IJTB VOLUME 2 (1) 2026

Indonesian Journal of Tropical Biology

https://journals.widhatulfaeha.id/index.php/ijtb

Original Research Paper

The Effect of Volume and Surface Area Ratio of Medium on Bacterial Cellulose Production at Different Fermentation Times by *Gluconacetobacter sp. SAL53*

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Article Info

Recived: May 4, 2026
Revised: May 14, 2026
Accepted: May 15, 2026
Published: May 15, 2026

ISSN **3108-9801**
ESSN: **3109-0842**
DOI: **10.65622/ijtb.v2i1.278**

Abstract

Bacterial cellulose is an extracellular polysaccharide synthesized by several bacterial genera, including Gluconacetobacter sp. SAL53, and has broad potential applications in biotechnology, food, and biomedical industries. Since this bacterium requires aerobic conditions, oxygen availability becomes a key factor influencing cellulose biosynthesis. The surface area of the fermentation medium affects oxygen transfer, while fermentation time determines the duration of bacterial metabolic activity and cellulose accumulation. This study aimed to evaluate the effects of different fermentation medium surface areas and fermentation durations on BC production. The experiment used four container diameters corresponding to surface areas of 78.5, 176.6, 314.0, and 490.6 cm², combined with fermentation periods of 5, 10, and 15 days. The results demonstrated that both surface area and fermentation time significantly influenced BC yield. Increased surface area and prolonged fermentation led to higher BC thickness, wet weight, and dry weight. The highest production was obtained under treatment P4W3, with a surface area of 490.6 cm² and a fermentation period of 15 days, yielding a thickness of 1.33 cm, wet weight of 415.5 g, and dry weight of 4.85 g. This condition was also characterized by a final pH of 3.89, the lowest reducing sugar content (5.22%), and the lowest remaining volume (350 mL), indicating optimal bacterial metabolism. These findings confirm that fermentation surface area and duration are critical factors for optimizing BC production.

Keywords: Bacterial cellulose, Fermentation surface area, Gluconacetobacter sp.

INTRODUCTION

Cellulose is a natural complex polysaccharide that is non-toxic (Cui et al., 2019)and biodegradable, making it widely used in various industrial sectors, particularly as a raw material for paper production (Ayu et al., 2025). Global demand for paper continues to increase, with consumption reaching hundreds of millions of tons and showing steady annual growth (Sharma & Bhende, 2024). In Indonesia, the pulp and paper industry has also demonstrated positive growth of approximately 4.52% in 2023 and is projected to reach up to 10% in 2024 (Serrano et al., 2024). This increasing demand directly drives the need for cellulose derived from wood-based resources (Jarre et al., 2020). However, heavy reliance on forest resources contributes to rising deforestation rates, which reached 1.18 million hectares in Indonesia in 2023 (Satyakti, 2026). This condition highlights that conventional cellulose utilization imposes significant ecological pressure, thereby necessitating the development of more sustainable alternatives.

As an alternative, bacterial cellulose (BC) has emerged as a promising innovation due to its superior properties compared to plant-derived cellulose (Ujjwal & Slaughter, 2025). BC exhibits high purity, a finer fibrillar structure, and improved mechanical strength and biodegradability (Y.-Y. Wang et al., 2023). In addition, its production process is relatively fast and cost-effective, and it does not rely on forest exploitation, making it more environmentally friendly (Rautio et al., 2023). One of the most widely studied BC-producing bacteria is Gluconacetobacter, which can synthesize cellulose via a biosynthetic process using glucose as a substrate in fermentation media (Yassine et al., 2016). This biosynthesis involves cellulose synthase enzymes that convert glucose into high-polymer cellulose chains. Therefore, the use of Gluconacetobacter is an important strategy for developing innovative and sustainable cellulose-based materials.

However, BC production is strongly influenced by complex fermentation conditions that must be carefully optimized. Factors such as nutrients, pH, temperature, and oxygen availability play crucial roles in determining bacterial growth and the quality of the produced cellulose (Bhatia et al., 2024). As an aerobic bacterium, Gluconacetobacter relies heavily on oxygen availability for its metabolic processes, making the surface area of the fermentation medium a critical factor in enhancing oxygen diffusion (Turganova et al., 2025). In addition, fermentation time significantly affects the thickness and yield of BC, with longer fermentation periods producing thicker, heavier cellulose (Tsouko et al., 2024). Nevertheless, studies that integrate the effects of the volume-to-surface-area ratio of the fermentation medium and varying fermentation time on BC production remain limited. This indicates a clear research gap that requires further investigation.

Citacion: Puspita, C. W., Sarkono, Kusuma, A. A. N. N., & Zana, M. Z. N. 2026. The Effect of Volume and Surface Area Ratio of Medium on Bacterial Cellulose Production at Different Fermentation Times by Gluconacetobacter sp. SAL53. *Indonesian Journal of Tropical Biology*, 1(2), 26-32. https://doi.org/10.65622/ijtb.v2i1.278

Based on the above considerations, the novelty of this study lies in the simultaneous evaluation of the volume-to-surface-area ratio of the fermentation medium and fermentation time for bacterial cellulose production using Gluconacetobacter sp. SAL53, which has not been extensively reported in previous studies. This study aims to analyze the effect of the volume-to-surface area ratio on BC production and to examine the influence of fermentation time under different treatment conditions. The urgency of this research is not only scientific but also practical, as it contributes to the development of more efficient and environmentally friendly cellulose production technologies. The findings of this study are expected to provide a foundation for optimizing fermentation processes and to support the advancement of sustainable biomaterial-based industries.

RESEARCH METHODS

Time and place

This study was conducted at the Advanced Biology Laboratory, Microbial Technology Room, Faculty of Mathematics and Natural Sciences, Universitas Mataram, from October 2024 to January 2025.

Research design

This study employed an experimental method aimed at determining the cause–effect relationship between treatments and observed outcomes (Y. Wang et al., 2026). External variables such as bacterial strain, medium composition and concentration, inoculum volume, incubation temperature, fermentation conditions, and initial pH of the medium were controlled to ensure experimental consistency. The study focused on independent variables, namely the ratio of medium volume to surface area and fermentation time (Singh et al., 2024), and dependent variables, namely bacterial cellulose (BC) production measured through several parameters (Srivastava & Mathur, 2024). The data collected consisted of both quantitative and qualitative data (Sandelowski, 2000).

Population and research sample

The population in this study consisted of all fermentation systems using Gluconacetobacter sp. SAL53 for bacterial cellulose production (Gomes et al., 2013). The samples included fermentation units subjected to different treatments based on the ratio of medium volume to surface area and fermentation time. The sampling technique used was a completely randomized design with factorial treatment combinations (Couper et al., 2005). The study applied two independent variables: (1) surface area of the fermentation medium (78.5; 176.6; 314; and 490.6 cm²) and (2) fermentation time (5, 10, and 15 days). Each treatment was repeated three times, resulting in a total of 36 experimental units (Gurevitch & Chester, 1986). Data collection was conducted through direct measurement and laboratory analysis. The instruments and materials used in this study included laboratory glassware, analytical instruments, and biological materials such as Gluconacetobacter sp. SAL53, coconut water medium, sucrose ($C_{12}H_{22}O_{11}$) 2% (w/v), yeast extract 0.5% (w/v), ammonium sulfate ($(NH_4)_2SO_4$) 0.5% (w/v), and other supporting reagents. Laboratory equipment consisted of fermentation containers with different diameters, digital balances, pH meters, measuring cylinders, and drying ovens used for monitoring and analyzing BC production.

Research procedure

Preparation of Tools and Materials

All tools and materials were prepared and sterilized to prevent contamination. Sterilization was carried out using an autoclave at 121°C and 1 atm pressure for 15 minutes, while equipment surfaces were disinfected using 70% alcohol.

Rejuvenation of Gluconacetobacter *sp. SAL53*

The bacterial isolate was obtained from the collection of the Advanced Biology Laboratory, Universitas Mataram. The isolate was rejuvenated using filtered coconut water medium enriched with sucrose 2% (w/v), ammonium sulfate 0.5% (w/v), and yeast extract 0.5% (w/v). The medium was heated, sterilized, cooled, and inoculated with 10% (w/v) starter culture (Sawatari et al., 2006), then incubated for 5–7 days until cellulose formation occurred (Reese et al., 1950).

Preparation of Starter Culture

The rejuvenated isolate was cultured in a larger fermentation medium (3.6 L) with the same composition. After sterilization and cooling, 10% (w/v) inoculum was added and incubated for 5–7 days until sufficient cellulose formation was achieved (Z.-G. Wang et al., 2016).

Fermentation for Bacterial Cellulose Production

A total of 10.8 L fermentation medium was prepared and distributed into containers with a volume of 900 mL each. After sterilization, 10% (w/v) inoculum was added to obtain a total volume of 1 L per container. The treatments consisted of four container diameters: P1 (10 cm), P2 (15 cm), P3 (20 cm), and P4 (25 cm), corresponding to surface areas of 78.5, 176.6, 314, and 490.6 cm². Fermentation was conducted for 5, 10, and 15 days with three replications per treatment. The treatment design is presented in Table 1. Observations and harvesting were conducted on days 5, 10, and 15 to evaluate differences in BC production across treatments.

Table 1. Experimental Design

Fermentation Time	P1 (78.5 cm²)	P2 (176.6 cm²)	P3 (314 cm²)	P4 (490.6 cm²)
5 days	P1W1	P2W1	P3W1	P4W1
10 days	P1W2	P2W2	P3W2	P4W2
15 days	P1W3	P2W3	P3W3	P4W3

*Note: Total experimental units: 12 treatments × 3 replications = 36 units.

Parameter Analysis

The observed parameters included morphology, thickness, remaining medium volume, pH, wet weight, dry weight, and reducing sugar content. Morphological observations were conducted visually. Thickness was measured using a caliper, while pH was measured using a calibrated pH meter (Z.-G. Wang et al., 2016). Dry weight measurement followed oven-drying at 50°C until constant weight (Candeiro et al., 2012). Reducing sugar content was determined using the Nelson–Somogyi method (Sudarmadji et al., 1984).

Research data analysis

The data obtained are presented in graphs and tables. The dry weight data of BC are presented in graphs and tables. Analyzed using ANOVA (Analysis of Variance) statistical analysis to determine the significance between treatments

(Sawyer, 2009). The data on thickness, remaining medium volume, pH, wet weight, and reducing sugar content are presented in graphs. The analysis was conducted descriptively and quantitatively to understand the influence of variables on cellulose production by Gluconacetobacter sp. SAL53 bacteria (Santoso et al., 2020).

RESULTS

Morphology of Bacterial Cellulose

Wet and dry bacterial cellulose (BC) exhibit distinct morphological characteristics. Freshly harvested wet BC appears milky white with a smooth, slippery surface. A uniform layer of BC evenly covers the entire surface of the fermentation medium. Its texture is soft, flexible, and elastic. Wet BC feels chewy and slightly slimy, indicating a high water content in its fiber structure. After drying, BC exhibits significant morphological changes. Dry BC appears more opaque with a yellowish-white color. Its surface becomes stiffer and rougher, with a dense, hard, wrinkled, and inflexible texture. The BC layer also shrinks and thins due to water loss during drying. More details can be seen in Figure 1.

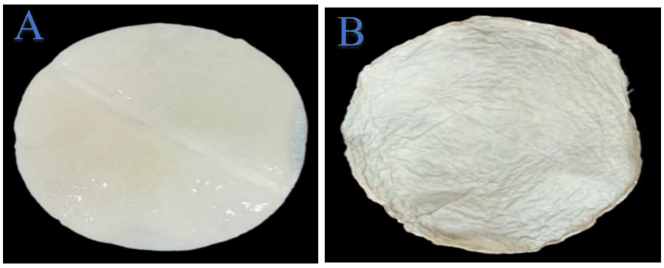

Figure 1. Morphology of bacterial cellulose: (a) Wet cellulose and (b) Dry cellulose.

Results of Bacterial Cellulose Thickness Measurement

The thickness of bacterial cellulose (BC) increased linearly with both surface area and fermentation time (Figure 2). Among the surface area treatments, P1 (78.5 cm²) produced the lowest BC thickness, followed by P2 (176.6 cm²) and P3 (314 cm²), while P4 (490.6 cm²) showed the highest thickness, indicating that a larger surface area enhances BC formation. Similarly, BC thickness increased with fermentation time, from day 5 (W1) to day 10 (W2), and reached the highest value on day 15 (W3). Overall, the highest average BC thickness produced by Gluconacetobacter sp. SAL53 was observed in P4W3, whereas the lowest was in P1W1.

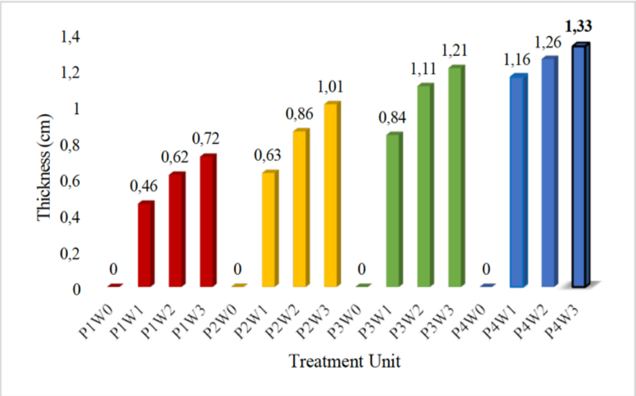

Figure 2. Average bacterial cellulose thickness produced by Gluconacetobacter sp. SAL53 in each treatment unit (PnWm = surface area and fermentation time).

Results of the Remaining Fermentation Medium Volume Measurement

The remaining volume of the fermentation medium decreased across all treatments as both surface area and fermentation time increased (Figure 3). In terms of surface area variation, treatment P1 had the highest remaining medium volume, which gradually decreased in P2 and P3, reaching the lowest value in P4. This indicates that a larger surface area leads to greater consumption of the fermentation medium. Regarding fermentation time, the remaining volume decreased linearly from W1 to W3. On day 5 (W1), a relatively large volume of medium remained; this volume decreased on day 10 (W2) and reached its lowest level on day 15 (W3). Based on the overall results across all treatments, the lowest average remaining medium volume indicates the optimal condition for Gluconacetobacter sp. SAL53 Figure. 3 was observed in treatment P4W3, while the highest average was found in treatment P1W1.

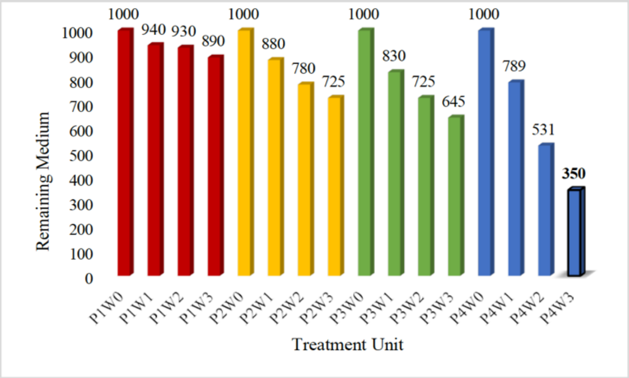

Figure 3. Average residual volume of fermentation medium by Gluconacetobacter sp. SAL53 in each treatment unit.

Results of Fermentation Medium pH Measurement

The pH value of the fermentation medium decreased across all treatments as both the surface area and fermentation time increased (Figure 4).

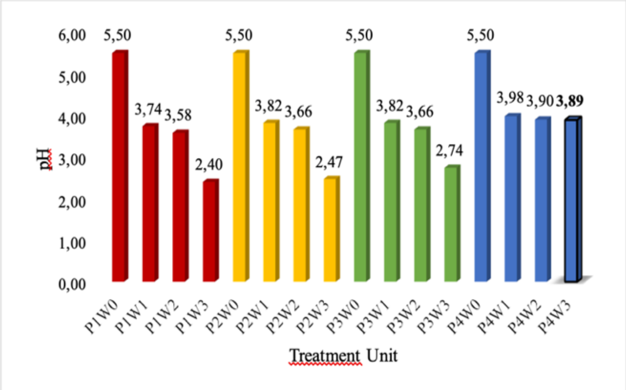

Figure 4. Average pH results of fermentation medium by Gluconacetobacter sp. SAL53 in each treatment unit.

In terms of surface area variation, treatment P1 had the highest pH, followed by P2 and P3, while P4 had the lowest. This indicates that a larger surface area promotes more active bacterial metabolism, leading to increased accumulation of acidic compounds. Regarding fermentation time, the pH decreased linearly from day 5 (W1) to day 15 (W3). The highest pH value was observed on day 5 (W1), followed by a decrease on day 10 (W2), and the lowest value was reached on day 15 (W3). Based on the overall results, the most optimal decrease in fermentation medium pH by Gluconacetobacter sp. SAL53 (Figure 4), was observed in treatment P4W3.

Results of Wet Weight Measurement of Bacterial Cellulose

The wet weight of bacterial cellulose (BC) increased in all treatments with larger surface area and longer fermentation time (Figure 5). P1 produced the lowest wet weight, followed by P2 and P3, while P4 showed the highest, indicating a positive effect of surface area on BC production. Wet weight also increased from day 5 (W1) to day 15 (W3), with the highest value observed at W3. Overall, the highest average wet weight of Gluconacetobacter sp. SAL53 was found in P4W3, while the lowest was in P1W1. This shows that wet weight, representing biomass and water content, increased proportionally with fermentation time and medium surface area.

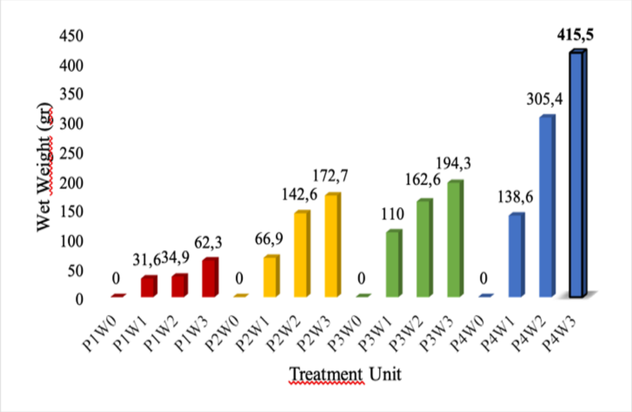

Figure 5. Average wet weight results of bacterial cellulose by Gluconacetobacter sp. SAL53 in each treatment unit.

Results of Dry Weight Measurement of Bacterial Cellulose

The dry weight of bacterial cellulose (BC) increased across all treatments with larger surface area and longer fermentation time (Figure 6).

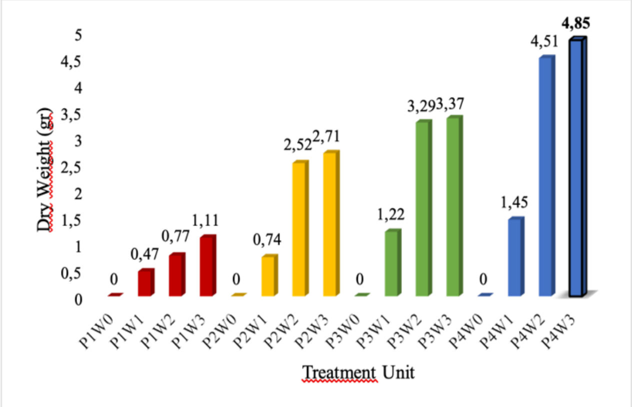

Figure 6. Average dry weight of bacterial cellulose produced by Gluconacetobacter sp. SAL53 in each treatment unit.

P1 showed the lowest dry weight, followed by P2 and P3, while P4 produced the highest, indicating a positive effect of surface area on BC production. Dry weight also increased from day 5 (W1) to day 15 (W3), reaching the highest value at W3. Overall, the highest average dry weight of Gluconacetobacter sp. SAL53 was observed in P4W3, while the lowest was in P1W1. As dry weight reflects the actual cellulose mass without water content, the results indicate greater biomass accumulation with longer fermentation time and larger medium surface area.

The results of the ANOVA analysis of variance (ANOVA) in Table **2** show that the dry weight of BC consistently increased with increasing fermentation time and surface area of the fermentation medium. This increase was statistically significant for each treatment, with optimal results obtained in the P4W3 treatment. Therefore, both factors (length of time and surface area of the fermentation medium) significantly influence BC production by Gluconacetobacter sp. SAL53.

Table 4.1 Results of ANOVA analysis of variance on the dry weight of bacterial cellulose by Gluconacetobacter sp. SAL53 in each treatment unit

Fermentation Time	P1 (78.5 cm^2)	P2 (176.6 cm^2)	P3 (314 cm^2)	P4 (490.6 cm^2)
5 days	P1W1	P2W1	P3W1	P4W1
10 days	P1W2	P2W2	P3W2	P4W2
15 days	P1W3	P2W3	P3W3	P4W3

***Note:** Different superscript letters indicate significant differences ($P<0.05$).

Results of Reducing Sugar Content Measurement in the Fermentation Medium

The reducing sugar content decreased across all treatments as both the surface area and fermentation time increased (Figure. 7).

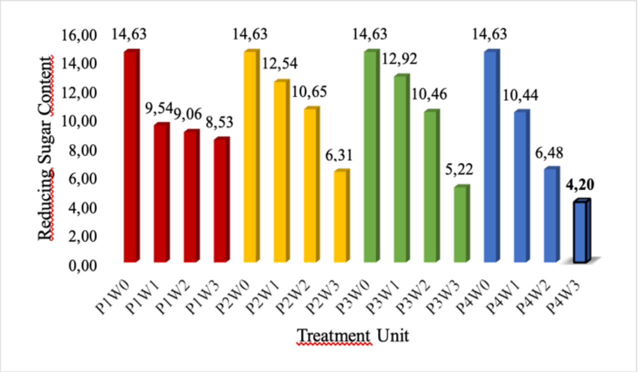

Figure 7. Average reducing sugar content of the fermentation medium by Gluconacetobacter sp. SAL53 in each treatment unit.

In terms of surface area variation, treatment P1 maintained relatively high reducing sugar levels throughout fermentation. In contrast, treatments P2, P3, and P4 showed a faster decline in reducing sugar content. This indicates that larger surface areas promote more rapid substrate utilization. Regarding fermentation time, the reducing sugar content decreased linearly from day 5 (W1) to day 15 (W3). The highest value was observed on day 5 (W1), followed by a decrease on day 10 (W2), and the lowest value was reached on day 15 (W3). Based on the overall results across all treatments, the lowest average reducing sugar content represents the optimal condition for Gluconacetobacter sp. SAL53 (Figure. 7) was observed in treatment P4W3.

DISCUSSION

The bacterial isolate used in this study was *Gluconacetobacter* sp. SAL53, which is classified as a Gram-negative, rod-shaped bacterium, aerobic, catalase-positive, and belonging to the family *Acetobacteriaceae* (Hamad et al., 2017). Through aerobic fermentation, *Gluconacetobacter* sp. SAL53 converts glucose in an acidic fermentation medium into cellulose. The production of cellulose is influenced by several key factors that must be controlled to optimize bacterial growth and product quality, including nutrients, pH, temperature, and oxygen availability (Bancin, 2021).

Bacterial cellulose (BC) production is categorized as primary metabolism, as it is directly formed from glucose metabolic pathways during the active growth phase of the bacteria. BC plays an important role in supporting physiological needs and structural stability of the cells, in contrast to secondary metabolites that are typically produced during the stationary phase and function in ecological interactions such as antibiosis (Demain & Adrio, 2008; Demain, 2014; Nayak et al., 2024). According to Palash et al. (2019), BC production is strongly influenced by oxygen availability and fermentation time, which are the two main factors examined in this study.

The parameters measured in this study included thickness, remaining medium volume, pH, wet weight, dry weight, and reducing sugar content. Thickness, wet weight, and dry weight showed increasing trends with greater surface area and longer fermentation time. In contrast, parameters such as remaining medium volume, pH, and reducing sugar content decreased. This pattern indicates a direct relationship between bacterial metabolic activity and BC production. Among these parameters, dry weight was used as the primary indicator of production efficiency, as it represents the pure cellulose mass without water content.

Quantitative analysis of BC production by *Gluconacetobacter* sp. SAL53 showed that the interaction between medium surface area and fermentation time is a crucial factor affecting biomass yield. This is closely related to bacterial growth kinetics and metabolic activity. As an obligate aerobic acetic acid bacterium, *Gluconacetobacter* sp. SAL53 requires oxygen for optimal productivity (Palash et al., 2019). A larger surface area increases dissolved oxygen availability, thereby accelerating glucose metabolism, as indicated by decreased reducing sugar content and pH. Meanwhile, longer fermentation time allows cellulose fibrils to accumulate, forming thicker and denser layers (Fernandes et al., 2020).

At the initial stage of fermentation, all treatments produced BC with relatively low thickness. However, treatment P4W1 already exhibited a thicker and denser layer compared to P1W1. This is due to the larger surface area in P4, which enhances oxygen diffusion and accelerates fibril formation. Conversely, P1W1, with a smaller surface area, experienced limited oxygen availability, resulting in thinner cellulose formation. By day 10, the differences among treatments became more pronounced. P4W2 produced thicker BC, higher wet and dry weights, and lower reducing sugar content compared to P1W2, P2W2, and P3W2. This indicates that better oxygenation in P4 accelerated glucose consumption and cellulose production. In contrast, P1W2 still had relatively high residual sugar and pH, indicating limited metabolic activity. The most significant differences were observed on day 15. P4W3 achieved the highest thickness, wet weight, and dry weight, along with the lowest reducing sugar content, pH, and remaining medium volume, indicating optimal substrate utilization. Conversely, P1W3 showed low thickness and yield, with relatively high residual sugar and medium volume, suggesting less efficient fermentation. Treatments P2W3 and P3W3 showed intermediate results, following the increasing trend with larger surface areas. Overall, P4W3 was identified as the most optimal treatment, while P1W1 represented the least optimal condition due to limited oxygen availability and shorter fermentation time.

The results also indicate that each parameter is interconnected within the BC production process. First, morphological observations (Figure 1) qualitatively support the quantitative data, showing structural changes in BC with increasing fermentation time and surface area. On day 5, BC appeared thin, semi-transparent, and fragile, indicating loosely arranged nanofibrils with low mechanical strength. By day 10, BC became thicker and denser, reflecting increased fibril organization. On day 15, particularly in treatment P4W3, BC exhibited superior characteristics, being thick, compact, dense, and elastic. After drying, BC showed a rough surface, wrinkles, and a more opaque color due to the loss of water molecules, which caused fibril collapse and volume shrinkage (Panjaitan et al., 2024).

Second, BC thickness is directly proportional to wet and dry weight. Thicker BC layers have higher water-holding capacity (wet weight) and greater accumulation of pure cellulose (dry weight). Based on Table 2, treatment P4W3, which combined the largest surface area (490.6 cm²) and longest fermentation time (15 days), produced the highest average dry weight of 4.85 g, which was statistically significant compared to other treatments ($P<0.05$). The increase in fermentation time from 5 days (W1) to 15 days (W3) also resulted in significant differences. The highest thickness (Figure 4.2) and wet weight (Figure 4.5) were also observed in P4W3. High wet weight reflects the high water-holding capacity of BC, where water can constitute more than 99% of its total mass (Panjaitan et al., 2024; Ullah et al., 2020). This hydrophilic property is advantageous for biomedical applications such as wound dressing and as a low-calorie food additive (Lahiri et al., 2021).

These findings can be explained using bacterial growth curve theory. On day 5 (W1), the culture was in the exponential phase, where energy is primarily used for cell replication rather than BC production. On day 10 (W2), the culture transitioned to the early stationary phase, where BC production increased as a protective response (Wibowo & Isroi, 2015). By day 15 (W3), the culture reached the late stationary phase, where metabolism focused on maintenance and cellulose production, resulting in maximum BC yield (Wibowo & Isroi, 2015; Fijalkowski et al., 2016).

Third, metabolic by-products in the fermentation medium were reflected by changes in remaining volume, pH, and reducing sugar content. A decrease in medium volume, particularly in treatment P4W3, indicated active bacterial metabolism and water absorption into the Bacterial cellulose matrix. The decline in pH showed organic acid production by Gluconacetobacter sp. SAL53, while the lowest pH in P4W3 indicated the highest metabolic activity. Reducing sugar content also decreased as BC yield increased, showing efficient glucose conversion into cellulose. Overall, treatment P4W3, with the largest surface area (490.6 cm²) and longest fermentation time (15 days), was the optimal condition, producing the highest BC yield, greatest thickness, and most efficient metabolic performance.

CONCLUSION

Different ratios of medium volume to surface area have a significant positive effect on bacterial cellulose production by Gluconacetobacter sp. SAL53, with the largest surface area treatment (P4 = 490.6 cm²) showing the most optimal BC production compared to other treatments. Different fermentation times also have a significant positive effect on bacterial cellulose production by Gluconacetobacter sp. SAL53, with the longest fermentation time (W3 = 15 days) resulting in the most optimal BC production compared to other treatments.

ACKNOWLEDGMENTS

The author team would like to thank all parties who have contributed to this research and all sources who have provided important information for this research.

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