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Research Paper

Performance Comparison of Static and Rotating Bioreactor for Bacterial Cellulose Production from Tofu Wastewater

Choerudin, Dadi Arif Viliando, Dyah Setyo Pertiwi*

Department of Chemical Engineering, Institut Teknologi Nasional, PH. H. Mustofa No. 23, Bandung. 40124, Indonesia

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ABSTRACT: *Tofu industry wastewater is a potential substrate for bacterial cellulose (BC) production due to its residual nutrient content. However, conventional static culture has limited oxygen transfer and substrate diffusion, resulting in low substrate utilization efficiency. This study presents a comparative evaluation of static and rotating bioreactor (RB) systems, with a primary focus on substrate utilization, bacterial growth, and BC yield. In addition, the effect of inoculum volume (10% and 30%) was investigated to assess its influence on process performance. The results demonstrate that the RB system significantly outperforms static culture, achieving 1.6 – 2 times higher substrate utilization and 53.3 - 61.3% higher BC yield on initial substrate. The highest BC product is 5 g dry mass BC/ L medium in the RB system. Furthermore, the RB system enhances substrate conversion toward bacterial growth, indicating improved metabolic efficiency under dynamic conditions. A lower inoculum volume, 10% inoculum with 1-10 million CFU/mL, resulted in better overall performance compared to 30% inoculum, suggesting that excessive inoculum does not improve process efficiency. These findings confirm that the rotating bioreactor is a more effective system for intensifying BC production, particularly in terms of substrate utilization, and highlight its potential for scalable and sustainable bioprocess applications.*

Keywords: *microbial cellulose; waste-to-value; Acetobacter; whey tofu; nata de soya*

1. INTRODUCTION

Wastewater generated from tofu industry (whey tofu) represents an abundant nutrient source that remains underutilized. The tofu production can produce wastewater up to 21 L/kg of soybean [1], containing residual organic compounds such as proteins and carbohydrates that are still suitable for microbial utilization [2], [3]. In Indonesia, particularly in the Cibuntu industrial area in Bandung, tofu production activities generate approximately 16.8 million m^3 of wastewater per year, much of which is discharged without proper treatment. This condition highlights the urgent need for sustainable strategies to valorize this agro-industrial waste into value-added products [4], as concept of waste-to-value (WtV).

One promising approach to valorizing this waste is through the production of bacterial cellulose (BC) [2], [3]. Bacterial cellulose, also known as microbial cellulose (MC), synthesized through glucose polymerization by one of three group species, namely *Gluconacetobacter, Acetobacter*, as well as *Komagataeibacter* [5], [6]. BC consists of a network of cellulose nanofibers with high purity, excellent crystallinity, and superior mechanical and biocompatibility properties, making it highly promising for applications in environmentally friendly packaging, filtration membranes, and biomedical materials [7], [8]. The use of low-cost substrate as agro-industrial wastewater has also been explored to reduce production costs and improve process sustainability [2], [9], [10].

Despite its advantages, the large-scale production of BC remains limited by low process efficiency [11], particularly when using conventional static culture systems. In static culture, oxygen transfer occurs only at the air–liquid interface, leading to significant mass transfer limitations and substrate concentration gradients within the medium [12]. As BC formation occurs primarily at the interface, the accumulation of the cellulose layer further restricts oxygen diffusion, ultimately reducing bacterial activity and overall productivity [13].

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Department of Chemical Engineering
Faculty of Industrial Technology
Universitas Muslim Indonesia, Makassar
Address
Jalan Urip Sumohardjo km. 05 (Kampus 2 UMI) Makassar- Sulawesi Selatan
e-mail : jcpe@umi.ac.id

Corresponding Author *
dsp@itenas.ac.id

These limitations result in inefficient substrate utilization and prolonged fermentation times, making static systems less suitable for process intensification. To overcome these limitations, various approach have been developed using bioreactor [14], microbial [15], [16], substrate [17], or environmental modification [18].

Dynamic and engineered bioreactor systems have been developed, including agitated, airlift, sprayed, and rotating bioreactors [7], [19]. Among these, rotating bioreactor systems offer a unique advantage by periodically exposing the growing biofilm to both air and liquid phases, thereby enhancing oxygen transfer and nutrient distribution without excessive shear stress [13], [20]. This mechanism is expected to improve substrate utilization efficiency and promote more uniform microbial activity compared to static systems.

Although several studies have investigated BC production using different bioreactor configurations, the application of rotating bioreactor systems for BC production from tofu wastewater remains limited. In particular, there is still a lack of systematic evaluation comparing static and rotating systems in terms of substrate utilization efficiency, biomass growth, and product yield when using agro-industrial wastewater as substrate. This gap indicates the need for a comprehensive comparative study from a process engineering perspective.

Therefore, this study aims to evaluate and compare the performance of static and rotating bioreactor systems for bacterial cellulose production from tofu wastewater. The analysis focuses on substrate utilization, bacterial growth, and product yield as key performance indicators. In addition, the effect of inoculum volume is investigated to understand its influence on process efficiency. This study offers a novel comparative analysis of static and rotating bioreactor systems, focusing on substrate utilization efficiency in bacterial cellulose production from tofu wastewater. The findings of this study are expected to contribute to the development of more efficient and scalable bioprocess systems for sustainable BC production.

2. MATERIAL AND METHOD

2.1 Raw Material Preparation

The primary substrate used in this study was tofu industry wastewater, specifically collected from the soybean cooking process (precipitation of soybean protein by a precipitating agent) in Cibuntu, Bandung. The wastewater was freshly collected to avoid compositional change [1], [2], [3]. Raw material preparation for BC production includes sterilization by heating, sedimentation, and filtration. Several parameters, including protein, carbohydrate, COD, and BOD levels, were analyzed before and after preparation at the Indonesian Institute of Sciences (LIPI) in Bandung.

2.2 Inoculum Preparation

Pure cultures of *Acetobacter xylinum* were obtained from the Microbiology and Bioprocess Technology Laboratory, Department of Chemical Engineering, Institut Teknologi Bandung. The cultures were stored on slant agar media containing Glucose Yeast Extract Agar (GYEA). GYEA consists of glucose (20 g), yeast extract (5 g), peptone (10 g), agar (20 g), and distilled water (1 L). The culture is stored in the refrigerator and can be stored for 3-4 weeks. This culture is then prepared as a microbial suspension using a similar medium without agar.

A starter is then prepared from the pure culture [1]. The starter is made using settled coconut water, which is then filtered using filter paper and heated to boil while stirring. Once boiling, acetic acid (10 mL/L) and sucrose (75 g/L) are added. This mixture is stirred until the sucrose dissolves. This solution is called sucrose acidified coconut water. In another container, urea (3 g/L) is dissolved in a small amount of coconut water (20 mL/g). This solution is brought to a boil and then poured into the sucrose acidified coconut water. While still hot, the medium is transferred to 150 mL Erlenmeyer flasks. The flasks are plugged with sterile cotton. Once cooled, 15 mL of the microbial suspension is added. Afterward, the medium was incubated at 30°C for 4 days (or until a white layer formed on the surface of the medium).

To improve substrate adaptability, the inoculum was gradually acclimatized to tofu wastewater in four stages. The ratios of tofu industry wastewater to coconut water used in each stage were 50:50, 75:25, 90:10, and 100:0, respectively. The acclimatized starter was analyzed for bacterial counts using total plate count (TPC) before being used for fermentation.

2.3 Rotating Bioreactor Design and Setup

The rotating bioreactor (RB) components consist of a container, a permeable lid, a container support, a pivoting cylinder, a DC motor, a cylinder support, and a DC power source. A diagram of the designed and used equipment is shown in Figure 1. The container used is prism-shaped and made of 5 mm thick acrylic. The working volume of the fermentation medium is 1500 mL. The cylinder used is made of polyvinyl chloride (PVC) with a diameter of 12.4 cm and a length of 20 cm. The shaft is made of stainless steel with a diameter of 10 mm. The cylinder rotation speed used in the experiment was 6 rpm, lower than other report (10-16 rpm) [21].

During operation, approximately 26.7% of the cylinder diameter was submerged in the fermentation medium, allowing periodic exposure to air and liquid phases. This configuration was designed to enhance oxygen transfer and reduce mass transfer limitations compared to static culture.

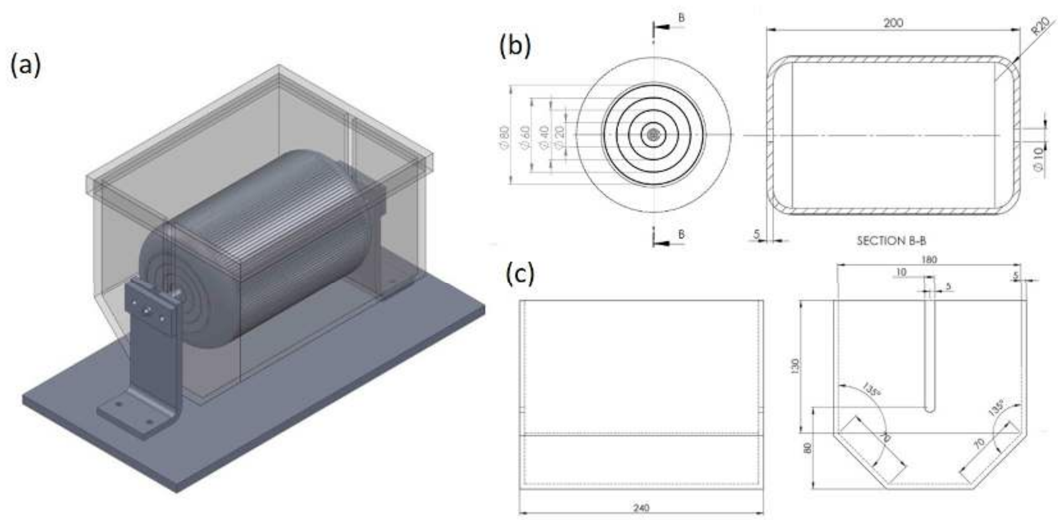

Figure 1. Sketch of rotating bioreactor for bacterial cellulose fermentation: (a) 3D picture of the rotating bioreactor, (b) the rotating drum, (c) the container

2.4 Experimental Design and Fermentation Procedure

This study employed a two-factor experimental design, consisting of culture type (static culture and rotation bioreactor) and inoculum volumes (10% and 30% v/v). The performance of the static and RB cultures was assessed using several parameters: bacterial growth (days 0, 1, and 2), substrate utilization (days 7 and 14), and product yield (after post-harvest treatment) [1], [22].

The fermentation medium was prepared by supplementing tofu wastewater with dextrose (100 g/L), acetic acid (10 mL/L) and urea (5 g/L). After cooling to room temperature, the medium was inoculated with acclimatized culture (10% and 30% of the total volume) and transferred to the fermentation vessels.

Fermentation was conducted at 30°C for 14 days under both static and RB conditions. The 14-days fermentation period was chosen as the optimum period for BC production was 14 days than other longer period [23]. Sampling was performed at days 0, 1, 2, 7, and 14 to monitor bacterial growth and substrate consumption.

At the end of the fermentation period, the bacterial cellulose (BC) pellicle was harvested. The BC was treated with 0.1 M NaOH at 80°C for 20 minutes, neutralized with 5% acetic acid, washed with distilled water, dried, and weighed to obtain dry mass [1].

2.5 Process Performance Evaluation

To evaluate and compare process performance, three parameters were quantified, i.e. biomass growth, substrate utilization, and product yield.

(1) Biomass Growth. Bacterial growth determined using is the Total Plate Count (TPC) method and expressed as colony-forming unit (CFU/mL) represents the number of viable bacteria in the fermentation medium.

(2) Substrate Utilization. Substrate consumption was determined based on the reduction of glucose concentration using the Nelson–Somogyi method (standard carbohydrate analysis method widely used in fermentation studies [20]).

$$Substrate\ Utilization\ (\%) = \frac{S_0 - S_t}{S_0} \times 100 \quad (1)$$

Where S_0 is the initial substrate concentration (g/L) and S_0 is the residual substrate concentration at time t (g/L).

(3) Product Yield. The efficiency of BC production was evaluated using two yield parameters.

Yield on initial substrate:

$$Y_{P/S_0} = \frac{P}{S_0} \quad (2)$$

Yield on consumed substrate:

$$Y_{P/S} = \frac{P}{S_0 - S_t} \quad (3)$$

Where P is the dry mass of bacterial cellulose (g).

All experiments were conducted in duplicate and average values were reported. The performance of static and RB systems was compared based on biomass formation, substrate utilization efficiency, and BC yield. This evaluation was used to assess the effectiveness of the rotating bioreactor in enhancing mass transfer and improving overall process performance as culture conditions significantly affect BC productivity and structure [3], [12], [20].

3. RESULT AND DISCUSSION

3.1 Raw Material Characteristics and Implications for Fermentation

The characterization of tofu wastewater before and after preparation (Table 1) indicates that, although the substrate contains residual nutrients such as proteins and carbohydrates, its concentration is relatively low to support optimal bacterial cellulose (BC) production. Therefore, external supplementation (additional carbon and nitrogen sources) is required to enhance microbial activity .

Table 1. Characteristics of tofu wastewater before and after preparation

Parameter	Before preparation	After Preparation
Protein (% m/v)	0.23	0.23
Carbohydrate (% m/v)	0.27	0.28
BOD_5 (mg/L)	1718.4	1270.08
COD (mg/L)	11508	5924.8

A significant observation is the high chemical oxygen demand (COD) and biological oxygen demand (BOD) values of the wastewater. These parameters indicate a high level of biodegradable organic matter, which can intensify microbial competition for oxygen during fermentation. This condition is particularly critical in static culture systems, where oxygen transfer is inherently limited.

From a process perspective, this finding highlights that oxygen availability becomes a key limiting factor, especially when using wastewater with high organic load. Insufficient oxygen supply may lead to incomplete substrate conversion and reduced BC formation. Therefore, improving oxygen transfer is essential to enhance substrate utilization efficiency and overall process performance.

3.2 Hydrodynamic Behavior in the Rotating Bioreactor

During operation of the rotating bioreactor (RB), a distinct hydrodynamic pattern was observed at the liquid surface surrounding the rotating cylinder (Figure 2). This phenomenon indicates the presence of localized mixing and periodic surface renewal caused by cylinder rotation.

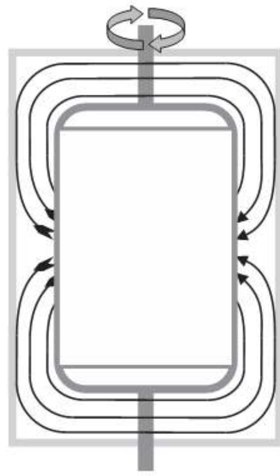

Figure 2. Hydrodynamic pattern observed at the fermentation medium surface

From a fluid mechanics perspective, this behavior enhances the liquid–air interfacial area and promotes continuous oxygen transfer into the medium. Unlike static systems, where oxygen diffusion occurs only through a stagnant interface, the RB system generates dynamic exposure of the medium to air. This alternating exposure (immersion–aeration cycle) increases oxygen availability and reduces diffusion resistance. Consequently, the RB system is expected to improve mass transfer efficiency, which directly influences microbial metabolism and substrate conversion. Although further quantitative hydrodynamic analysis is required, this observation supports the hypothesis that reactor configuration plays a critical role in overcoming mass transfer limitations.

3.3 Effect of Bioreactor Type on Bacterial Growth

The comparison of bacterial growth (Figure 3) shows that the RB system consistently produces higher population than static culture. This indicates that the RB environment is more favorable for microbial growth. The primary scientific finding here is that bioreactor configuration directly influences microbial growth kinetics. The improved growth observed in the RB system can be attributed to enhanced oxygen transfer, which is essential for aerobic bacteria such as *Acetobacter*.

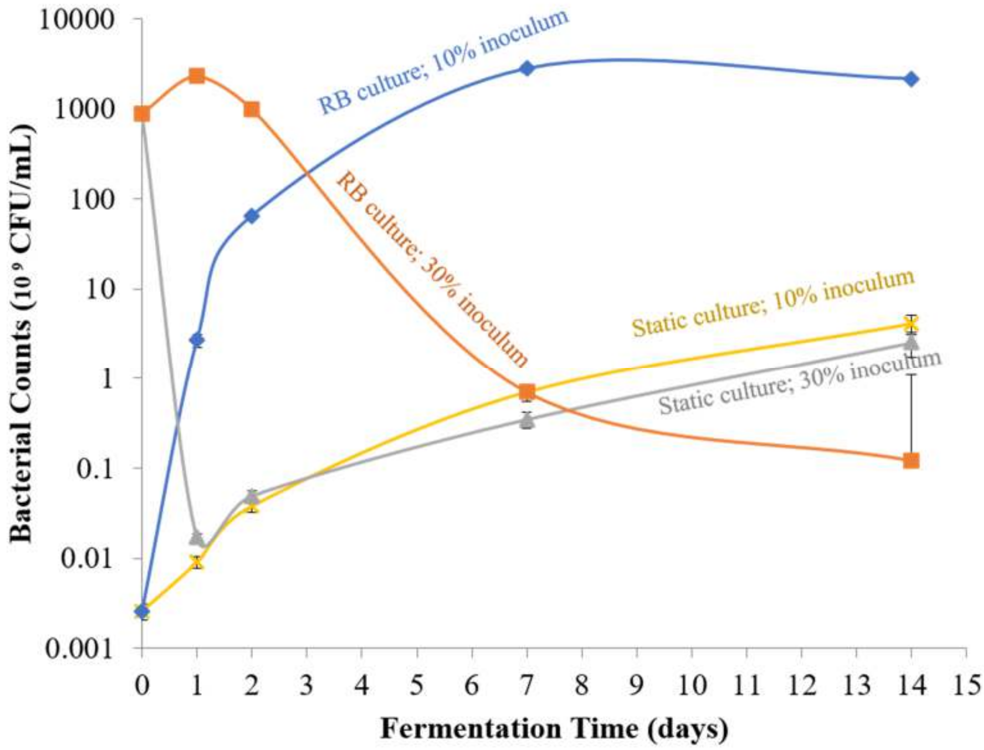

Figure 3. Bacterial growth in static and rotating bioreactor (RB) culture

However, an interesting trend is observed with respect to inoculum volume. Although a higher inoculum (30%) provides a larger initial cell population up to 10^{12} CFU/mL, it does not lead to improved growth performance. Instead, a decline in bacterial population is observed after the second day of fermentation in the RB system. This phenomenon suggests that substrate limitation becomes dominant at higher cell densities. While oxygen availability is improved in the RB system, the available substrate is rapidly consumed, leading to competition among microorganisms. As a result, cell death increases, reducing the overall bacterial population. This finding is in line with other research that a value of 10^8 CFU/mL gives better BC yield than higher population because of less competition for nutrients and better bacterial growth [23].

This finding confirms that optimal balance between substrate availability and biomass concentration is essential for efficient bioprocess operation, and excessive inoculum does not necessarily improve system performance.

3.4 Substrate Utilization: Key Performance Indicator

Substrate utilization represents the primary indicator of process efficiency in this study. The results (Figure 4) shows that the increase in substrate utilization from day 7 to day 14 indicates that fermentation time plays a critical role in determining process efficiency. This trend reflects the transition from early exponential to late exponential growth phase, where microbial metabolic activity reaches its maximum. This finding is consistent with previous work [23], which demonstrated that 14 days of incubation resulted in significantly higher BC yield compared to longer fermentation periods. This suggests that 14 days represents an optimal balance between microbial activity and substrate availability.

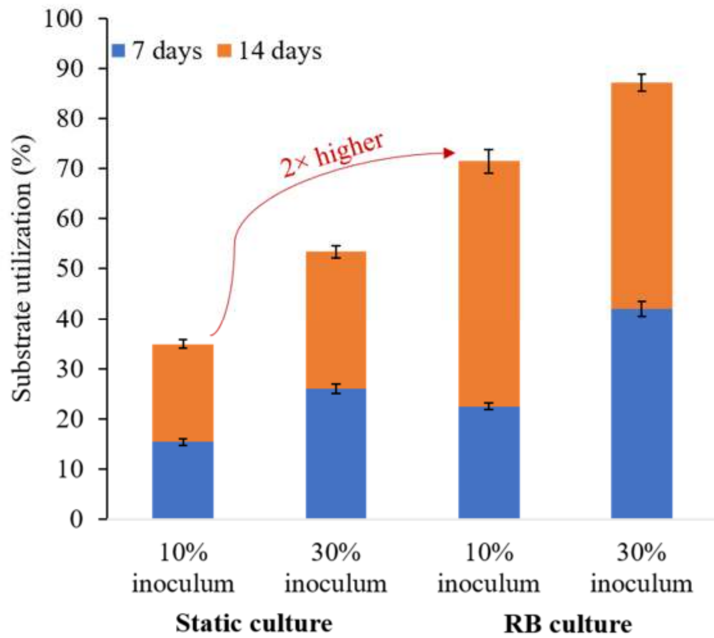

Figure 4. Substrate utilization in static and rotating bioreactor culture

The results also clearly show that the RB system significantly enhances substrate consumption compared to static culture under all experimental conditions. The most important scientific finding is that substrate utilization in the RB system is substantially higher, reaching up to 2 times higher with 10% inoculum and 1.6 times higher with 30% inoculum compared to static culture. This improvement can be explained by the following mechanisms: (1) Enhanced oxygen transfer supports higher metabolic activity, (2) Reduced diffusion limitation allows more uniform substrate distribution, (3) Increased surface renewal improves contact between microorganisms and substrate.

In contrast, static culture suffers from substrate gradients and limited mass transfer [12], resulting in inefficient substrate conversion. The formation of a BC layer at the air–liquid interface further restricts oxygen diffusion, leading to lower substrate utilization. Therefore, it can be concluded that the RB system promotes more efficient substrate-to-biomass conversion, which is a key factor in improving overall process performance.

3.5 Product Formation and Yield Analysis

The BC dry mass product and its yield for both static and RB cultures are provided in Figure 5. The BC dry mass in static culture are 4.9 g (10% inoculum) and 3.9 g (30% inoculum) while in RB are 7.5 g (10% inoculum) and 6.3 g (30% inoculum) as can be seen in Figure 5a. Highest BC product is produced by RB system with 10% inoculum which is 5 g BC / L medium. These results are not much different from another research using *Komagataeibacter* which resulting 4.77 g BC / L medium [24].

The yield on initial substrate is provided in Figure 5b. The results shows that the RB system produces relatively higher product than static culture with increases of 53.3% (10% inoculum) and 61.3% (30% inoculum). This confirms that improved substrate utilization in the RB system translates into higher product formation [12]. However, when yield is expressed relative to substrate consumption (Figure 5c), an inverse trend is observed, where static culture shows higher yield efficiency. This indicates that, although RB consumes more substrate, not all of it is converted into BC.

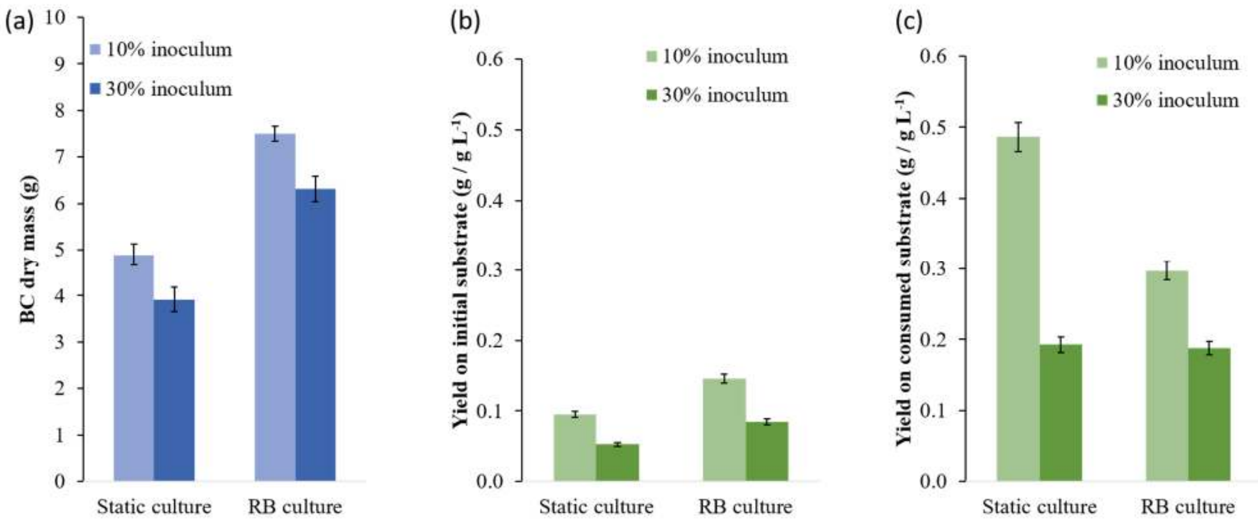

Figure 5. Bacterial cellulose product (a) and yield on (b) initial substrate and (c) consumed substrate in static and rotating bioreactor culture

The scientific implication of this finding is that the RB system directs a larger fraction of substrate toward biomass growth rather than product formation. A higher yield is normally achieved by culturing bacterial cells in static conditions rather than with dynamic condition, e.g. agitation [11]. In other words, the RB system is the growth-dominated system while static culture is product-oriented system (under limited conditions). This behavior is consistent with microbial metabolism in aerobic systems, where increased oxygen availability promotes cell growth [12], [25]. Since BC is a growth-associated product, higher biomass still contributes to increased production, but not necessarily higher conversion efficiency. Thus, the overall process can be interpreted as RB system improves total productivity (g BC produced) while static culture may show higher conversion efficiency per substrate used.

3.6 Overall Process Interpretation

Table 2 compares the BC production performance obtained in this study with several systems reported in previous studies. Under the same substrate conditions, the rotating bioreactor outperformed static culture, increasing BC productivity from 0.35 to 0.54 g L^{-1} day^{-1} and improving yield from 0.09 to 0.15 g BC g^{-1} initial substrate. This corresponds to productivity and yield improvements of approximately 54% and 67%, respectively. The enhanced performance is consistent with the higher substrate utilization observed in the rotating system [21], suggesting that improved oxygen transfer and reduced diffusion resistance promoted more effective microbial metabolism and cellulose biosynthesis. Although the productivity obtained in this study remains lower than that reported for optimized media such as corn steep liquor (CSL) and molasses, the results demonstrate that reactor configuration can substantially improve BC production even when using a low-cost substrate such as tofu wastewater.

From an engineering perspective, the findings demonstrate that reactor configuration significantly affects mass transfer, substrate utilization, and product formation. Preliminary two-way ANOVA confirmed that bioreactor type significantly influenced microbial growth, substrate utilization, BC production, and yield performance ($p < 0.05$, provided in Supplementary Material). Significant interaction effects were observed for most response variables, indicating that the influence of inoculum volume depended on the culture system employed. The RB system offers clear advantages in improving oxygen availability, reducing diffusion limitations, and enhancing substrate utilization. However, it also shifts the metabolic pathway toward biomass growth, which may reduce yield efficiency. These results confirm that process intensification through reactor design is a critical strategy in improving BC production, particularly when using low-cost and complex substrates such as tofu wastewater.

Table 2. Comparison of some previous studies to this study

No.	System	Substrate	Species	Productivity, g BC L^{-1} day^{-1}	Yield, g BC / g initial substrate	Refs.
1	Static	Tofu ww.	*Acetobacter xylinum*	0.57	0.09	[1]
2	Static	Tofu ww. + added sugar	*Acetobacter xylinum*	1.79	0.32	[1]
3	Static	HS medium + papaya extract	*Komagataeibacter europeaus*	0.50	0.09	[17]
4	Rotating (disk)	CSL medium + molasses	*Acetobacter xylinum*	1.66	0.08	[21]
5	Static	Tofu ww.	*Acetobacter xylinum*	0.35	0.09	This study
6	Rotating (drum)	Tofu ww.	*Acetobacter xylinum*	0.54	0.15	This study

4. CONCLUSIONS

This study confirms that bioreactor configuration significantly influences the efficiency of bacterial cellulose production. The rotating bioreactor enhances substrate utilization and overall process performance by improving oxygen transfer and reducing mass transfer limitations compared to static culture.

Substrate utilization emerges as the key parameter distinguishing both systems, where the rotating system promotes more intensive microbial activity. However, this also shifts substrate conversion toward biomass growth rather than maximizing product yield efficiency.

Overall, the rotating bioreactor presents a promising strategy for process intensification in bacterial cellulose production. Further optimization is required to improve conversion efficiency and support scale-up for industrial applications.

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