting step is crystallisation. The next part will go into more detail on the crystallinity of BC that has undergone CMC modification. The enhancement of bacterial cellulose (BC) production varies depending on the degree of polymerisation (DP) and degree of substitution (DS) when carboxymethyl cellulose (CMC) is added. In addition, CMC proved its ability to produce BC in small pellet forms that stay in the suspension of the media. In the media with the addition of CMC, less BC can be seen attached to the walls of the conical flask and instead floating in the suspension. In contrast to that, BC produced in the control medium (0.0%) was mostly seen attached to the walls of the conical flask. This phenomenon can be supported by a previous study by Costa *et al.* (2017), which stated that CMC boosts the production of BC in small pellets. This may be explained by an increase in BC's solubility in the presence of the highly soluble CMC, which may bind to the BC fibrils' surface (Cheng *et al.*, 2009a). Moreover, the addition of CMC increases the medium viscosity and causes *K. xylinus* to experience less shear stress, so the BC stays in the suspension. These characteristics are useful in industry prospects as BC can be continuously produced and make it possible for real-time sampling (Krempel *et al.*, 2019).

B. The Influences of Carboxymethyl Cellulose (CMC) on BC Properties

BC produced with the addition of CMC at different concentrations was observed under SEM. The surfaces of the freeze-dried BC samples captured are shown in Figure 2. The difference in CMC concentration seems to have an impact on BC morphology. BC without the addition of CMC (0.0% (w/v)) appeared less dense and porous compared to BC with the addition of CMC. As the concentration of CMC increases, BC samples also become denser and more rigid. An increase in CMC concentration also caused the BC surface structure to change, and the images showed that it has some debris-like structure on the surface contrary to the negative control BC. This is due to CMC being incorporated into BC microfibrils (Tsouko *et al.*, 2015). The debris-like structure was CMC being attached to the BC microfibrils and formed a multi-layered network of joined CMC and BC fibrils (Daya *et al.*, 2016). According to Haigler *et al.* (1982), CMC addition can alter the shape of BC into distinct, interweaving bundles of microfibrils but does not stop the crystallisation of microfibrils. This discovery is similar with the result of a study that was conducted by Cheng *et al.* (2009a). He stated that the thickness of BC fibres seemed to have decreased as CMC concentration increased.

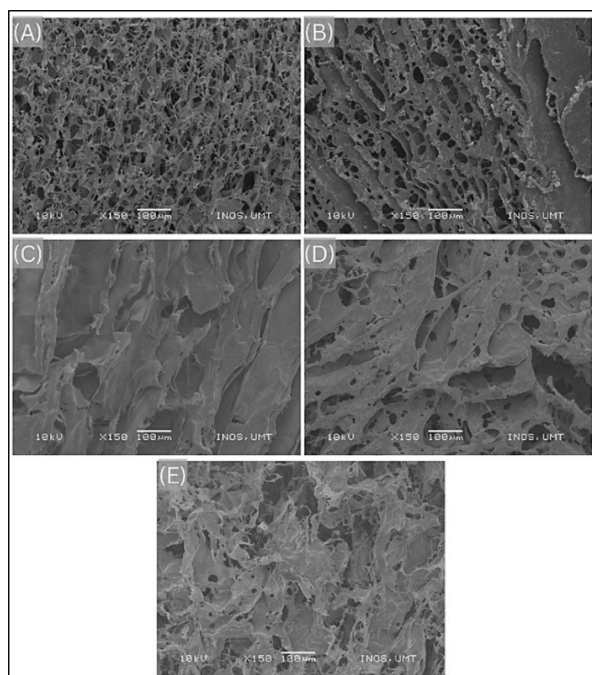


Figure 2. SEM images of freeze-dried CMC-altered BC samples. (A) 0.0% CMC-BC (negative control), (B) 1.3% CMC-BC, (C) 1.5% CMC-BC, (D) 1.7% CMC-BC and (E) 1.9% CMC-BC

The addition of CMC influenced the crystallinity and crystallite size of BC, even though it has been demonstrated that CMC cannot block the crystallisation process of BC microfibrils (Cheng *et al.*, 200a). The X-ray diffraction patterns produced from freeze-dried BC samples are shown in Figure 3. The crystallinity indices were computed using the data from the XRD examination. The outcome demonstrated that as CMC concentration increases, BC crystallinity likewise appears to decrease. BC was synthesised with 0.0% (w/v) of CMC and had an 89.1% crystallinity. Meanwhile, BC with 59% crystallinity was synthesised using 1.5% (w/v) of CMC. It's likely that CMC in the media was absorbed and combined with BC fibrils during crystallisation, which resulted in decreased crystallinity.

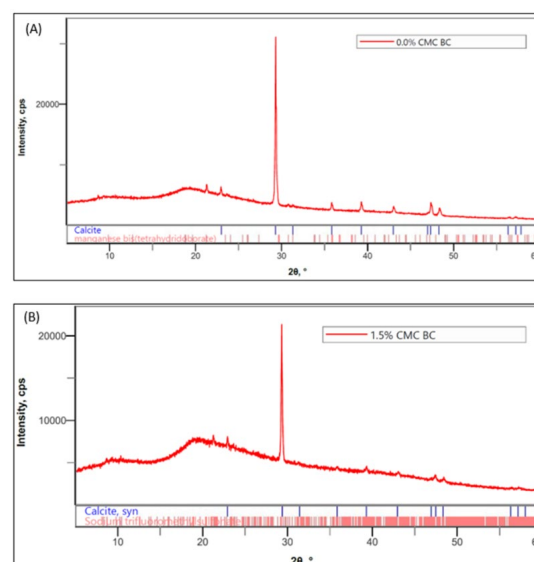


Figure 3. Peaks of BC produced by *K. xylinus* in HS-medium (A) without the addition of CMC (0.0%) and (B) with the addition of 1.5% of CMC.

The intra- and intermolecular hydrogen interactions between the hydroxyl groups and oxygen atoms during BC synthesis are hindered by this CMC incorporation in BC fibrils, as well as the van der Waals force-driven self-assembly process (Zhong, 2020). According to Zhong (2020), the addition of CMC affects the ratios of cellulose I β and I β in BC. As a result of CMC integration, thinner nanofibers will be formed, principally BC of cellulose I β coated with CMC. Furthermore, because of this, the yield of BC rose. The decreased crystallinity of the CMC-BC was equivalent to that seen by Cheng *et al.* (2011), who added

CMC to fermentation media and observed the same trend of decreased crystallinity.

To identify the functional group that occurs in the generated BC, FTIR analysis was conducted. All FTIR spectra were displayed in Figure 4.

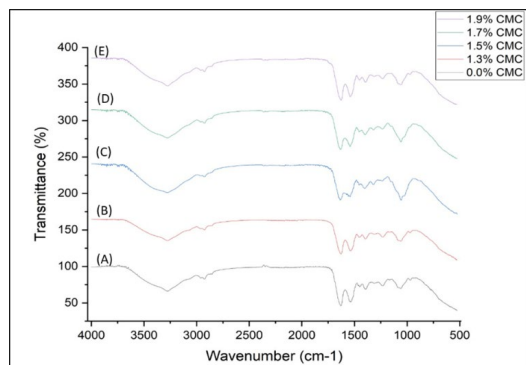


Figure 4. FTIR Spectra for (A) 0.0% CMC-BC, (B) 1.3% CMC-BC, (C) 1.5% CMC-BC, (D) 1.7% CMC-BC, (E) 1.9% CMC-BC.

FTIR analysis was conducted to identify the functional groups present in the produced bacterial cellulose (BC). Figure 4 displays the FTIR spectra for all samples, revealing broad absorption peaks of hydroxyl groups (OH-) in the range of 3500-3000 cm⁻¹ across all BC samples. The abundance of hydroxyl groups along the polymer chain contributes to unique properties in BC, such as a high water-holding capacity. Consequently, the hydroxyl groups and oxygen atoms in anhydroglucose units form numerous hydrogen bonds (Pororelova *et al.*, 2020). Additionally, the spectra indicate that carboxyl groups are present in CMC-BC, a feature absent in pure BC, as evidenced by an absorption peak in the 1800-1600 cm⁻¹ range (Cheng *et al.*, 2011). The carboxyl peak which was attributed to CMC is proof that CMC integrated into BC microfibrils during crystallisation. This discovery is in line with what was found by Cheng *et al.* (2011), who stated that the carboxyl group being present is the result of CMC's contribution. These spectra characteristics can serve as evidence that CMC is incorporated with BC during crystallisation.

C. Evaluation of Xanthan Gum (XG) Concentrations on BC Yield

Under aerobic conditions, the introduction of hydrocolloids had a remarkable impact on the production of bacterial

cellulose (BC) when *K. xylinus* was cultured. We explored the influence of different amounts of xanthan gum (XG) on BC-XG productivity, as varying XG quantities could yield distinct outcomes. As depicted in Figure 5, an increase in the supplied amount of XG led to a significant rise in BC productivity ($p < 0.05$). Because improved bacterial adherence and biofilm formation are essential for effective BC synthesis, researchers reported this improvement to XG's capacity to alter the medium's rheological characteristics (Gao *et al.*, 2020).

In this study, BC was generated using 75 mL of HS medium, resulting in dry weights of 0.0509 g, 0.0513 g, 0.0700 g, 0.0797 g, and 0.0893 g for samples containing 0.0% XG, 0.05% XG, 0.1% XG, 0.15% XG, and 0.2% XG, respectively (Figure 5).

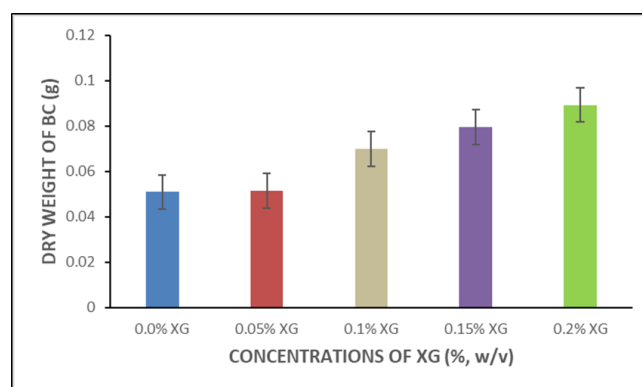


Figure 5. Production of BC cultured with or without XG at various concentrations (0.0%, 0.05%, 0.1%, 0.15% and 0.2% (w/v)) by *K. xylinus*.

Although there were no significant differences in bacterial cellulose (BC) production between the negative control and the medium containing 0.05% XG, with only a marginal variance of 0.0004 g, the inclusion of hydrocolloids in all media resulted in BC formation. Prior research on the in-situ manipulation of BC using additives that dissolves in water revealed that as the concentration of added XG increased, the productivity of BC-XG also increased. However, once it increased beyond 0.2% (w/v), yield started to decline. This observation suggests that the addition of XG positively affects BC production within a specific range but hampers it when the amount surpasses this range.

Taking all factors into account, 0.2% XG (w/v) was identified as the optimal additive amount, as it yielded the highest BC productivity. The enhanced solubility of BC in

the presence of this concentration of XG contributed to the improved efficiency of cellulose production with the addition of hydrocolloids (Nguyen *et al.*, 2022).

As documented by Kim and colleagues (2012), the strain utilised for BC production is susceptible to shear force and necessitates a high oxygen transfer rate. Therefore, minimising shear force during BC production is crucial. Previous studies have suggested potential mechanisms for enhancing BC, such as decreasing shear force by augmenting the viscosity of the medium. Consequently, agar, a water-soluble polysaccharide, is introduced to mitigate shear force and improve BC yield. The findings indicate that increasing agar concentration, up to 0.4%, leads to enhanced BC productivity. Thus, it can be inferred that substances inducing soluble viscosity, such as xanthan gum, prove more effective in BC production for shear force reduction.

D. The Influences of Xanthan Gum (XG) On BC Properties

More direct evidence of the above assumption is from the SEM result. BC produced with the addition of XG at various concentrations was observed under SEM. The SEM images shown in Figure 6 revealed that all the BC samples have a network, interwoven structure, and a microporous structure, with smooth surfaces and highly interconnected open pores. The use of different concentrations of XG seems to have an influence on BC morphology. Based on the surface morphology, BC without the addition of XG (0.0% w/v) appeared relatively less dense and rigid. On the other hand, the SEM images in Figure 6 (b), (c) and (d) showed some differences in surface structures as XG concentration increased, which the BC samples become more dense and rigid. The images showed that it has some debris-like structure on the surface contrary to the negative control. Since XG is being incorporated into BC microfibrils, the reticulated structure may be attributed to the presence of XG in the pellicles. According to Cheng and coworkers (2009a), the hydrocolloid during BC formation attached to the surface of the BC fibrils to improve the solubility as well as the structure of the BC layer.

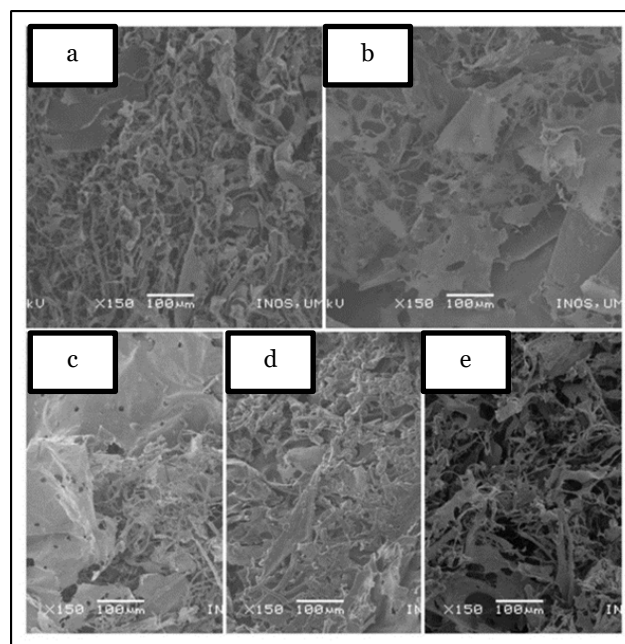


Figure 6. SEM images of BC samples that were produced using various concentrations of XG. (a): 0.0% XG; (b): 0.05% XG; (c): 0.1% XG; (d): 0.15% XG; (e): 0.2% XG.

XRD was used to examine the freeze-dried pure BC and XG incorporated BC membranes. Figure 7 depicts the X-ray diffraction peaks of two BC samples with varying XG concentrations. CrI was computed using XRD data. According to the XRD analysis data presented in Table 1, the crystal structure of BC fell considerably from 79.6% to 30% when 0.2% (w/v) XG was added. The lower crystallinity of BC-XG samples suggests that the presence of xanthan gum influences with the self-assembly of BC fibres marginally. Additionally, XG deposited on the surface hinders the formation of hydrogen linkages between fibres and microfibrils, further decreasing crystallinity. This reduction in crystal structure is similar to that achieved by the exogenous addition of hydrocolloids to the fermentation broth during in-place modification. In situ modification is generally conducted by adding chemicals to the brewing broth, allowing the morphology and characteristics of BC fibres to be modified by influencing the arrangement style and crystallites making during BC synthesis, according to Liu *et al.* (2019). BC's application prospects can be expanded by decreasing its crystallinity.

Table 1. Crystallinity indices of BC samples with different concentrations of XG.

Properties	0.0% XG	0.05% XG	0.1% XG	0.15% XG	0.2% XG
Crystallinity index (CrI) (%)	79.6	72.1	53	41.9	30

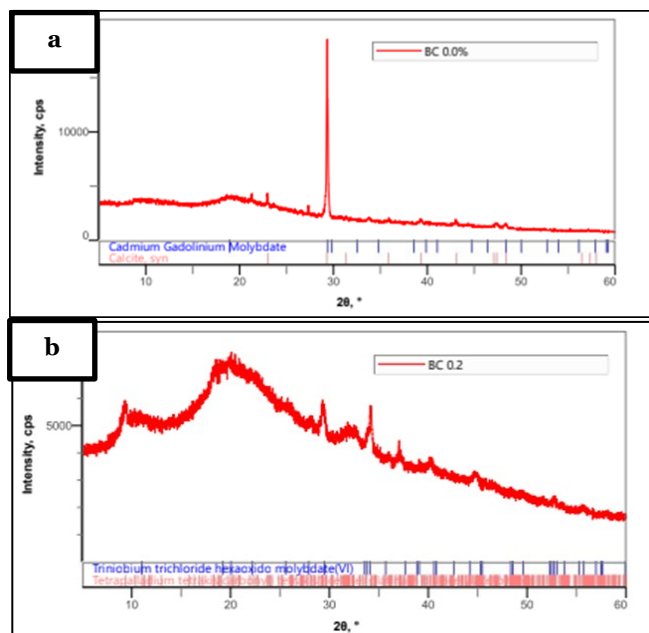


Figure 7. XRD patterns of the two samples of BC with different concentrations of XG. (a): 0.0% XG; (b): 0.2% XG.

FTIR was employed to further evaluate and describe the effect of adding hydrocolloid, XG on BC molecular entities. The FTIR spectra of BC cultured with or without XG at various concentrations are displayed in Figure 8 below.

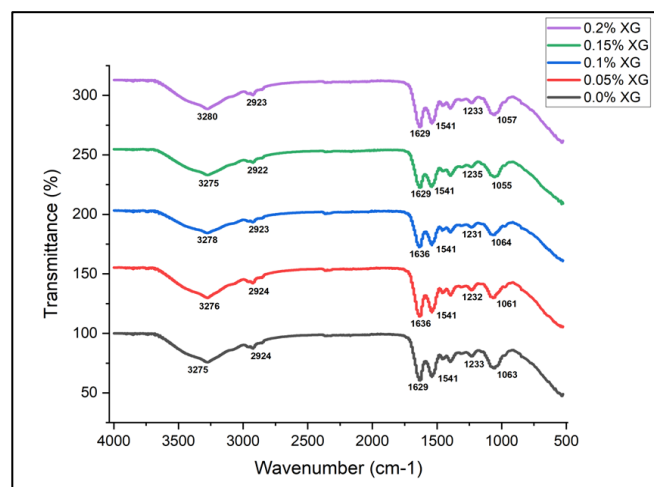


Figure 8. FTIR spectra of BC at different percentage of XG.

The spectra of all the samples displayed a prominent, wide absorption peak attributed to hydrogen-bonded OH- groups in the range of 3500-3000 cm⁻¹. This is a direct result of the high concentration of hydroxyl groups within XG, and the greater abundance of these hydroxyl functional groups leads to a smoother OH- peak.

The CH- stretching band appeared in the spectra of both pure BC and BC-XG. In the region between 2912-2924 cm⁻¹, the observed bands corresponded to the stretching frequencies of CH- in cellulose. The peaks, as shown in Figure 8, were nearly identical due to the similar backbone of BC and XG, despite their different assembly methods. The peak arrangement of BC-XG closely resembled that of BC, indicating similar fibrils organisation mode. The carbonyl peak (1600-1900 cm⁻¹) in BC-XG was found at a similar frequency as in BC. In the IR spectrum, the fingerprint region, which spans from 400 to 1400 cm⁻¹, showed distinctive features. The BC-XG sample's spectrum indicated characteristic BC peaks, implying that XG may acquire some of the superior properties of BC materials.

IV. CONCLUSION

The investigation aimed to investigate the impacts of hydrocolloids on cellulose using the well-known *Komagataeibacter xylinus* ATCC 53524. The findings from this study demonstrated that the bacterial strain *K. xylinus* can achieve high BC production when supplemented with varied concentrations of hydrocolloids during the cultivation phase. Generally, the incorporation of these components has a good influence on BC production within a specific radius. However, exceeding this range can impede production. Consequently, BC shows promise in boosting output and offers advantages for various industrial applications. Nevertheless, a more comprehensive investigation is required before BC can entirely replace traditional cellulose materials in most industrial applications. Therefore, future research efforts should primarily focus on developing cost-effective, readily available, and renewable culture media, as well as identifying new microorganisms capable of producing BC.

V. ACKNOWLEDGEMENTS

The authors acknowledged the Faculty of Science and Marine Environment and Universiti Malaysia Terengganu (UMT), Malaysia for supporting this research.

VI. CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest

VII. REFERENCES

- Aswini, K, Gopal, NO, Uthandi, S 2020, 'Optimized culture conditions for bacterial cellulose production by *Acetobacter senegalensis* MA1', BMC Biotechnology, vol. 20, p. 46.
- Auta, R, Adamus, G, Kwiecień, M, Radecka, I, Hooley, P 2017, 'Production and characterization of bacterial cellulose before and after enzymatic hydrolysis', African Journal of Biotechnology, vol. 16, pp. 470-482.
- BeMiller, JN 2019, '8 - Cellulose and Cellulose-Based Hydrocolloids, in BeMiller, J.N. (Ed.), Carbohydrate Chemistry for Food Scientists (Third Edition)', AACC International Press, pp. 223-240.
- Campano, C, Balea Martin, A, Blanco, A, Negro, C 2016, 'Enhancement of the fermentation process and properties of bacterial cellulose: a review', Cellulose, vol. 23.
- Cheng, K-C, Catchmark, JM, Demirci, A 2009a, 'Effect of different additives on bacterial cellulose production by *Acetobacter xylinum* and analysis of material property', Cellulose, vol. 16, pp. 1033-1045.
- Cheng, K-C, Catchmark, JM, Demirci, A 2009b, 'Enhanced production of bacterial cellulose by using a biofilm reactor and its material property analysis', Journal of Biological Engineering, vol. 3.
- Cheng, K-C, Catchmark, JM, Demirci, A 2011, 'Effects of CMC Addition on Bacterial Cellulose Production in a Biofilm Reactor and Its Paper Sheets Analysis', Biomacromolecules, vol. 12, pp. 730-736.
- Ciecholewska-Juśko, D, Broda, M, Żywicka, A, Styburski, D, Sobolewski, P, Gorący, K, Migdał, P, Junka, A, Fijałkowski, K 2021, 'Potato Juice, a Starch Industry Waste, as a Cost-Effective Medium for the Biosynthesis of Bacterial Cellulose', International Journal of Molecular Sciences, vol. 22, no. 19, p. 10807.
- Costa, AFS, Almeida, FCG, Vinhas, GM, Sarubbo, LA 2017, 'Production of Bacterial Cellulose by *Gluconacetobacter hansenii* Using Corn Steep Liquor as Nutrient Sources', Frontiers in Microbiology, vol. 8, p. 2027.
- Dickinson, E 2003, 'Hydrocolloids at interfaces and the influence on the properties of dispersed systems', Food Hydrocolloids, vol. 17, pp. 25-39.
- Gao, G, Cao, Y, Zhang, Y, Wu, M, Ma, T, Li, G 2020, 'In situ production of bacterial cellulose/xanthan gum nanocomposites with enhanced productivity and properties using *Enterobacter* sp. FY-07', Carbohydrate Polymers, vol. 248, p. 116788.
- Hungund, BS, Gupta, SG 2010, 'Improved Production of Bacterial Cellulose from *Gluconacetobacter persimmonis* GH-2', Journal of Microbial and Biochemical Technology, vol. 2, pp. 127-133.
- Jacel, P, Dourado, F, Gama, M, Bielecki, S 2019, 'Molecular aspects of bacterial nanocellulose biosynthesis', Microbial Biotechnology, vol. 12, pp. 633-649.
- Jian, H, Zhu, L, Zhang, W, Sun, D, Jiang, J 2012, 'Galactomannan (from *Gleditsia sinensis* Lam.) and xanthan gum matrix tablets for controlled delivery of theophylline: In vitro drug release and swelling behavior', Carbohydrate Polymers, vol. 87, pp. 2176-2182.
- Krempel, M, Griffin, K, Khouryieh, H 2019, '13 - Hydrocolloids as Emulsifiers and Stabilizers in Beverage Preservation, in: Grumezescu, A.M., Holban, A.M. (Eds.), Preservatives and Preservation Approaches in Beverages', Academic Press, pp. 427-465.
- Kumar, A, Rao, KM, Han, SS 2018, 'Application of xanthan gum as polysaccharide in tissue engineering: A review', Carbohydrate Polymers, vol. 180, pp. 128-144.
- Nguyen, Q-D, Nguyen, T-V-L, Nguyen, T-T-D, Nguyen, N-N 2022, 'Effects of different hydrocolloids on the production of bacterial cellulose by *Acetobacter xylinum* using Hestrin-Schramm medium under anaerobic condition', Bioresource Technology Reports, vol. 17, p. 100878.
- Petri, DFS 2015, 'Xanthan gum: A versatile biopolymer for biomedical and technological applications', Journal of Applied Polymer Science, vol. 132.
- Rachtanapun, P, Jantrawut, P, Klunklin, W, Jantanasakulwong, K, Phimolsiripol, Y, Leksawasdi, N,

- Seesuriyachan, P, Chaiyaso, T, Insomphun, C, Phongthai, S, Sommano, SR, Punyodom, W, Reungsang, A, Ngo, TMP 2021, 'Carboxymethyl Bacterial Cellulose from *Nata de Coco*: Effects of NaOH', *Polymers*, vol. 13, p. 348.
- Rosalam, S & England, R 2006, 'Review of xanthan gum production from unmodified starches by *Xanthomonas compestris* sp.', *Enzyme and Microbial Technology*, vol. 39, pp. 197–207.
- Sperotto, G, Stasiak, LG, Godoi, JPMG, Gabiatti, NC, De Souza, SS 2021, 'A review of culture media for bacterial cellulose production: complex, chemically defined, and minimal media modulations', *Cellulose*, vol. 28, pp. 2649–2673.
- Vasconcellos V, Farinas, C 2018, 'The effect of the drying process on the properties of bacterial cellulose films from *Gluconacetobacter hansenii*', *Chemical Engineering Transactions*, vol. 64, pp. 145–150.
- Zhong, C 2020, 'Industrial-Scale Production and Applications of Bacterial Cellulose', *Frontiers in Bioengineering and Biotechnology*, vol. 8.