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# Bacterial Cellulose Production by *Gluconacetobacter* sp. RKY5 in a Rotary Biofilm Contactor

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## Abstract

A rotary biofilm contactor (RBC) inoculated with *Gluconacetobacter* sp. RKY5 was used as a bioreactor for improved bacterial cellulose production. The optimal number of disk for bacterial cellulose production was found to be eight, at which bacterial cellulose and cell concentrations were 5.52 and 4.98 g/L. When the aeration rate was maintained at 1.25 vvm, bacterial cellulose and cell concentrations were maximized (5.67 and 5.25 g/L, respectively). The optimal rotation speed of impeller in RBC was 15 rpm. When the culture pH in RBC was not controlled during fermentation, the maximal amount of bacterial cellulose (5.53 g/L) and cells (4.91 g/L) was obtained. Under the optimized culture conditions, bacterial cellulose and cell concentrations in RBC reached to 6.17 and 5.58 g/L, respectively.

**Index Entries:** Bacterial cellulose; bioreactor; fermentation; *Gluconacetobacter*; optimization; rotary biofilm contactor.

## Introduction

Cellulose is one of the most abundant biological macromolecules in nature, wherein it plays a crucial role in the integrity of plant cell walls *(1)*. Cellulose is a linear insoluble biopolymer, made up of the repeated unit of β-1,4 glycosidic bonds *(2)*. Cellulose molecules are chain or microfibrils, of up to 14,000 units of D-glucose that occurs in twisted rope-like bundles held together by hydrogen bond *(3)*. Bacterial cellulose synthesized by microorganisms differs from plant cellulose in its structure. Bacterial cellulose is extremely pure and exhibits a higher degree of polymerization and crystallization in respect of the fibrous with lignin, hemicellulose, and waxy aromatic substances *(4)*. On account of these physicochemical properties, there

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has been recently interest in new fields of application and development of new methods for mass production of bacterial cellulose *(5–7)*.

A rotary biofilm contactor (RBC) has become a popular method for the treatment of domestic and industrial wastewater during the last decades. Tyagi et al. *(8)* previously investigated biodegradation of petroleum refinery wastewater in a polyurethane-attached RBC, and they found that RBC was capable of retaining considerable amounts of attached biomass, which when coupled with a good oxygen transfer capability of the system, could provide successful performance. The RBC generally consists of a series of circular disks mounted on a horizontal shaft *(9)*. The disks within the RBC are rotated, and alternatively exposed to the fermentation medium and air space *(8)*. Bacterial cellulose has been conventionally produced by static culture method, which requires a long culture period and intensive manpower, thus resulting in a low productivity. An agitated culture method converts bacterial cellulose-producing strains into cellulose-negative (*Cel*$^-$) mutants, which become more enriched than wild-type strain because of their rapid growth, thereby resulting in the lower productivity of bacterial cellulose *(10)*.

A cultivation of bacterial cellulose-producing bacteria in an RBC may not have a strong shear stress and an air bubble at the surface of liquid medium, which seems to be very excellent in terms of oxygen transferability by which the microorganisms can be readily contacted with air in comparison with stirred-tank bioreactor. In this study, the cultivation of *Gluconacetobacter* sp. RKY5 in RBC was attempted to improve the bacterial cellulose production, which might be a first trial for the production of bacterial cellulose using RBC as a bioreactor. This work mainly aimed at maximization of bacterial cellulose production through optimization of fermentation conditions in the RBC.

## Materials and Methods

### *Microorganism*

Microorganism used in this study was *Gluconacetobacter* sp. RKY5 KCTC 10683BP, which was previously isolated from persimmon vinegar *(11)*. *Gluconacetobacter* sp. RKY5 belongs to the group of Gram-negative aerobic bacterium, and it is rod-shaped without motility. It was maintained on 2% (w/v) agar plates containing Hestrin and Shramm (HS) medium *(12)*.

### *Composition of Medium*

The standard medium for regeneration of the strain and precultivation was HS medium, which consisted of 20.0 g/L of glucose, 5.0 g/L of peptone, 5.0 g/L of yeast extract, 2.7 g/L of $Na_2HPO_4$, and 1.2 g/L of citric acid monohydrate. A modified HS medium, consisted of 15.0 g/L of glycerol, 8.0 g/L of yeast extract, 3.0 g/L of $K_2HPO_4$, and 3.0 g/L of acetic

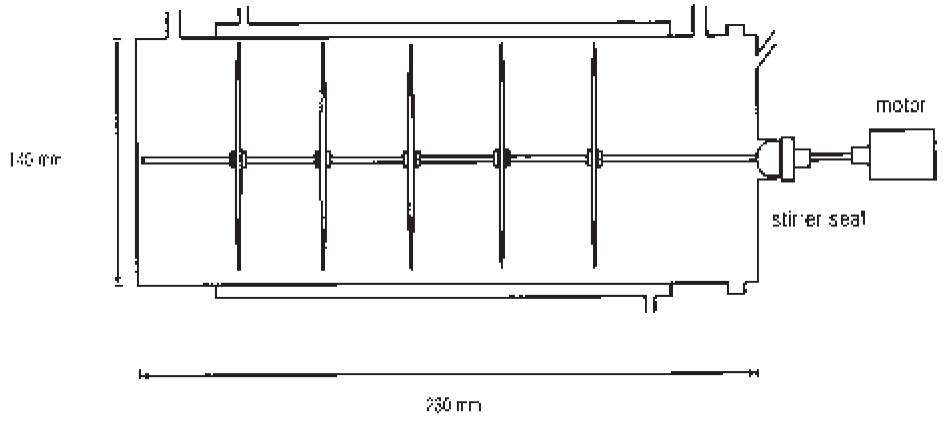


**Fig. 1.** Schematic diagram of a RBC.

acid was used for main fermentation. Before sterilization of each medium at 121°C, the pH value of the medium was adjusted to 6.0.

### *Cultivation Conditions*

Precultivation was carried out by inoculation of a single colony into 50 mL of HS medium in a 250-mL Erlenmeyer flask, and then incubated at 30°C and 150 rpm for 1 d in a shaking incubator (KMC-8480SF; Vision Scientific, Daejeon, Korea). Fifty milliliters of the preculture broth were homogenized by a homogenizer (X520D; CAT Ingenieurbüro M. Zipperer GmbH, Staufen, Germany) at 10,000 rpm for 1 min, and then 2% (v/v) of the homogenate was used as an inoculum.

### *Experimental Setup*

A schematic diagram of RBC used in this study is shown in Fig. 1, whereas the specifications of RBC are documented in Table 1. All disks were made of polypropylene and 34% of the disk was immersed in the medium. Disk diameter was 12 cm and disk thickness was 0.3 cm. A surface area of each disk was 226.2 $cm^2$, whereas an effective surface area of each disk was 221.1 $cm^2$. The RBC used in this study was made of pyrex, which was constructed with a double jacket to maintain the temperature through the circulation of constant temperature water. Total volume of the RBC was 3.5 L. Five to nine disks were mounted on a horizontal steel shaft and rotated with a variety of speeds from 15 to 35 rpm using a direct driven digital stirrer with a 50 W electric motor (SS-200; Global Lab, Seoul, Korea). Gas aeration was provided by an air pump through a filter (0.45-μm pore size) and controlled by an airflow meter. The overall experimental setup of RBC for bacterial cellulose production is schematically illustrated in Fig. 2. Bacterial cellulose fermentation was conducted in a 3.5-L RBC containing 1.0 L of working volume at 30°C for 96 h.

Table 1
Summary of the Dimensions of a RBC

| Parameter | Unit | Specification |
|---|---|---|
| Disk diameter | cm | 12 |
| Disk thickness | cm | 0.3 |
| Total surface area | $cm^2$ | 1131–2035.8 |
| Submergence | % | 34 |
| Effective area | $cm^2$ | 1105.6–2021.2 |
| Total volume | $cm^3$ | 3540 |
| Working volume | $cm^3$ | 1000 |
| Rotation speed | rpm | 15–35 |
| Temperature | °C | 30 |

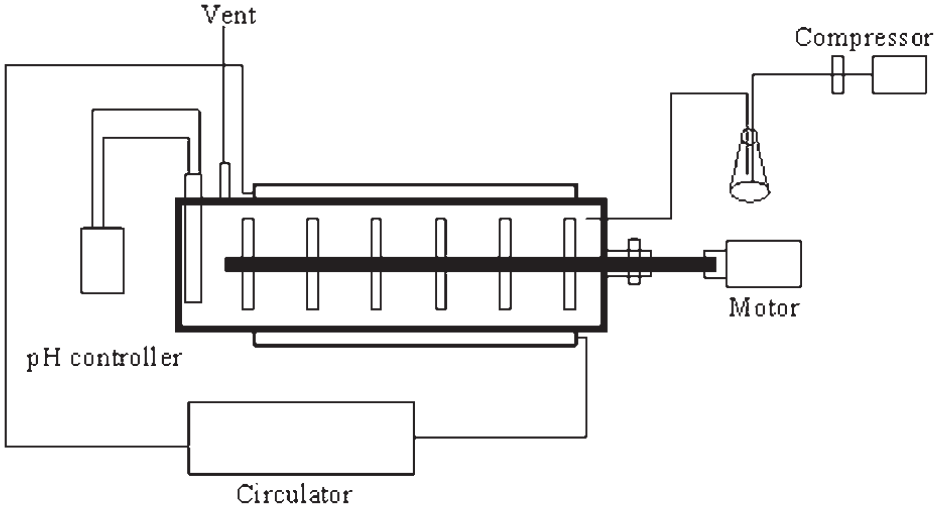


**Fig. 2.** Experimental setup of a RBC for bacterial cellulose production.

## *Analytical Methods*

Cell concentration was determined by measuring the optical density at 660 nm ($OD_{660}$) using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The $OD_{660}$ was measured after the culture broth containing cellulose pellicle was treated with 0.1% (v/v) cellulase (Celluclast 1.5 L; Novozymes A/S, Bagsvaerd, Denmark) at 50°C with shaking at 150 rpm for 1 h. Dry cell weight was then calculated by using a predetermined calibration curve *(13,14)*. The thick cellulose membrane formed on the surface of the disk was flaked with tweezers, which was washed with distilled water several times to remove the medium components and then treated with 0.1 *N* NaOH at 80°C for 30 min in order to dissolve the microorganisms *(3)*. After these treatments were done, bacterial cellulose was rinsed

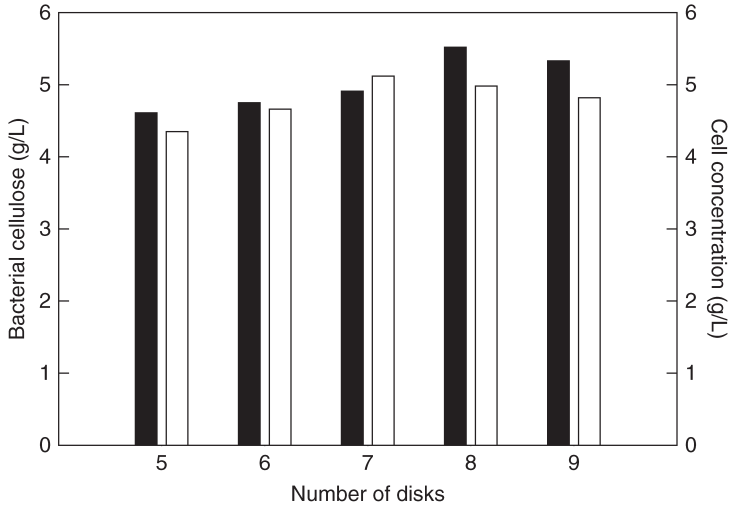


**Fig. 3.** Effect of number of disks on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 cultured in a RBC. (Culture conditions: pH, uncontrolled; temperature, 30°C; rotation speed, 15 rpm; aeration rate, 1 vvm; fermentation time, 96 h.) Symbols: ■, bacterial cellulose and □, cell concentration.

again with distilled water until the pH of water became neutral. Purified bacterial cellulose was dried at 80°C until constant weight was obtained, and then weighed.

## Results and Discussion

### *Influence of the Number of Disks*

In order to determine the optimal number of disk, fermentation was conducted with different number of disks using a modified HS medium. *Gluconacetobacter* sp. RKY5 was cultured in a 3.5-L RBC, containing 1 L working volume, at 30°C, 15 rpm, and 1 vvm for 96 h. Before sterilization at 121°C, the pH value of medium was adjusted to 6.0. During the cultivation, the pH of culture broth was not controlled. Figure 3 shows the amount of bacterial cellulose produced and cell concentration at different number of disks. As shown in Fig. 3, the amount of bacterial cellulose gradually increased with the number of disks up to eight disks, but then somewhat decreased beyond eight disks. Therefore, the optimal number of disks seemed to be eight, at which the amount of bacterial cellulose produced and cell concentration was 5.52 and 4.98 g/L, respectively.

### *Influence of Aeration Rates*

To investigate the effects of aeration rate on bacterial cellulose production, fermentation was conducted with 0–1.5 vvm in a 3.5-L RBC, containing 1 L working volume, at 30°C, 15 rpm, and eight disks for 96 h.

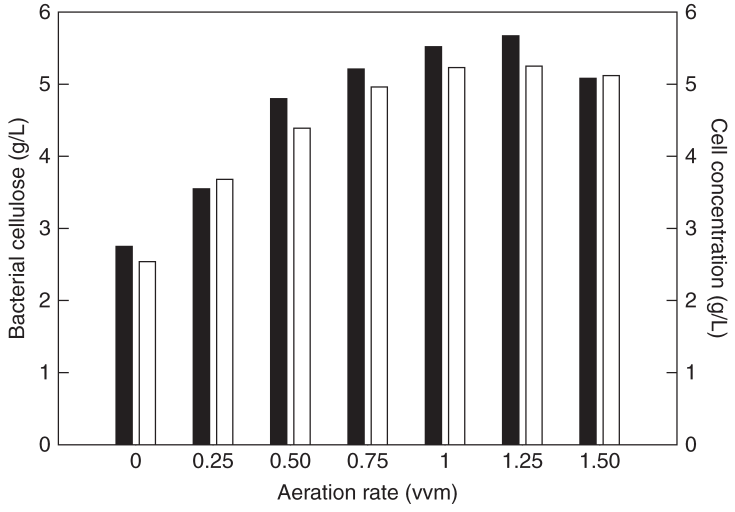


**Fig. 4.** Effect of aeration rates on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 cultured in a RBC. (Culture conditions: pH, uncontrolled; temperature, 30°C; rotation speed, 15 rpm; number of disk, 8; fermentation, 96 h.) Symbols: ■, bacterial cellulose and □, cell concentration.

During the cultivation, culture pH was not controlled. Figure 4 shows the amount of bacterial cellulose produced and cell concentration according to the respective aeration rates. As shown in Fig. 4, the amount of bacterial cellulose produced and cell concentration increased in accordance with increases in aeration rate. When aeration rate was 1.25 vvm, the maximal bacterial cellulose was 5.67 g/L and cell concentration was 5.25 g/L. In contrast, the excessive air supply higher than 1.25 vvm lowered both bacterial cellulose production and cell concentration.

## *Influence of Rotation Speeds*

Fermentations were conducted with 15–35 rpm in a 3.5-L RBC, containing 1 L working volume, at 30°C, 1.25 vvm, and eight disks for 96 h, in order to find the optimal rotation speed for enhancement of bacterial cellulose production. Before sterilization, the pH value of medium was adjusted to 6.0. During the cultivation, the pH of culture broth was uncontrolled. Figure 5 shows the amount of bacterial cellulose produced and cell concentration at various rotation speeds of the impeller. As shown in Fig. 5, both the amount of bacterial cellulose produced and cell concentration showed quite similar trends between 15 and 25 rpm, but then slightly decreased beyond this value. The highest amount of bacterial cellulose (5.41 g/L) and cell concentration (5.15 g/L) were obtained when the rotation speed was 15 rpm.

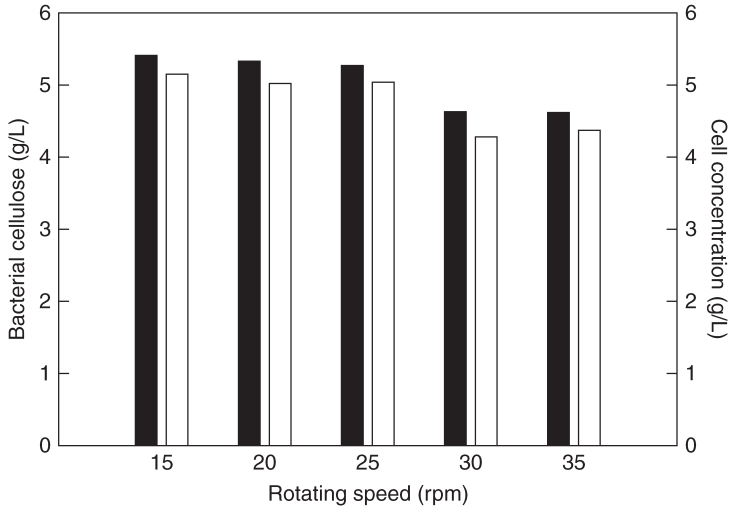


**Fig. 5.** Effect of rotation speeds on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 cultured in a RBC. (Culture conditions: pH, uncontrolled; temperature, 30°C; aeration rate, 1.25 vvm; number of disk, 8; fermentation time, 96 h.) Symbols: ■, bacterial cellulose and □, cell concentration.

## *Influence of Culture pH*

To investigate the effect of culture pH on bacterial cellulose fermentation, fermentation was conducted in a 3.5-L RBC, containing 1 L working volume, at 30°C, 15 rpm, 1.25 vvm, and eight disks for 96 h. The pH of culture broth was maintained at a constant value by automatic addition of 5 *N* NaOH and 5 *N* HCl. Figure 6 shows the amount of bacterial cellulose produced and cell concentration at pH values of 5.0, 6.0, 7.0, 8.0, and uncontrolled. As shown in Fig. 6, when the culture pH was not controlled, both bacterial cellulose production and cell concentration were considerably higher than those of the pH controlled fermentations. This was probably because of the inhibitory effect of nonuniformly mixed alkali and/or acid solution used for pH control, because the culture broth in the RBC was almost static. The maximal amount of bacterial cellulose produced (5.53 g/L) and cell concentration (4.91 g/L) was obtained at uncontrolled pH.

The optimal conditions for bacterial cellulose production in a RBC were established from the aforementioned results, and the amounts of bacterial cellulose produced and cell concentration were 6.17 and 5.58 g/L, respectively, under the aforementioned optimal conditions. Furthermore, bacterial cellulose concentration produced was generally associated with cell growth. From this observation, it can be deduced that bacterial cellulose fermentation should be severely associated with the growth of microorganism. Figure 7 shows a photograph of the thick cellulose membrane formed on the surface of the disk in a RBC.

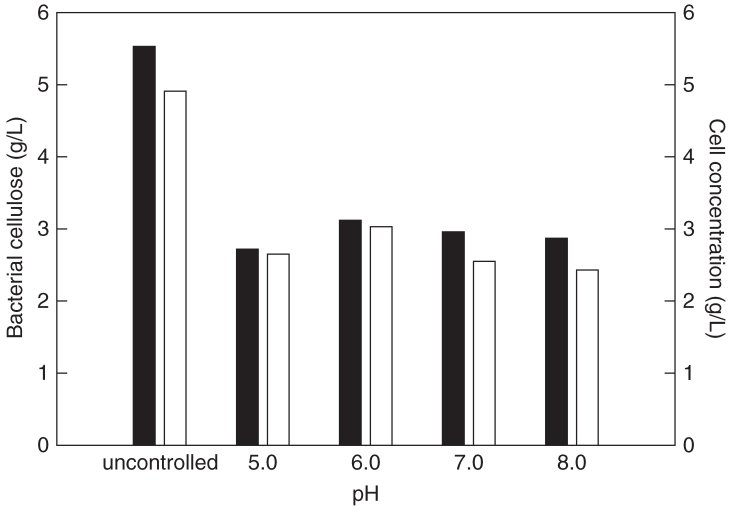


**Fig. 6.** Effect of culture pHs on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 cultured in a RBC. (Culture conditions: temperature, 30°C; aeration rate, 1.25 vvm; rotation speed, 15 rpm; number of disk, 8; fermentation time, 96 h.) Symbols: ■, bacterial cellulose and □, cell concentration.

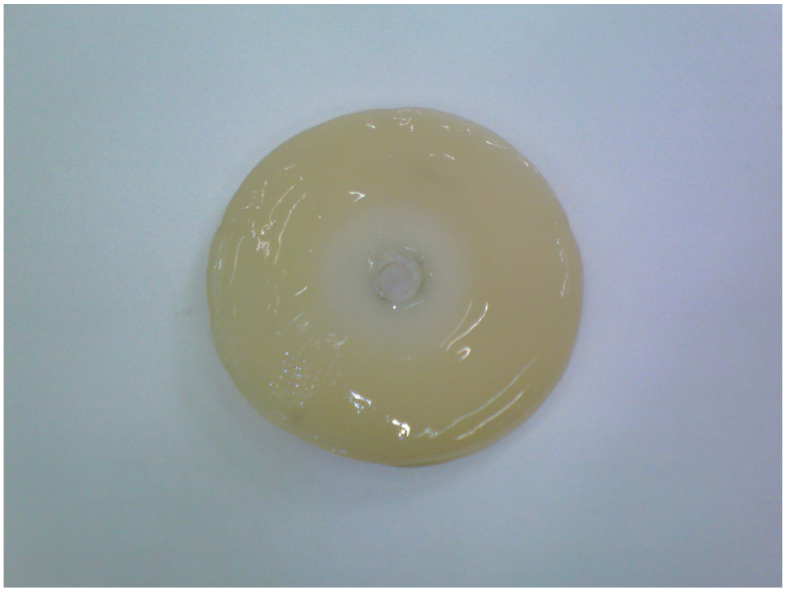

**Fig. 7.** Photograph of bacterial cellulose membrane formed on the surface of the disk during the fermentation using a RBC.

## Conclusions

The optimal fermentation conditions were investigated by using *Gluconacetobacter* sp. RKY5 in a RBC in order to improve bacterial cellulose production. The optimal number of disks was eight, at which the amount of bacterial cellulose produced and cell concentration was 5.52 and 4.98 g/L, respectively, and the aeration rate was 1.25 vvm with the maximal bacterial cellulose and cell concentration of 5.67 and 5.25 g/L, respectively. The highest amount of bacterial cellulose (5.41 g/L) and cell concentration (5.15 g/L) was obtained when the rotation speed was 15 rpm. The maximal amount of bacterial cellulose (5.53 g/L) and cell concentration (4.91 g/L) was obtained when the culture pH was not controlled at fixed value. After optimizing the operational parameters of RBC for bacterial cellulose production, the amounts of bacterial cellulose and cell concentration could be increased to 6.17 and 5.58 g/L, respectively.

## Acknowledgments

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