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

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Abstract

In this study, fermentation using a rotary biofilm contactor was conducted to improve bacterial cellulose production. We investigated the optimal fermentation conditions by using a newly isolated *Gluconacetobacter* sp. RKY5 in the rotary biofilm contactor. The optimal total area of discs was found to be 1,769 cm^2 at which bacterial cellulose and cell concentration was obtained to 5.52 g/L and 4.98 g/L, respectively. In case of aeration experiment, when the aeration rate was 1.25 vvm, the maximal bacterial cellulose (5.67 g/L) was obtained and cell concentration was 5.25 g/L.

Introduction

Cellulose is one of the most abundant biological macromolecules in nature, where it plays a crucial role in the integrity of plant cell walls[1)]. Cellulose is a linear insoluble biopolymer composed 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 occur in twisted rope-like bundles held together by hydrogen bonding[3)]. Bacterial cellulose has been produced conventionally by a static culture, which requires a long culture period and intensive manpower, thus resulting in a low productivity. An agitated culture converts microbial strains into Cel^- mutants, which become more enriched than the wild type because of their rapid growth, thereby

causing the lower productivity of bacterial cellulose in a continuous culture[4]. Fermentation using the rotary biofilm contactor does not have strong shear stress and an air bubble at the surface of liquid medium. It is also very excellent in terms of oxygen transfer ability by which the microorganisms can be readily contacted with air in comparison with stirred-tank reactor.

Materials and Methods

Microorganism

The microorganism used in this study was *Glucoacetobacter* sp. RKY5 KCTC 10683BP, which was isolated from traditional persimmon vinegar.

Cultivation conditions

The cells were kept on 2% agar plates containing HS medium[5]. The plate was then incubated at 30℃ until colonies formed. The cultivation was carried out by inoculation of a single colony into 50 mL of HS medium in a 250 mL Erlenmeyer flask, and cultivated at 30℃ and 150 rpm for 1 day in a shaking incubator (KMC-8480SF; Vision Scientific, Daejon, Korea). The pre-culture broth of 50 mL was homogenized by a homogenizer (CAT Homogenizer X520D; M.Zipperer GmbH, Germany) at 10,000 rpm for 1 min, and then 2% (v/v) of the homogenate was used as inoculum. The fermentation medium used in this study was a modified HS medium, consisted of 15 g/L glycerol, 8 g/L yeast extract and 3 g/L acetic acid. Prior to sterilization at 121℃, the pH value of medium was adjusted to 6.0.

Rotary biofilm contactor

The rotary biofilm contactor consisted of a series of circular discs mounted on a horizontal shaft. All discs were made of polypropylene and 34% of the discs was immersed in the medium. Disc diameter was 6 cm and disc thickness was 0.3 cm. A surface area of each disc was 226.2 cm^2, whereas a effective surface area of each disc was 221.1 cm^2. Total volume of the rotary biofilm contactor was 3.5 L.

Analytical methods

The cell concentration was determined by measuring the absorbance at 660 nm (OD_{660}) using a UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). The OD_{660} was measured in diluted broth, after the culture broth was treated with 0.1% (v/v) cellulase (Celluclast 1.5L, Novozymes A/S, Denmark) at 50℃ with shaking at 150 rpm for 1 hour. The dry cell weight is then calculated by using a pre-determined calibration curve. The thick cellulose membrane formed on the surface of the disc 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℃ for 30 min in order to dissolve the microorganisms[3]. After these treatments were done, bacterial cellulose was rinsed again with distilled water until the pH of water became neutral. The purified bacterial cellulose was dried at 80℃ until constant weight was obtained.

Results and Discussion

In order to determine the optimal surface area of discs, experiment was conducted at the different number of discs in a modified HS medium. The fermentation was carried out in a 3.5 L rotary biofilm contactor with 1 L working volume at 30℃, 15 rpm, and 1 vvm (v/v) for 96 hours. Prior to sterilization at 121℃, the pH value of medium was adjusted to 6.0. During the fermentation, the pH of culture broth was uncontrolled. As shown in Fig. 1, the amount of bacterial cellulose gradually increased with the number of discs up to 8 discs, but then somewhat decreased beyond 8 discs. Therefore, the optimal number of discs was seemed to be eight, at which the amount of bacterial cellulose produced and cell concentration was obtained to 5.52 g/L and 4.98 g/L, respectively. To investigate the effect of aeration rate on bacterial cellulose production, experiment was conducted with 0 to 1.5 vvm in a 3.5 L rotary biofilm contactor with 1 L working volume at 30℃, 15 rpm, and 8 discs for 96 hours, and the pH of culture broth was uncontrolled. As shown in Fig. 2, the amount of bacterial cellulose produced and cell

concentration increased in accordance with increases in aeration rate. When the aeration was 1.25 vvm, maximal bacterial cellulose (5.67 g/L) was obtained and cell concentration was 5.25 g/L. Fig. 3 shows the thick cellulose membrane formed on the surface of the disc.

References

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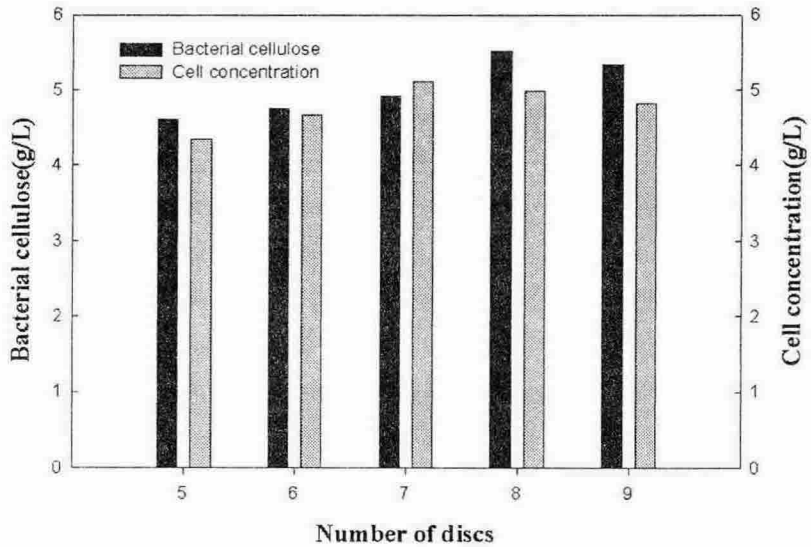

Fig. 1. Effect of number of discs on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 in a rotary biofilm contactor.

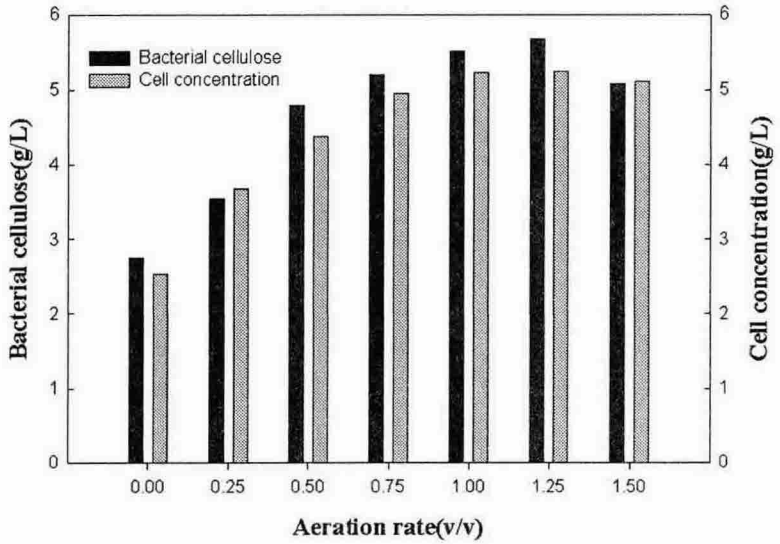

Fig. 2. Effect of aeration rates on bacterial cellulose production and cell concentration by *Gluconacetobacter* sp. RKY5 in a rotary biofilm contactor.

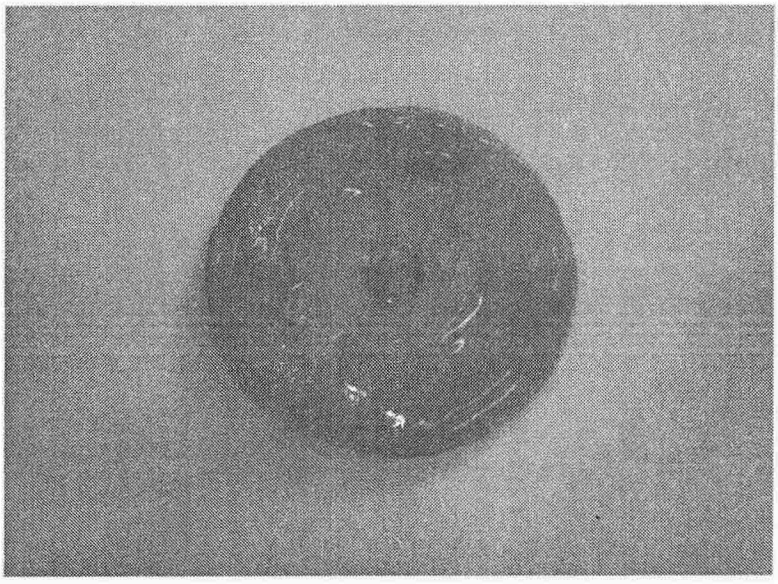

Fig. 3. Photograph of cellulose membrane formed on the surface of the disc in a rotary biofilm contactor.