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Variation of Tea Waste Addition on Bacterial Cellulose Production from Kombucha Fermented Sago Liquid Waste

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Abstract. *Sago liquid waste contains high levels of carbohydrates and has potential as a fermentation medium for bacterial cellulose production. However, its nutritional content requires enhancement by supplementing it with tea waste to achieve optimal yields. This study investigated the effect of varying tea waste additions on the characteristics of bacterial cellulose produced from kombucha fermentation based on sago liquid waste. The research method used a completely randomized design with five treatments (2, 4, 6, 8, and 10 g of tea dregs) and three replications. The observed parameters included yield, thickness, moisture content, cellulose content, and Fourier Transform Infrared (FTIR) spectroscopy. The results showed that increasing tea waste levels significantly affected all parameters ($p < 0.05$). The best treatment (10 g) resulted in a yield of 18.21%, a thickness of 13.47 mm, a moisture content of 47.57%, and a cellulose content of 52.43%. FTIR spectral analysis confirmed the characteristic peaks of bacterial cellulose, indicating the presence of crystalline β-1,4-glucan structures. The identification of cellulose type I is significant because it represents the native crystalline form of bacterial cellulose, which is associated with high mechanical stability, strong hydrogen bonding, and potential suitability for biopolymer applications. This study demonstrates that tea waste is an effective nutrient supplement that enhances the quality and quantity of bacterial cellulose derived from kombucha fermentation of sago liquid waste.*

Keywords: *Cellulose; kombucha; sago; tea; waste.*

Type of the Paper: Regular Article.

1. Introduction

Bacterial cellulose (BC) is a biomaterial that has attracted considerable research attention due to its advanced three-dimensional nanostructure, high crystallinity, elevated degree of polymerization, excellent water-holding capacity, high liquid and gas permeability, remarkable purity, good biocompatibility, non-toxicity, and biodegradability [1]. One of its main advantages over plant-derived cellulose is its exceptional purity, as it lacks lignin and hemicellulose, typically present in plant fibers [2]. In addition, BC possesses outstanding water absorption capabilities, making it suitable for applications in wound dressings and cosmetic products [3]. Its mechanical strength, especially under wet conditions, ensures high stability across various environments [4]. A primary source of bacterial cellulose is the pellicle formed by the Symbiotic Culture of Bacteria and Yeast (SCOBY) during kombucha fermentation.

The SCOBY pellicle is a by-product of acetic acid bacteria metabolism, including genera

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such as *Acetobacter*, *Gluconobacter*, *Gluconacetobacter*, and *Komagataeibacter* [5]. *Gluconacetobacter xylinus* (now reclassified to *Komagataeibacter xylinus*) is known as the most efficient cellulose producer due to its close association with yeasts in symbiotic culture. The members of *Gluconacetobacter* (now *Komagataeibacter*) dominate kombucha fermentation, even under suboptimal temperature conditions [6]. This strain produces water-soluble brown pigments and specific metabolites such as 2,5-diketo-D-gluconate and γ-pyrone from glucose [7]. *Komagataeibacter* species are known for producing D-saccharic acid-1,4-lactone, a compound with hepatoprotective effects against acetaminophen-induced toxicity [8]. During fermentation, acetic acid bacteria form cellulose fibers approximately 1.5 nm in diameter and 9 nm in length, which interweave to form a compact, hydrated cellulose network after several days of culturing [9].

Carbon and nitrogen availability in the fermentation medium strongly influence SCOBY yield. Utilizing low-cost carbon sources such as agro-industrial waste enhances bacterial cellulose production's economic and environmental feasibility. Developing sustainable waste utilization strategies reduces production costs and adds value to waste, aligning with circular economy principles and environmental protection goals [10].

Sago liquid waste, which contains high levels of carbohydrates and acidic pH, presents a promising alternative as a fermentation medium. Generated from starch extraction processes, this waste is rich in organic matter and has a carbon-to-nitrogen ratio of 105:0.12 [11]. Approximately 20,000 liters of water are used to process 1 ton of sago starch, of which 94% becomes liquid waste, contributing to an annual release of 5.8 million m^3 of wastewater into aquatic ecosystems [12]. However, industrial waste is a complex, undefined, and unstable substrate, often leading to inconsistencies in bacterial cellulose quality [13].

To address this issue, external nutrient enrichment is necessary. Tea waste, which contains residual phenolics and nitrogenous compounds, offers potential as a supplementary nutrient source to enhance microbial activity and stabilize bacterial cellulose production. Tea waste contains crude protein, fat, ash, moisture, fiber, hemicellulose, polyphenols, and free amino acids [14]. Black and green tea residues retain compounds such as gallocatechin (GC), epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and gallic acid [15]. These factors influence cellulose biosynthesis by modulating pH, promoting microbial symbiosis, and inhibiting contaminants [16]. Kombucha fermented with green tea residues reportedly contains higher total polyphenols and caffeine than black tea or without residues [17]. Thus, incorporating tea waste into kombucha fermentation reduces environmental burden and benefits the tea industry.

Although various studies have investigated liquid waste substrates for bacterial cellulose production, such as sago or tofu wastewater, combining sago liquid waste with tea waste in

kombucha fermentation remains underexplored. Reported that using sago liquid waste with *Acetobacter xylinum* LKN6 for 14 days yielded up to 62.73% bacterial cellulose [18]. Adding 12% sucrose to the kombucha fermentation of tofu wastewater produced a maximum bacterial cellulose yield of 6.64% [19]. Green tea kombucha (nata de ocha) fermented with 15% sugar for 21 days produced 5.72% bacterial cellulose [20]. Other research results identified 10 g/L as the optimal tea concentration for bacterial cellulose production [21]. Excessive tea can inhibit microbial growth and reduce SCOBY quality, while too little tea inhibits optimal pellicle formation [22].

Considering the limited research on kombucha fermentation utilizing both sago liquid waste and tea waste, this study aims to evaluate the effect of different levels of tea waste supplementation on the characteristics of bacterial cellulose produced from kombucha fermented sago liquid waste.

2. Materials and Methods

2.1. Materials

The materials used were a 2-week-old kombucha starter previously cultivated from black tea, commercial sucrose, tea waste obtained from the Danau Kembar tea factory in Solok, homemade sago liquid waste, and all chemical materials purchased from the local chemical store.

2.2. Research Design

An experimental method was employed using a completely randomized design (CRD), followed by Duncan's New Multiple Range Test (DNMRT). The experiment consisted of five treatments with three replications. The research treatments are presented in Table 1.

Table 1. Treatment matrix

Material	P_1	P_2	P_3	P_4	P_5
Tea waste (g)	2	4	6	8	10
Sucrose (%)	12	12	12	12	12
Sago liquid waste (ml)	300	300	300	300	300

2.3. Bacterial cellulose production

Sago liquid waste was sterilized at 121°C for 15 minutes and then used to brew the tea waste according to the treatment design. The resulting infusion was filtered and supplemented with sucrose. The mixture was stirred until homogeneous and cooled to room temperature (28-30°C). Once cooled, 10% (v/v) kombucha starter was added to the solution, and the mixture was fermented in a clean fermentation chamber using 750 mL glass jars for 14 days. After fermentation, the resulting bacterial cellulose pellicle was removed and soaked in distilled water for 24 hours. The pellicle was then boiled in 2% NaOH solution at 80°C for 1 hour to remove non-cellulosic components and residual microbes. Finally, the bacterial cellulose was soaked in 3% H_2O_2 solution for 1 hour to remove the brownish coloration that appeared during fermentation.

2.4. Yield of bacterial cellulose

Yield was determined following the method of [23]. The bacterial cellulose pellicle was repeatedly washed, drained for approximately 30 minutes to remove excess water, and weighed to determine its wet weight. The yield was calculated using Eq. (1) below.

$$\text{Yield (\%)} = \left(\frac{\text{Wet weight (g/L)}}{\text{Substrate volume (mL)}}\right) \text{x } 100 \quad (1)$$

2.5. Thickness of bacterial cellulose

Thickness measurements were taken at five different points, and the average value was calculated to determine the final thickness of the bacterial cellulose [24].

2.6. Moisture content and cellulose content

Moisture and cellulose contents were determined following the method of [25]. The difference between the wet and dry weights of the bacterial cellulose was used to calculate the moisture content. The bacterial cellulose was dried at 60°C for 3 hours, cooled in a desiccator for 15 minutes, and re-dried for 1 hour until a constant weight was obtained. Moisture content was calculated using Eq. (2) below.

$$\text{Moisture content (\%)} = \left(\frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}}\right) \text{x } 100 \quad (2)$$

The cellulose content was calculated using Eq. (3) below.

$$\text{Cellulose content (\%)} = 100\% - \text{moisture content} \quad (3)$$

2.7. FTIR spectroscopy

This analysis was conducted only on the best treatment, referring to the method of [26]. The bacterial cellulose was washed three times with distilled water, cut into small pieces, and dried. The dried bacterial cellulose pellicle was analyzed at room temperature. The infrared spectrum was recorded in the range of 4000-400 cm^{-1}.

3. Results and Discussion

The results of kombucha fermentation using sago liquid waste with varying amounts of tea waste addition are presented in Fig. 1.

3.1 Yield

Yield is the percentage of final product obtained relative to the initial material used and is expressed either in dry mass or wet weight per substrate volume. In kombucha production, bacterial cellulose yield refers to the biomass produced during fermentation by the symbiotic culture of bacteria and yeast in a specific nutrient medium [25].

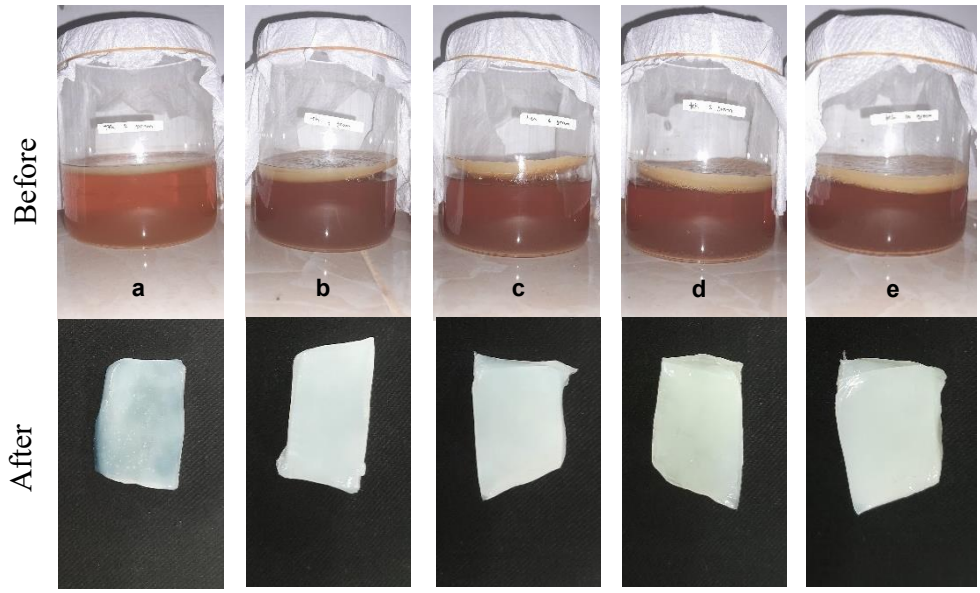

Fig. 1. BC with tea waste addition of 2 g (a), 4 g (b), 6 g (c), 8 g (d), and 10 g (e) on day 14 before-after NaOH and H_2O_2 treatment

The analysis of variance showed that different levels of tea waste addition had a significant effect ($P<0.05$) on the yield of bacterial cellulose from kombucha fermented with sago liquid waste. The average yield values for each treatment are presented in Table 2. Table 2 clearly shows that all five treatments consistently increased the yield of bacterial cellulose. Yield is an initial indicator of the amount of bacterial cellulose that can be isolated and purified. Since bacterial cellulose possesses high economic value and functional potential, yield analysis is essential in evaluating the feasibility of a substrate as a raw material for biomaterial industry applications [27].

Table 2. Yield of bacterial cellulose

Sample Code	Yield (%)
P_1	8.6077 ± 0.18 [a]
P_2	13.7686 ± 0.20 [b]
P_3	16.7978 ± 0.18 [c]
P_4	17.5170 ± 0.17 [d]
P_5	18.2089 ± 0.19 [e]

Treatment P_1 (2 g) yielded the lowest yield at 8.61%, while P_5 (10 g) yielded the highest at 18.21%. This result indicates a statistically significant difference in bacterial cellulose biomass productivity due to the variation in tea waste addition. The increase in yield is directly related to the number of bioactive compounds retained in tea waste. As a by-product of tea processing, tea waste still contains polyphenols, tannins, and aromatic compounds that can support microbial metabolism in kombucha, particularly those of the *Komagataeibacter* genus, which is well known as the primary bacterial cellulose producer [28]. These components may enhance the activity of

acetic acid bacteria in converting sugars into cellulose through the metabolic pathway of glucose into β-1,4-glucan chains.

According to Pradhan *et al.* [29], the availability of nitrogen and carbon compounds from tea waste can accelerate the growth of cellulose-producing bacteria and enhance the rate of macromolecular polysaccharide synthesis. Furthermore, sago wastewater, which is used as the primary carbon source, makes a positive contribution. The hydrolyzed starch in sago wastewater can be directly converted into free glucose, facilitating efficient cellulose biosynthesis [18]. The use of green tea in kombucha production also resulted in lower bacterial cellulose yields than black tea. Despite being waste-based, the combination of sago liquor and tea dregs still produced competitive yields, highlighting the potential of utilizing local waste resources to replace conventional fermentation media such as Hestrin-Schramm [30].

The antioxidant content in tea not only supports microbial growth but also contributes to the stability of the fermentation environment. Antioxidants such as catechins and theaflavins are known to neutralize free radicals formed during aerobic fermentation, thus preserving the health of cellulose-producing bacterial cells [31]. This dual function of tea waste, enhancing yield while improving the chemical stability of the produced cellulose, offers added value for future industrial applications.

3.2 Thickness

The thickness of bacterial cellulose produced from kombucha fermentation using sago liquid waste is one of the key physical parameters that reflects the biosynthesis rate of cellulose during the fermentation process. This thickness indicates the accumulation of cellulose biomass formed on the surface of the liquid medium, synthesized by *Komagataeibacter* species. The thicker the cellulose layer of kombucha bacteria, the greater the total cellulose produced. Various factors, such as the type of carbon substrate, fermentation time, and environmental conditions, including pH, temperature, and oxygen availability, greatly influence the thickness of the bacterial cellulose pellicle [28].

Table 3. Thickness of bacterial cellulose

Sample Code	Thickness (mm)
P_1	5.0667 ± 0.21[a]
P_2	6.1667 ± 0.06[b]
P_3	8.4667 ± 0.15[c]
P_4	12.5000 ± 0.89[d]
P_5	13.4667 ± 0.31[e]

Analysis of variance results showed that variation in tea waste addition had a significant effect ($P < 0.05$) on the thickness of bacterial cellulose produced from sago liquid waste kombucha. The average thickness values for each treatment are shown in Table 3. The table indicates that all

five treatments consistently led to an increase in cellulose thickness. A higher quantity of tea waste provides additional nutrients and acts as a thermal stabilizer and pH buffer, maintaining optimal conditions for microbial activity during fermentation. Stable pH conditions are crucial to ensure maximum nata de coco or bacterial cellulose production [32].

Based on the results, P_1 (2 g) produced the thinnest pellicle with an average thickness of 5.1 mm, while P_5 (10 g) produced the thickest pellicle at 13.47 mm. These findings indicate that increasing amounts of tea waste significantly enhance the thickness of the bacterial cellulose biomass. Thickness is often used as a quality indicator, representing the consistency and reproducibility of the fermentation process. A uniform and consistent thickness implies that fermentation conditions were favorable and bacterial activity was optimal. The increase in thickness reflects the vertical accumulation of bacterial cellulose during fermentation. The cellulose produced by *Komagataeibacter xylinus* forms a three-dimensional nanofiber network that grows upward on the liquid surface, resulting in progressively thicker layers of nata over time [33].

According to Fernandes et al. [34], fermentation media containing a complete composition of nutrients enhance the biosynthesis of exopolysaccharides, directly influencing the cellulose network's microstructure. The more tea dregs added, the more the availability of micronutrients and minerals such as magnesium and calcium increases. These micronutrients are known to support the activity of cellulose synthase enzymes, which are responsible for building the polymeric structure of bacterial cellulose. Thus, the measurement of thickness becomes a rapid and practical method for evaluating the quality of bacterial cellulose before proceeding to more complex chemical or mechanical characterization.

Using sago liquid waste as the primary fermentation medium also supports the structural growth of cellulose by supplying readily available glucose. As a simple monosaccharide, glucose plays a crucial role in enhancing cellulose synthase activity in forming vertically aligned nata fibers [18]. The naturally acidic pH of sago liquid waste (around 4-5) also creates a favorable environment for *Komagataeibacter* growth, supporting the optimal formation of a thick cellulose pellicle.

3.3 Moisture content

Moisture content analysis of bacterial cellulose from sago liquid waste kombucha is an essential parameter in evaluating the quality and physical characteristics of the final product. Moisture content indicates the level of water retained in the polymeric matrix of cellulose, which in turn can influence its structural integrity, microbial stability, post-processing efficiency (such as drying and purification), and further application suitability [35]. A high moisture content may indicate an imperfect fiber network or weak water binding within the matrix, leading to reduced

physical stability and increased risk of microbial contamination during storage.

The analysis of variance showed that the variation in tea waste addition had a statistically significant effect ($P<0.05$) on the moisture content of bacterial cellulose produced from sago liquid waste kombucha. The average moisture content for each treatment is presented in Table 4. All five treatments resulted in a consistent decrease in water content as the amount of tea dregs increased. At lower levels of tea dregs, the nata structure tended to be looser due to suboptimal cellulose biosynthesis, resulting in more water being retained within the cellulose network through capillary action [36]. In contrast, higher tea waste levels promote denser and more organized fiber formation, reducing the cellulose structure's interstitial spaces, leading to decreased water retention.

Table 4. Moisture content of bacterial cellulose

Sample Code	Moisture Content (%)
P_1	54.1824 ± 0.66 [c]
P_2	52.1512 ± 0.72 [b]
P_3	51.2368 ± 0.40 [b]
P_4	49.3295 ± 0.28 [a]
P_5	47.5708 ± 1.99 [a]

Based on Table 4, P_1 (2 g) exhibited the highest moisture content at 54.18%, while P_5 (10 g) had the lowest moisture content at 47.57%. This trend illustrates a significant reduction in moisture content as tea waste increases. The reduction in water content can be attributed to the densification of the fermentation matrix, which results from the increased concentration of dissolved solids from tea waste. It directly concerns the density and compactness of the cellulose network. As a result, the bacterial cellulose formed is less capable of retaining water through capillary forces or weak hydrogen bonding [37].

The increase in available nutrients from tea waste enhances the rate and directionality of cellulose biosynthesis, producing a more crystalline and less hydrophilic polymer matrix that retains less water [29]. According to Fernandes et al. [34], the microfibrillar structure of bacterial cellulose typically holds large amounts of water due to the porosity between nanofiber bundles. However, when this structure becomes denser because of a more nutrient-rich substrate, its porosity decreases and consequently lowers its moisture content.

Tea waste from processing industries still contains bioactive compounds such as polyphenols, tannins, amino acids, and micronutrients that act as metabolic stimulants for acetic acid bacteria and yeasts during kombucha fermentation. The presence of phenolic and nitrogenous compounds in tea waste enhances the growth rate of the microbial symbiosis. It accelerates bacterial cellulose biosynthesis via the acetyl-CoA and UDP-glucose metabolic pathways [38]. With the increasing tea waste addition, the concentration of available nutrients in the substrate

rises, accelerating the formation of a denser and thicker cellulose layer. This matrix becomes stiffer and holds less water, indirectly reducing the moisture content of BC produced from sago liquid waste.

Sago liquid waste contains simple carbohydrates like glucose and minerals such as magnesium and potassium, which serve as energy sources for fermentative microorganisms [18]. When used to brew tea waste, sago liquid waste dissolves bioactive compounds from the tea residue and enriches the fermentation medium with additional nutrients. This combination creates a complex fermentation substrate that supports waste reduction while offering a cost-effective and environmentally friendly strategy to enhance the efficiency of bacterial cellulose production.

3.4 Cellulose content

Cellulose content analysis of bacterial cellulose from sago liquid waste kombucha is a critical parameter for evaluating the quality and potential utility of the fermented product. Cellulose synthesized by acetic acid bacteria such as *Komagataeibacter xylinus* is known for its highly pure nanofibrillar structure, making it suitable for various industrial applications, including biomedical materials, food packaging, and composite manufacturing [37].

The analysis of variance showed that the variation in tea waste addition significantly influenced the cellulose content ($P<0.05$) of bacterial cellulose produced from sago liquid waste kombucha. The average cellulose content values are shown in Table 5. All five treatments showed a consistent increase in cellulose content as tea waste increased.

Table 5. Cellulose content of bacterial cellulose

Sample Code	Moisture Content (%)
P_1	45.8176 ± 0.66 [a]
P_2	47.8488 ± 0.72 [b]
P_3	48.7632 ± 0.40 [b]
P_4	50.6705 ± 0.28 [c]
P_5	52.4292 ± 1.99 [c]

Based on Table 5, P_1 (2 g) produced the lowest cellulose content at 45.82%, while P_5 (10 g) produced the highest cellulose content at 52.43%. These results demonstrate a significant effect of tea waste addition on the final cellulose concentration in bacterial cellulose. The increased cellulose content indicates that the nata network becomes denser and richer in pure cellulose fibers, rather than containing residual water or other components. This improvement is directly related to the availability of macro- and micronutrients from tea waste, which stimulates bacterial growth and enhances the biosynthesis of β-1,4-glucan polysaccharide structures that constitute the cellulose matrix [29].

Enriching the nutrient substrate gives the acetic acid bacteria greater energy to synthesize extracellular polysaccharides, particularly cellulose. Higher amounts of tea waste increase the

availability of soluble bioactive compounds in the fermentation medium, affecting both the rate and structure of cellulose biosynthesis. Natural antioxidants from tea plants can modulate the expression of genes involved in cellulose biosynthesis in *Komagataeibacter* spp [32]. Therefore, increasing the quantity of tea waste results in a greater contribution to supporting cellulose formation, thus increasing cellulose content in bacterial cellulose produced from sago liquid waste.

Industrial tea waste from dry tea processing still contains residual bioactive compounds such as polyphenols and tannins, which act as nutrient sources for microbial fermentation. These components serve as co-substrates that support the growth and activity of acetic acid bacteria in synthesizing bacterial cellulose. Phenolic compounds like catechins and theaflavins provide antioxidant activity and accelerate bacterial cellulose synthesis by increasing membrane permeability and stimulating the activity of key enzymes involved in the cellulose biosynthesis pathway [39].

The observed increase in cellulose content also reflects the efficiency of the fermentation system using sago liquid waste. The sugar content in sago liquid waste provides an adequate substrate for yeast to produce ethanol, which acetic acid bacteria oxidize into acetic acid, eventually contributing to cellulose formation. According to Yanti et al. [18], fermentation using sago-based liquid media can still yield bacterial cellulose with competitive physical and chemical characteristics compared to conventional substrates.

3.5 FTIR spectroscopy

Fourier transform infrared spectroscopy (FTIR) is an analytical technique used to identify functional groups in a sample based on the absorption of infrared radiation at specific wavelengths corresponding to the vibrational modes of chemical bonds. FTIR analysis was carried out on the bacterial cellulose sample P_5, which had the best overall performance, with yield (18.21%), thickness (13.47 mm), water content (47.57%), and cellulose content (52.43%). The spectra were recorded at room temperature in the 4000-400 cm-1 range, and the resulting absorption bands are presented in Fig. 2.

The absorption band area between 3600-3000 cm^{-1} corresponds to the hydrogen bonding region, which is associated with the inter- and intra-molecular –OH groups present in bacterial cellulose molecules. This region reflects the stretching vibrations of hydroxyl groups commonly found in water and carbohydrates [30]. The FTIR spectrum of bacterial cellulose derived from kombucha fermented with sago liquid waste exhibited a distinct and characteristic peak for bacterial cellulose, notably a broad absorption at 3339 cm^{-1}. This peak is a primary indicator of type I cellulose, which is exclusively found in bacterial cellulose and typically appears within the specific range of 3350–3340 cm^{-1} [28,40,41].

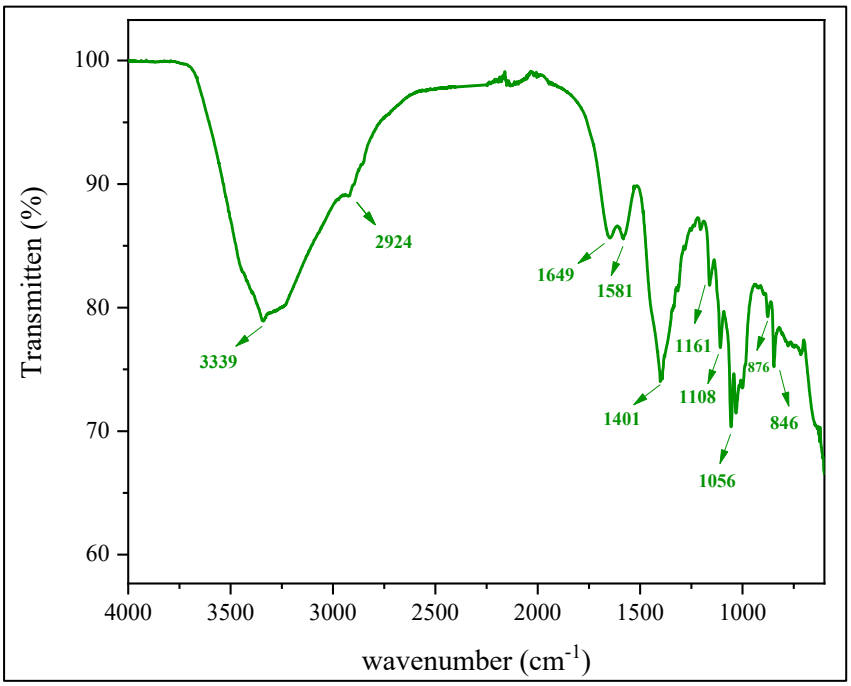

Fig. 2. FTIR spectrum of bacterial cellulose from sago liquid waste kombucha using 10 g of tea waste (P_5)

The next region, between 3000-2800 cm^{-1}, corresponds to C–H stretching vibrations, indicating differences in stretching of methyl (CH_3) and methylene (CH_2) groups within the cellulose structure [30]. A small peak observed at 2924 cm^{-1} in the bacterial cellulose sample indicates asymmetric CH_2 stretching, a marker of the aliphatic polysaccharide backbone. This finding is consistent with the results of [42], who reported that asymmetric CH_2 stretching typically occurs within the 2935-2915 cm^{-1} absorption range.

The region between 1800-1500 cm^{-1} indicates the presence of organic acids produced during kombucha fermentation, such as acetic, gluconic, and glucuronic acids [30]. The absorption band at 1649 cm^{-1} in the bacterial cellulose spectrum reflects bending vibrations of O–H bonds from phenolic compounds trapped within water molecules or C=O stretching of tea residue compounds. Meanwhile, the peak at 1581 cm^{-1} suggests N–H stretching, potentially associated with amide I bands from bacterial proteins or aromatic rings [36,40,41,43,44].

The fingerprint region (1500-400 cm^{-1}) presents specific functional group signals unique to bacterial cellulose. The absorption at 1401 cm^{-1} indicates symmetric CH_2 bending vibrations, confirming the crystalline structure of type I cellulose [43,45].

The region from 1200-900 cm^{-1} corresponds to stretching vibrations of macromolecular carbohydrates, including C–O–C, C–OH, C–H, C–O, and C–C linkages [30,36,43,44]. A sharp peak at 1161 cm^{-1} signifies asymmetric C–O–C stretching of β-1,4-glycosidic bonds [41,45]. Absorptions at 1108 cm^{-1} and 1056 cm^{-1} are associated with C–C linkages of polysaccharide monomers and skeletal C–O and C–O–H vibrations of glucose rings, corresponding to primary

and secondary alcohols [30,35]. These three bands represent major fingerprint regions of bacterial cellulose, distinguishing it from plant-derived cellulose.

The low-wavenumber region (<900 cm^{-1}) corresponds to out-of-plane C–H bending in aromatic rings, reflecting characteristic out-of-plane carbohydrate vibrations [45]. Peaks at 876 cm^{-1} and 846 cm^{-1} indicate C–H out-of-plane vibrations and asymmetric out-of-phase stretching of β-1,4-glycosidic rings, further confirming the presence of type I cellulose [35,44,45]. The spectral bands observed in bacterial cellulose from kombucha fermented with sago liquid waste confirm the presence of a crystalline type I cellulose structure, characterized by β-1,4-glycosidic linkages and distinct –OH, C–O, and C–H patterns. Although minor contaminants are indicated by the 1649 cm^{-1} and 1581 cm^{-1} bands, the main structure of the bacterial cellulose remains intact and chemically well-defined.

3.6 Study Comparative

A comparative analysis was carried out between the present study and previous research on bacterial cellulose production to strengthen the discussion and contextualize the findings. The comparison focuses on key parameters, including yield, thickness, moisture content, and cellulose content, as these characteristics are crucial indicators of the quality and applicability of bacterial cellulose. This comparison also highlights the novelty of utilizing sago liquid waste supplemented with tea residues as a sustainable fermentation medium.

Table 6. Comparison of the characteristics of bacterial cellulose with some earlier reported studies

Medium/Condition	Yield	Thickness	Moisture Content	Cellulose Content	Reference
BNC production from kombucha used tea leaves	12.8±0.25 g/L (dry weight)	29±2 mm	92.5±0.5%	-	[22]
BC production from kombucha black tea broth	25 g/L (dry weight)	-	-	-	[21]
BNC production from kombucha green tea	6.5% (dry weight)	-	89.7%	-	[46]
BC production from kombucha-soy whey	42 g/L (dry weight)	-	92%	-	[36]
BNC production from kombucha acid whey	59.58 g/L (wet weight)	4.02 mm	-	-	[47]
BNC production from kombucha lemongrass tea	70 g/L (wet weight)	-	-	-	[40]

Table 6. Continued

Medium/Condition	Yield	Thickness	Moisture Content	Cellulose Content	Reference
BC production from green tea	5.723% (dry weight)	6.4 mm	85.90%	1.347%	[20]
BC production from kombucha-soy whey	6.89% (wet weight)	2.7 mm	82.21%	4.62%	[19]
BC production from kombucha used sago liquid waste and tea waste	18.21±0.19% (wet weight)	13.47±0.31 mm	47.57±1.99%	52.43±1.99%	Present study

As shown in Table 6, the yield of bacterial cellulose varies widely, ranging from 5.72% to 70 g/L, depending on whether the values are reported on a dry or wet weight basis. Thickness also differs considerably, with some studies reporting thin membranes (2.7-6.4 mm), while others, such as kombucha with used tea leaves, achieve up to 29 mm. Moisture content is typically high (82-93%) in most reports, reflecting the hydrophilic nature of bacterial cellulose.

Compared with these studies, the present work demonstrates a balanced performance. The production of bacterial cellulose from sago liquid waste supplemented with tea waste achieved a relatively high thickness (13.47 mm) and cellulose content (52.43%), while maintaining a lower moisture content (47.57%), indicating denser and potentially stronger membranes. This finding suggests that agro-industrial byproducts, such as sago liquid waste, can serve as sustainable and efficient media for bacterial cellulose production, thereby positioning this research as a valuable contribution to resource valorization strategies.

4. Conclusions

This study successfully demonstrated that adding tea waste during kombucha fermentation using sago liquid waste significantly affects the characteristics of the resulting bacterial cellulose. Increasing the tea waste concentration from 2 g to 10 g consistently improved yield, thickness, and cellulose content, decreasing the moisture content. The best performance was observed at 10 g tea waste addition, yielding 18.21% bacterial cellulose, with a thickness of 13.47 mm, a moisture content of 47.57%, and a cellulose content of 52.43%. FTIR spectral analysis confirmed the presence of functional groups characteristic of crystalline type I cellulose, including –OH (3339 cm-1), CH2 (2924 cm-1), glycosidic C–O–C (1161 cm-1), and C–OH (1056 cm-1), indicating the β-1,4-glucan structure typical of bacterial cellulose. These findings demonstrate that tea waste is an effective additional nutrient source, enhancing the quality and quantity of bacterial cellulose.

The results also support the synergistic and sustainable potential of utilizing agro-industrial waste for bacterial cellulose production.

Data availability statement

The data sets generated and/or described during the research are included in this paper.

CRediT authorship contribution statement

Fadla Binti Syarif: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Resources, Software, Visualization, Writing – original draft, and Writing – review and editing. **Deivy Andhika Permata**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft, and Writing – review and editing. **Ira Desri Rahmi:** Conceptualization, Data curation, Formal analysis, Supervision, Validation, and Writing – original draft.

Declaration of Competing Interest

The authors declare no conflict of interest in preparing this paper.

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