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Research article

Effects of Incorporation of *Aloe vera* Leaf Extract on the Synthesis and Characteristics of Bacterial Cellulose Produced by *Komagataeibacter xylinus*

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Abstract

Bacterial cellulose (BC) is a non-toxic, high-purity, and biocompatible polymer that is abundant in various bacteria and does not contain lignin, hemicellulose and pectin found in plant cellulose. High concentrations of carbon sources can limit yields of BC. One potential solution to enhance BC production is the use of elicitors like plant extracts; however, the use of plant extracts such as *Aloe vera* leaf extract remains limited. Therefore, the current study aimed to investigate the effects of *Aloe vera* leaves on BC properties, glucose consumption, and synthesis rate by *Komagataeibacter xylinus* to increase BC volumetric production. *Aloe vera* extract was incorporated into the culture medium (given the conditions used), and BC was analyzed after 5 to 7 days of fermentation. The fermentation process at 30°C showed that increasing *Aloe vera* extract volume to 30% (v/v) improved glucose consumption, resulting in 3.35 g/L of BC accumulated in culture flasks. Adding different amounts of *Aloe vera* leaf extracts to the bacteria culture altered the morphology of the BC film and trapped *Aloe vera* extract inside the BC fibers. The treatment with 30% (v/v) *Aloe vera* leaf extract also resulted in a less homogeneous Aloe-BC morphological surface structure. The X-ray diffractometer (XRD) crystallinity index of control BC improved from 83.70% to 93.70% compared to the 30% Aloe-BC. According to the IR spectral analysis, the presence of amine groups in dried BC samples is confirmed by the detected absorption in the 1536-1635 cm^{-1} range, which indicates N–H bending vibrations. In conclusion, including *Aloe vera* leaf increased glucose intake, enhanced BC biomass, and altered the shape of the BC.

Keywords: bacterial cellulose; fermentation; *Aloe vera* leaf; *Komagataeibacter xylinus;* volumetric productivity

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1. Introduction

Apart from cellulose originating from plants, such as wood, cotton, and bamboo, different types of bacteria, such as those belonging to the *genera Komagataeibacter, Lactiplantibacillus, Rhodococcus, Agrobacterium,* and *Leifsonia*, can also secrete cellulose (Sulaeva et al., 2020; Lupaşcu et al., 2022); that is known as microbial cellulose (MC) or bacterial cellulose (BC). BC's physical and mechanical characteristics are determined by its nanostructure, which is also shaped by the type of culturing method and bioreactor used. BC can be freeze-dried to produce a hydrogel or dry state product. The development of microorganisms, which react to environmental changes in numerous ways, depends critically on the optimal medium design. BC is much thinner than plant cellulose and has a higher surface-area-to-volume ratio thanks to its nanofiber woven 3D network, which enables strong contact with nearby components (Sulaeva et al., 2020). In addition, it possesses a high degree of polymerization and crystallinity (about 90%), exceptional mechanical strength, and the ability to retain water. It is also becoming more and more well-liked because of its biodegradable nature, lack of toxicity, and high purity (free from plant structural features). Its usage in a variety of industrial applications is made possible by these qualities. Moreover, it can be functionalized and employed in the creation of material composites by chemically hybridizing or grafting it with other biopolymers via its many hydroxyl functional groups along the polymer chain (Chaiyasat et al., 2018; Rol et al., 2019).

The productivity of BC production is low despite its high cost of production (Anguluri et al., 2022). Numerous investigators employed *Aloe vera* to enhance the composition and characteristics of BC (Rahman et al., 2017). The addition of *Aloe vera* enhances the composition and characteristics of BC for several reasons such as nutritional enhancement because *Aloe vera* provides a rich source of nutrients, including sugars and amino acids (Cuvas-Limón et al., 2022), which promote the growth of cellulose-producing bacteria. This can lead to increased production rates and yield of BC. *Aloe vera* can also enhance the tensile strength and elasticity of BC, making it more suitable for various applications. However, limited studies have focused on the optimization of the fermentation conditions for Aloe vera-mediated BC production despite its promising effects. Because *Aloe vera* has unique features that may improve BC's attributes and productivity, it can be added to the fermentation medium as an alternative nutritional supplement (Dai et al., 2019). Therefore, the addition of *Aloe vera* leaf extract and gel to the fermentation medium to optimize the synthesis of BC may have a favorable impact on the BC properties and greatly increase the yields of BC (Shi et al., 2014; Barshan et al., 2019). *Aloe vera* (*Aloe vera* Linn), a tropical plant, is easy to grow in hot, dry climates like Thailand. *Aloe vera* has long been used in Thailand to treat burns and is one of the medicinal plants used for various forms of wound care (Hai et al., 2019; Khanal et al., 2021). Previous studies have identified many active ingredients in *Aloe vera*, including flavonoids, lectins, terpenoids, fatty acids, tannins, polysaccharides, enzymes, minerals, and vitamins (Pigaleva et al., 2019; Bodea et al., 2021; Saha et al., 2023). When *Aloe vera* is introduced into the BC production environment, it may inhibit the growth of certain bacteria, potentially impacting the strains used for cellulose production. This could lead to reduced yields or altered cellulose characteristics. Optimizations of the culture conditions such as concentration control, pH adjustment and temperature control would be necessary to balance the benefits of *Aloe vera* with the needs of the bacterial culture.

Our goal in this study was to investigate how *Aloe vera* leaf extract affected BC production and BC properties. The structure, feature of crystallinity, and relationship between BC and *Aloe vera* are explained.

2. Materials and Methods

2.1 Microorganism

For BC synthesis, a strain of *Komagataeibacter xylinus* ATCC 53524, obtained from plant-derived foodstuff from the American Type Culture Collection that came in lyophilized condition was utilized.

2.2 Media for fermentation and culture preservation

The Hestrin-Schramm (HS) medium discovered by Hestrin& Schramm (1954) was utilized as the designated culture medium for experiments. This liquid medium, used for both inoculation and fermentation, comprised various components, including 2.0% glucose (w/v), 0.5% yeast extract (w/v), 0.5% peptone (w/v), 0.27% disodium hydrogen phosphate (w/v), and 0.15% citric acid (v/v). For the preparation of a solid medium intended for storage and preservation, 1.5% agar (w/v) was added. The pH was adjusted to 5.0 using a 1.0M NaOH solution.

2.3 Fermentation conditions

Fermentation commenced by introducing an inoculum culture, prepared by transferring fresh *K. xylinus* culture to HS medium broth and subsequently culturing it with agitation at 30°C for 24 h (Hashim et al., 2021). The flasks were continuously agitated under incubation conditions at 30°C and 70 rpm for 5 to 7 days. The growing culture was agitated to ensure a uniform distribution of cells in the inoculum. Subsequently, the resulting cell suspension was inoculated into a 250 mL conical flask containing 100 mL of liquid medium.

2.4 Preparation of *Aloe vera* leaves extract

Fresh mature leaves of *Aloe vera* plants were collected from the local nursery located near the Universiti Malaysia Terengganu. The preparation of *Aloe vera* leaf extract involved several steps. The leaves were soaked in 20% (v/v) chlorine bleach for 10 min and then dried at room temperature (Atykyan et al., 2020). The rind and aloins of *Aloe vera* leaves from the gel were separated and sliced into small pieces. The leaves were soaked in a 0.06% Tween-20 mixture for 10 min to remove the remaining sap (Fuller et al., 2018). The leaves were ground and mixed with distilled water in a 1:1 ratio (100 g leaf in 100 mL distilled water). Under sterile conditions, the mixture was filtered using a fine mesh strainer. The strain was transferred into a 50 mL Falcon tube and centrifuged at 3000 x g for 10 min. Subsequently, the supernatant was filtered using a sterile vacuum filtration method through a filter paper (pore sizes 80-25 µm). The eluent was used in the treatment media.

2.5 Treatment medium and inoculation of mother culture

The treatment media were prepared by adding the *Aloe vera* leaves into HS liquid medium at concentrations of 1% (v/v), 10% (v/v), 20% (v/v), and 30% (v/v), respectively. Aloe vera-

free HS medium was used as control medium. The medium with added *Aloe vera* leaves was first shaken before inoculation to ensure the complete dissolution of *Aloe vera* leaf extract. All cultures were incubated at room temperature without agitation. For each treatment medium, three replications were randomly sampled after overnight cultivation. Sampling was repeated daily until 4 or 7 days of fermentation. The size of inoculum was 8% (v/v) (Sumardee et al., 2020).

2.6 Purification and drying of BC

The BC pellicles were collected by filtering using filter paper and rinsed with distilled water. Subsequently, the BC pellet was immersed in 0.1 M NaOH solution and incubated at 90°C for 15 min (Bang et al., 2021). The purpose of this step was to effectively remove, disrupt, and dissolve any microbial cells that might have adhered to the BC pellicles. The mixture was then centrifuged at 3000x *g*, 4°C for 20 min as previously described (Lahiri et al., 2021). The pellicle went through a series of filtered water washes and centrifuged again to ensure that all the alkali had been removed, bringing the pH level back to neutral. The washing procedure was repeated thrice, and the washed pellets were kept for BC concentration analysis (Andritsou et al., 2018). The washed BC pellets were collected and put into an oven-dryer for the drying process. To ensure uniform mass, the purified BC was subjected to oven drying (60^{o}C) for 24 h, if needed, until a stable mass was attained at 48 h.

2.7 Scanning electron microscopy (SEM) analysis

The appearance and features of the BC were examined using a scanning electron microscope (SEM), Japan Electron Optics Laboratory Company, Limited (JEOL) at an accelerating voltage of 15 kV at room temperature. During the coating process, gold (Au) was applied to the BC samples.

2.8 Determination of crystallinity

The crystallinity of the BC was assessed via X-ray diffraction (XRD) using a Rigaku Miniflex II diffractometer equipped with Cu Kα radiation. The operational settings were 40 kV for voltage and 30 mA for current. The XRD analysis was conducted at room temperature, scanning the 2θ angle range from 5° to 60° at a rate of 2° per min to assess the radiation reflection of the BC samples.

2.9 Fourier transform infrared (FTIR) spectroscopy analysis

FTIR analysis was performed using a Shimadzu model IRTracer-100 spectrophotometer with the attenuated total reflectance (ATR) technique (Sardjono et al., 2019). The diamond on the sample platform was cleaned with acetone using a piece of lens paper. A background scan without any sample on the diamond was done. Then, a small amount of BC dried sample was placed on the diamond. The pressure arm was set into position and locked over the diamond filled with sample. Force was applied to the sample, pushing it onto the diamond surface (Ul-Islam et al., 2021). Finally, the sample was analyzed with the software and the FTIR spectra were obtained. To investigate structural changes using FTIR at room temperature, the IR spectra of the samples were recorded. FTIR spectra were used to characterize the groups of the BC molecules. The triclinic (Iα) and monoclinic (Iβ)

unit allomorphs exhibit unique IR peaks at 710 cm^{-1} and 750 cm^{-1}, respectively, allowing for their differentiation. By quantifying the absorption signals at 710 cm^{-1} and 750 cm^{-1}, the relative ratio of cellulose Iβ to Iα allomorphs were calculated, enabling the determination of the proportion of Iβ present.

3. Results and Discussion

3.1 Effects of *Aloe vera* leaf extracts on BC synthesis

The effects of various concentrations of *Aloe vera* leaf extract on BC produced by *K. xylinus* are shown in Figure 1. The results showed that the incorporation of 20% (v/v) and 30% (v/v) increased the BC production after 2 days of fermentation. The highest BC was obtained after 4 days of fermentation from a culture containing 30% (v/v) *Aloe vera* extract, which was 3.3 g/L. This was followed with 20% (v/v) *Aloe vera* extract which produced 3.1 g/L BC. On the other hand, in the 1% (v/v) and 10% (v/v) *Aloe vera* extracts, the BC produced did not significantly differ among the treatments after 4 days of fermentation. Nonetheless, the results showed that the BC produced in these treatment media was higher than in the control. Th results also show that BC produced was higher in volume in the *Aloe vera* treatment medium compared to control due to the unique components such as polyphenols and polymannose. Polyphenols can act as antioxidants and may influence the metabolic processes of cellulose-producing bacteria. They can enhance cellulose synthesis by providing a protective effect against oxidative stress, potentially leading to increased BC production. As a polysaccharide, polymannose can serve as a carbon source for the bacteria. Its presence can stimulate cellulose production by providing the necessary sugars for bacterial metabolism. Additionally, it may enhance the structural integrity of the BC by promoting better interaction between the cellulose fibers and the surrounding matrix. Hence, it is a promising candidate material for use in the culture medium.

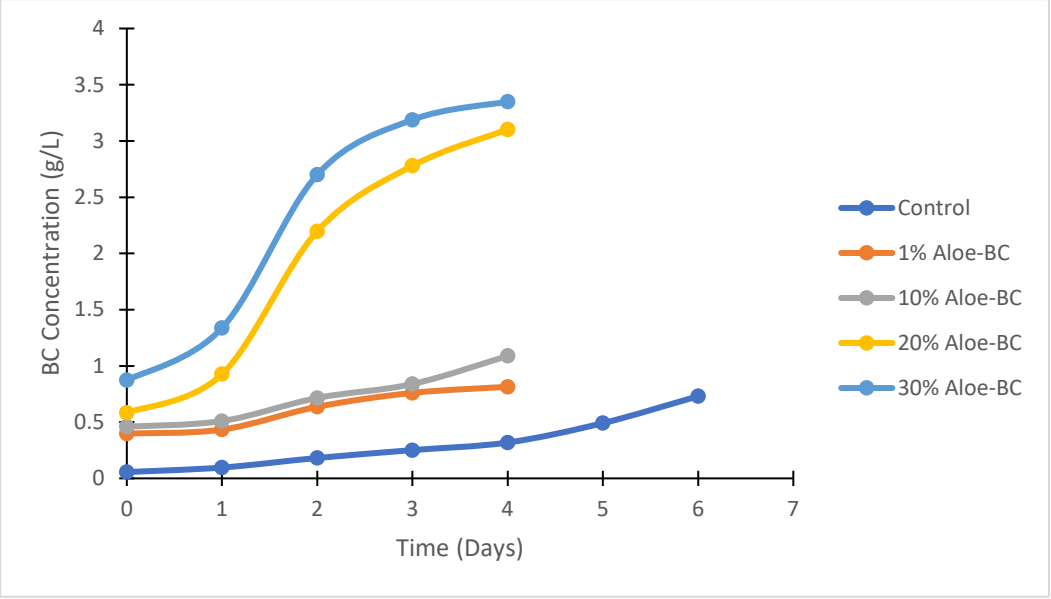

Figure 1. The effects of *Aloe vera* leaf extracts on BC concentrations (g/L) by *K. xylinus*

3.2 Effects of *Aloe vera* leaf extracts on BC and Aloe-BC morphology

The structural features of the produced BC were examined through SEM analysis, with the surface micrographs displayed in Figure 2. SEM imaging revealed that the BC pellicle exhibited a network composed of rod-shaped nanofibers, creating a compact and intricate structure with nano-scale diameters. These nanofibers constituting the BC were observed to be randomly oriented without a distinct pattern.

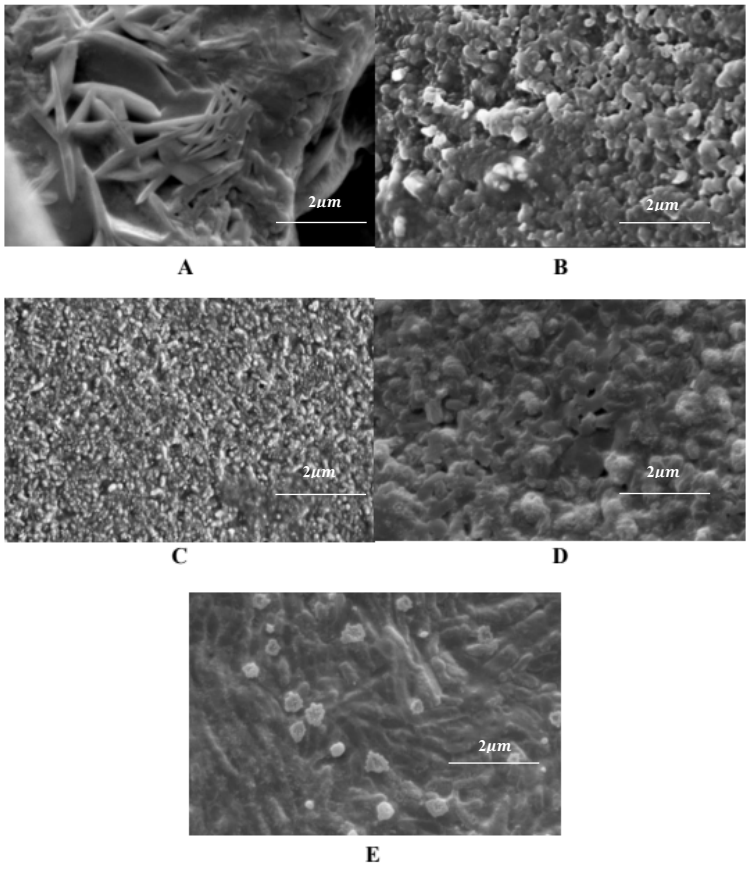

Figure 2. SEM images of surface morphology of A) Control BC, B) 1% Aloe-BC, C)10% Aloe-BC, D) 20% Aloe-BC, and E) 30% Aloe-BC at x6000 magnification

3.3 Effects of *Aloe vera* leaf extracts on degree of crystallinity

The control BC film exhibited characteristic diffraction peaks typical of pure BC, albeit with a slight shift towards lower 2θ values. The crystallinity index (CrI) was determined from the XRD data, as depicted in Figure 3. The XRD pattern of pure BC displayed prominent diffraction peaks at 2θ = 21.33° (main peak), 23.01°, and 29.30° corresponding to cellulose Iα, a naturally occurring crystalline form of cellulose.

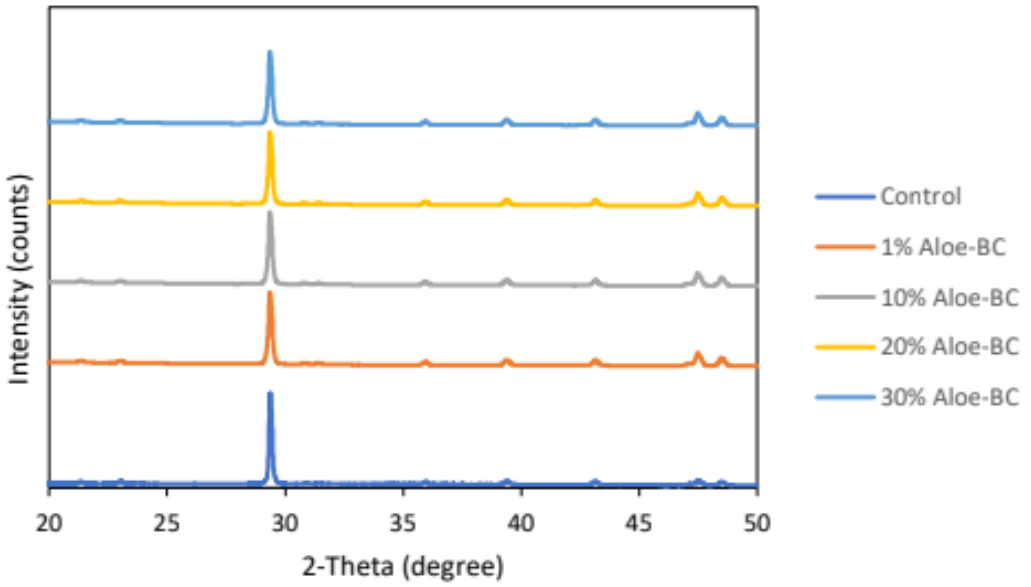

Figure 3. The crystallite indices determination of A) Control BC, B) 1% Aloe-BC, C)10% Aloe-BC, D) 20% Aloe-BC, and E) 30% Aloe-BC by XRD

By self-assembly, BC fibers create a highly tangled, randomly distributed network structure. In the case of BC fibers, self-assembly involves the cellulose molecules coming together to form long chains. These chains then align and intertwine with each other to create a highly tangled, randomly distributed network structure. The resulting fibrous network is stable and robust; however, its specific structure is created entirely through natural interactions without any external manipulation. The 10% Aloe-BC sample's decreased crystallinity suggests that *Aloe vera* leaf presence somewhat impedes the BC fibers' ability to self-assemble (Yosboonruang et al., 2023). Incorporating 10% *Aloe vera* extract into BC likely disrupted the regular alignment and crystallization of the BC fibers, resulting in lower crystallinity compared to pure BC. This decrease in crystallinity suggests that the *Aloe vera* interfered with the self-assembly of the cellulose fibrils, which typically align to form a crystalline structure in pure BC. The lower *Aloe vera* concentration in 1% Aloe-BC resulted in slight disruption, while maintaining higher crystallinity due to less interference with the BC structure. Further reduction in crystallinity may be attributed to the inhibition of hydrogen bond formation between cellulose fibers and microfibers, likely caused by *Aloe vera* compounds adsorbed on the BC surface. The 10% Aloe-BC's decreased crystallinity was comparable to results from earlier in-situ modification when modifiers were added exogenously to the fermentation medium (Ghozali et al., 2021). From this study, 10% (v/v) *Aloe vera* leaf altered the BC generated and had a crystallinity of 50.40%, whereas 1% Aloe-BC had a crystallinity of 95.00%. Higher concentrations of *Aloe vera*, such as 20% and 30% Aloe-BC lead to even further reduction in crystallinity, as more *Aloe vera* would prevent BC fibers from aligning as effective. Therefore, the 10% Aloe-BC sample sits between these two extremes, showing moderate crystallinity reduction, indicating partial interference with fiber assembly but maintenance of some degree of order. This indicates structural alterations in BC architecture caused by physical and chemical stimuli (González-García et al., 2022).

3.4 Effects of *Aloe vera* leaf extracts on functional group presence

The BC films, enriched with *Aloe vera* leaf extract concentrations of 1% (v/v), 10% (v/v), 20% (v/v), and 30% (v/v) within the culture medium, exhibited distinct changes in their Fourier-transform infrared (FTIR) spectra. Specifically, new peaks at 1530.26, 1533.97, 1534.50, and 1542.67 cm-1 were observed, indicating a shift in peak positions and the appearance of new absorption bands. This phenomenon suggests the occurrence of intermolecular interactions between the cellulose chain of BC and the amino groups present in *Aloe vera* leaf extract, corroborating findings from previous studies (Hęś et al., 2019). Furthermore, SEM analysis supports the notion that *Aloe vera* leaf extracts facilitated the formation of intermolecular bonds with cellulose fibrils (Saibuatong, 2011). *Aloe vera* contains polysaccharides such as acemannan, which can interact with cellulose. These polysaccharides can form hydrogen bonds with cellulose fibrils, enhancing the structural integrity of the cellulose network.

Figure 4 illustrates the infrared spectra of BC samples. In the FTIR spectrum of the BC control, distinct peaks are observed, including -NH stretching at 3274 cm^{-1}, -OH bending at 1646 cm^{-1}, broad water absorption due to hydrogen bonding at 1640 cm^{-1}, and a broad absorption peak of -OH stretching expanded by hydrogen bonding at approximately 3340 cm^{-1}. In FTIR spectroscopy, specific wavenumber ranges can indicate the presence of cellulose and compounds found in *Aloe vera* extract. For the treated BC, a broad peak of -NH stretching and -OH stretching at 3338 cm^{-1}. This broad peak related to hydroxyl groups, followed by overlapping peaks ascribed to C=O stretching and O-H bending vibrations, and water absorption due to hydrogen bonding at 1637 cm^{-1}, are evident. Notably, these characteristic peaks of *Aloe vera* leaf extract and BC exhibit a frequency shift towards lower wavenumbers, indicating an effect of their combined presence (Gorgieva & Trček, 2019).

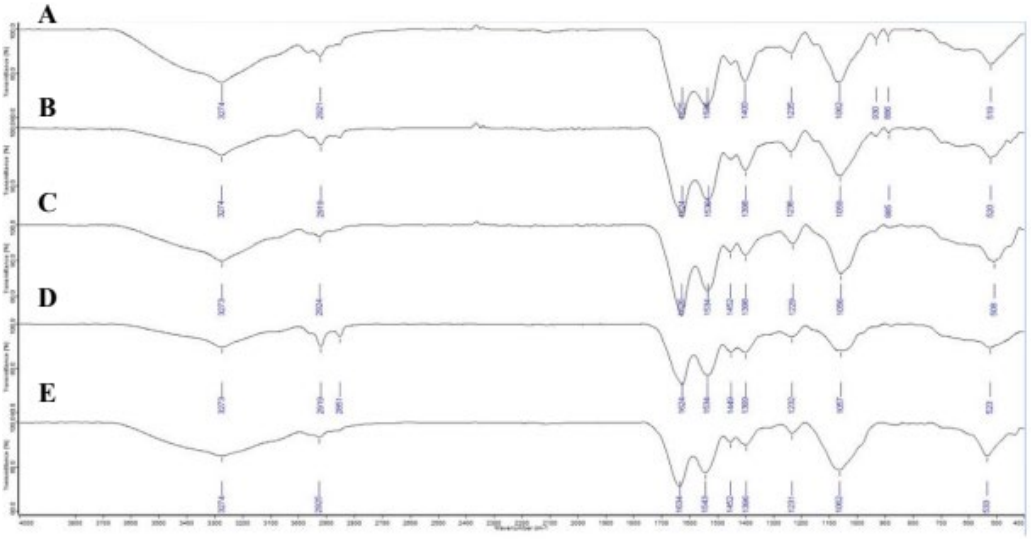

Figure 4. The functional group determination of A) Control BC, B) 1% Aloe-BC, C)10% Aloe-BC, D) 20% Aloe-BC, and E) 30% Aloe-BC by FTIR

The resulting BC spectra (Figure 4) did not differ in any noticeable way. Each peak showed up in the same location and with a comparable intensity. Chen et al. (2019) found that cellulose had the same functional groups and chemical bonds as the resulting BC. The polysaccharide that *K. xylinus* synthesized was BC, as demonstrated by the FTIR spectra. The peaks of the FTIR spectra showed that *K. xylinus* synthesized BC in a polymeric form (González-García et al., 2022). Table 1 displays the functional groups and FTIR adsorption bands that were assigned to those groups.

Table 1. The adsorption and assigned functional groups on IR spectra

Functional Groups	**Adsorption Band (cm^{-1})**
O-H group stretching	3600-3000
C-H stretching in cellulose skeleton	2895
-C=O stretch	1740-1700
Absorbed water	1645-1630
H-C-H, O-C-H in-plane bending	1493-1396
CH2 rocking vibration at C6 carbon	1315
Out-of-plane bending vibration of C-O-H at C6	1296-1219
Symmetrical stretching vibration from C-O-C	1205
Asymmetric stretching vibrations from C-O-C	1162
C-C, C-OH, C-H ring and side group vibrations	1140-926
C-O-C (1-4) glycosidic linkages	1107
C-O-C, CC-O, C-C-H deformation and stretching vibrations	898

3.5 Effect of *Aloe vera* leaf extracts on BC yield and BC volumetric productivity

The yield coefficient in each concentration of *Aloe vera* leaf extract was calculated to assess the efficiency of BC synthesis by *K. xylinus*. This metric evaluates the effectiveness of bacterial activity to determine the most efficient pathways to produce cellulose. Increased volumetric productivity indicates a more efficient method because it indicates higher cellulose production. By evaluating volumetric productivity across different concentrations of *Aloe vera* leaf extract, the study sought to determine the most efficient concentration for large-scale manufacturing (Añibarro-Ortega et al., 2021). Table 2 presents the yield coefficients and volumetric productivity for the different *Aloe vera* leaf extract concentrations. The analysis of yield coefficient (g/g) and volumetric productivity (g/L/D) shows that the 30% concentration was more efficient than the other concentrations.

Table 2. Effect of *Aloe vera* leaves on yield coefficient and volumetric productivity

Parameters	*Aloe vera* Leaf Concentrations				
	Control	**1%**	**10%**	**20%**	**30%**
BC (g/L)	0.304	0.609	0.723	1.918	2.290
BC yield (g/g)	0.031	0.082	0.087	0.281	0.307
BC volumetric productivity (g/L/D)	0.633	1.901	2.258	5.993	7.155

In this investigation, *Aloe vera* leaf was utilized as an additive to modify the properties of BC. The incorporation of *Aloe vera* leaf extract led to a notable enhancement in BC production compared to the control BC. For instance, with the introduction of 1% *Aloe vera* leaf extract, *K. xylinus* yielded 0.43 g/L of cellulose pellicles on the initial day of fermentation, escalating to 0.81 g/L on the final day of fermentation. BC synthesis exhibited a substantial increase as the concentration of *Aloe vera* leaf extract rose, peaking at 30% (v/v). Specifically, the inclusion of 30% (v/v) *Aloe vera* leaf extract resulted in the highest BC production of 3.3 g/L, representing a 4.5-fold increase compared to the control treatment. This was in agreement with the results of a previous study by Saibuatong (2011), who discovered that the addition of 30% BC-Aloe vera gel offered the most favorable conditions for film formation.

The utilization of *Aloe vera* extracts led to a notable enhancement in BC yield compared to the control BC (Figure 1). Supplementation with 1% *Aloe vera* extract resulted in *K. xylinus* generating 0.43 g/L of cellulose pellicles on the initial day of fermentation and 0.81 g/L on the final day. Notably, as the *Aloe vera* leaf extract concentrations increased up to 30% (v/v), a significant rise in BC synthesis was observed. Specifically, the inclusion of 30% (v/v) *Aloe vera* leaf extract yielded the highest BC production at 3.3 g/L, representing a 4.6-fold increase over the control group. This boost was attributed to improved incorporation or adsorption of additives and a reduction in crystallinity, a key factor influencing BC production rates. Furthermore, a gradual rise in BC synthesis proportional to *Aloe vera* leaf extract concentrations was evident across other treatments, affirming the positive impact of higher *Aloe vera* concentrations on BC production efficiency (Naomi et al., 2020).

The nanostructures of the *K. xylinus* control BC was a highly porous, three-dimensional network structure. Compared to Aloe-BC composites described in earlier research, such as those including Aloe vera gel or crude extract (e.g., Saibuatong, 2011; Ul-Islam et al., 2021), the Aloe-BC created in this study showed more homogeneous integration and adherence of Aloe vera compounds inside the BC matrix. Analysis of fiber diameter distribution revealed that control BC fibers were primarily between 50 and 110 nm, but Aloe-BC fibers were thicker, ranging between 90 and 190 nm. The addition of *Aloe vera* leaf extract also resulted in a decrease in inter-fiber separation and a significant rise in fibre diameter. The introduction of *Aloe vera* leaf extract led to a reduction in inter-fiber spaces and a notable increase in fiber diameter.

Furthermore, the BC structure may exhibit localized elemental enrichment, potentially influenced by the incorporation of *Aloe vera* constituents. The surface of the bacterial cellulose seemed to undergo successive modification by the *Aloe vera* leaf, with elements such as carbon, oxygen, and phosphate groups potentially contributing to the observed smaller pore sizes, likely due to *Aloe vera* leaf entrapment within the cellulose

fibrils. The presence of phosphate groups from the HS media and *Aloe vera* may have influenced the local concentration medium, leading to their embedding between the BC fibers (Ul-islam et al., 2021).

It is noticeable that with increasing concentrations of *Aloe vera* leaf extract, the intensity of the diffraction peak at 23.01° decreased significantly compared to the control BC. This indicates a transformation of the cellulose I structure into a different allomorph, leading to the conversion of some crystalline regions into amorphous structures due to the introduction of additional cellulose components during BC synthesis (Irham et al., 2020). Moreover, the inclusion of 10% (v/v) *Aloe vera* leaf extract resulted in a reduction in the crystallinity index of BC as determined by XRD analysis. For example, while 1% Aloe-BC exhibited a crystallinity of 95.00%, the incorporation of 10% (v/v) *Aloe vera* leaf extract altered the produced BC to possess a crystallinity index of 50.40%. The existence of amorphous polysaccharides like hemicellulose and pectin may influence the crystallinity index.

Based on FTIR patterns, the distinctive spectra of bacterial cellulose revealed a broad peak at a wavenumber of 3276 to 3339 cm-1, which was related to the stretching on intra- and inter-chain hydrogen-bonded hydroxyl groups (-OH). Meanwhile, the C–H stretching vibration of CH_2 of the hydroxymethyl groups is seen at 2925 to 2930 cm^{-1}. The presence of carboxylic and carboxylate groups was shown by the peak at wavenumber 1644 cm^{-1} and 1421 cm^{-1}. The sugar ring's C–O–C and C–O–H stretching vibrations may be linked to the peak at wavenumber 1036 cm^{-1}. On the other hand, the presence of a crystalline region within the structure was indicated by the peaks at wavenumber 1330 cm^{-1} and 1257 cm^{-1}, which were attributed to O–H in-plane bending and C–O stretching, respectively (Gorgieva & Trček, 2019).

The optimal substrate composition for cellulose synthesis was determined by the concentration of *Aloe vera* leaf extract. At a 30% concentration, *Aloe vera* leaf extract provided optimal nutrients and environments for bacterial growth and cellulose formation. The yield coefficient measures the efficiency of BC synthesis, indicating that more cellulose is produced per unit of substrate used (Godinho et al., 2016). The study revealed that the 30% (v/v) concentration of *Aloe vera* leaf extract was the most advantageous, exhibiting the best yield coefficient and volumetric productivity, indicating that *K. xylinus* functions more effectively in these conditions, demonstrating increased BC production.

When Aloe vera leaf extract was added, the BC yields were similar to those observed in earlier investigations. Glucose and maltose-based formulations produced the most volumetric BC (0.07 g/L/D) across evaluated media. While earlier studies investigated the impacts of various carbon sources, including up to eight types, this study is unique in that it focuses on the incorporation of *Aloe vera* leaf extract into the fermentation process. Unlike Molina-Ramírez et al. (2017), our study found that *Aloe vera* leaf extract can considerably increase the BC synthesis.

4. Conclusions

In this study, *Aloe vera* leaf extract was added to *Komagataeibacter xylinus* ATCC 53524 to generate a modified BC. FTIR, XRD, and SEM analyses were performed on the resultant BC. The FTIR spectra of the BC used in this investigation proved that the BC made by *K. xylinus* ATCC 53524 was cellulose. XRD analysis revealed the crystal structure of cellulose type I. SEM morphology was utilized to observe the three-dimensional structure of BC and BC integrated with *Aloe vera*. These findings indicate that *Aloe vera* leaf extract had the potential to serve as a cost-effective supplement and potentially beneficial nutrient for the

enhancement of BC development. Furthermore, *Aloe vera* is known for its eco-friendly properties and potential benefits for the environment. By incorporating *Aloe vera* into BC production, the study highlights a sustainable synergy between two natural materials. This approach reinforces the eco-friendly narrative of the research and underscores the potential for environmentally responsible manufacturing processes.

5. Acknowledgements

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6. Authors' Contributions

Azila Adnan and Aziz Ahmad: Conceptualization. Nuratiqah Ismail, Nur Fajrina Roslan, Vishnupriya A/P Murugam and Nurqamarina Abang Nasyhin: Writing and original draft preparation, performed research. Wan Syahiidah Wan Abd Aziz and Nurul Nadhirah Ruzelan: Contributed reagents/analytic tools. Nuratiqah Ismail, Nur Fajrina Roslan, Vishnupriya A/P Murugam and Nurqamarina Abang Nasyhin: Data collection, data analysis and data interpretation. Azila Adnan and Aziz Ahmad: Supervision. All authors discussed the results and contributed to the final manuscript.

7. Conflicts of Interest

The authors declare no conflict of interest.

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