Biotechnology and Bioprocess Engineering **2006**, 11: 26-31

Comparisons of Physical Properties of Bacterial Celluloses Produced in Different Culture Conditions Using Saccharified Food Wastes

Seung-Hyeon Moon[1], Ji-Min Park[1], Hwa-Young Chun[2], and Seong-Jun Kim[2]*

[1] Department of Environmental Science Engineering, Gwangju Institution of Science & Technology, Gwangju 500-712 Korea
[2] Department of Civil, Earth and Environmental Engineering, Chonnam National University, Gwangju 500-757, Korea

Abstract The saccharogenic liquid (SFW) obtained by the enzymatic saccharification of food wastes was used as a medium for production of bacterial cellulose (BC). The enzymatic saccharification of food wastes was carried out by the cultivation supernatant of *Trichoderma harziaum* FJ1 culture. *Acetobacter xylinum* KJ1 was employed for the BC production culture. The physical properties, such as polymerization, crystallinity, Young's modulus, and tensile strength, of BCs produced by three culture methods: the static cultures using HS (Hestrin-Schramm) as a reference medium (A) or the SFW medium (B), the shaking culture (C) or the air circulation culture (D) using the SFW medium, were investigated. The degrees of polymerization of BCs produced under the different culture conditions (A~D) showed 11000, 9500, 8500, and 9200, respectively. Young's modulus was 4.15, 5.0, 4.0, and 4.6 GPa, respectively. Tensile strength was 124, 200, 80, and 184 MPa, respectively. All of the BC had a form of cellulose I representing pure cellulose. In the case of the shaking culture, the degree of crystallinity was 51.2%, the lowest degree. Under the other culturing conditions, the trend should remain in the range of 89.7~84%. Overall, the physical properties of BC produced from SFW were similar to those of BC from HS medium, a commercial complex medium, and BC production by the air circulation culture mode brought more favorable results in terms of the physical properties and its ease of scale-up. Therefore, it is expected that a new BC production method, like air circulation culture using SFW, would contribute greatly to BC-related manufacturing.

Keywords: bacterial cellulose, physical properties, saccharification, food wastes, *Acetobacter xylinum*

INTRODUCTION

Bacterial cellulose (BC) produced by *Acetobacter xylinum* is a pure cellulose aggregate which does not include any impurities, such as hemicellulose, pectin, and lignin, and, differing from plant-derived cellulose, has no intracellular cavity. Additionally, BC has a microscopic reticular microfibrillar structure with the fiber thickness of about 0.1 μm and a higher degree of crystallinity than wood pulp. Therefore, BC has a very high surface area, a high water retention value, a high moldability, and a strong tensile strength. These excellent physical properties of BC make possible studies of its actual use in a speaker diaphragm, tourniquet, or dietary fiber, and because of its low toxicity and chemical stability, it is used in manufacturing an artificial skin, as well as paint used as a thickener for ink [1,2]. Particularly, considering that it is an environment-friendly material, it has unbounded versatility and potential for development.

However, if any shearing stress is applied to the *Acetobacter* strain during culture, a *Cel*$^-$ mutation inhibiting cellulose production occurs, resulting in a significant reduction in the productivity of BC. For this reason, the static culture, disadvantaged by low productivity, a long culturing time, and a great labor power, has been used to obtain bacterial cellulose. Therefore, in order to overcome this problem and produce BC on an industrial scale, studies on the enhancement of BC productivity in the selection of microbes which are genetically stable, and adding lactate, pyruvate, and ethanol to the culture medium, have been conducted [3]. Additionally, studies on microbial culture and operating conditions for cost reduction have been continued [4,5]. However, the productivity of BC is still too low and its production cost is too high for industrial use.

We have developed an optimized culture technique for mass production of BC which greatly reduces the unit cost of BC production, thanks to a low-cost sugar gener-

***Corresponding author**
Tel: +82-62-530-1864 Fax: +82-62-530-0864
e-mail: seongjun@chonnam.ac.kr

ated from the hydrolysis of macromolecule fibrinous substances included in food wastes, and used as a medium for BC production. In this study, saccharogenic liquid, which was obtained by enzymatic saccharification of food wastes, was used as a medium, and BC was produced through different methods, such as the static culture using a 30 L fermentor, the shaking culture using a 10 L fermentor, and the air circulation culture using a 10 L spherical airlift-type reactor. BC produced from the saccharogenic liquid and BC produced from a commercial complex medium, HS medium, were comparatively examined in terms of their productivity and physical properties. This study was ultimately designed to achieve the low-cost mass production technology using the saccharogenic liquid medium and to provide information on the physical properties of BC produced in various cultures.

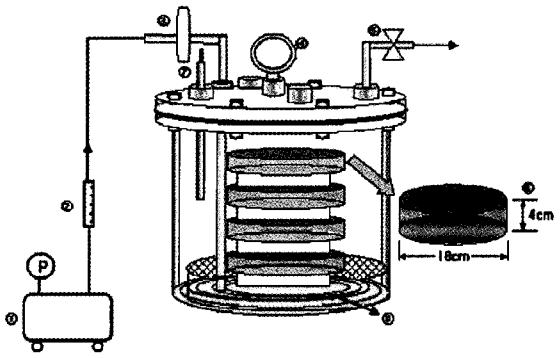

Fig. 1. Apparatus of the 30 L static culture system. 1: air compressor, 2: flow meter, 3: air inlet, 4: air pressure, 5: air outlet, 6: stainless dish, 7: thermometer, 8: air diffuser.

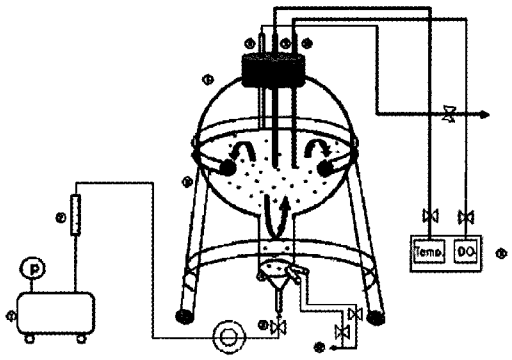

Fig. 2. Apparatus of the 10 L spherical air circulation culture system. 1: air compressor, 2: flow meter, 3: air inlet, 4: air diffuser, 5: fixing bar, 6: 10 L airlift fermenter, 7: stopper, 8: gas outlet, 9: temperature sensor, 10: DO meter, 11: recorder, 12: sampling nozzle.

MATERIALS AND METHODS

Production of Bacterial Cellulose

Enzyme Production

Trichoderma harzianum FJ1, separated by Kim *et al.* [6], was used in the enzyme production culture. *T. harzianum* FJ1, precultured according to the method of Yoo *et al.* [8], was inoculated at 2% in the 10 L jar fermentor with a 5 L working volume, in which fibrinous wastes (rice straw, waste paper) were used at 1%, respectively, in place of CMC and Avicel used as a carbon source in the Mandel's medium [7]. It was then cultured by shaking at 30°C, 200 rpm, 0.6 vvm, and non-controlled pH for 5 days.

Enzymatic Saccharification of Food Wastes

The food wastes used for enzymatic saccharification were collected from the 1st Student Hall of Chonnam National University, and the moisture content of the waste was about 80~85%. The composition of the food wastes used in the experiments showed 45 ± 2% as TOC (total organic carbon), 44.5 ± 0.3% as carbon, and 2.4 ± 0.2% as nitrogen in elementary analysis. For the enzymatic saccharification reaction, 4 kg of food waste was mixed with 1 L of the enzyme liquid, and the mixture was enzymatically hydrolyzed by reacting in the 10 L fermentor at 50°C and 150 rpm for 24 h [9]. The enzyme activity used in the saccharification was adjusted at 0.22 U/mL of FPase, 5.40 U/mL of CMCase, and 2.80 U/mL of amylase. The enzyme activity was measured by the method used by Kim *et al.* [6].

Method for BC Production

A. xylinum KJ1, separated from decayed grapes by Son *et al.* [10], was used for BC production. As for respective culturing methods for BC production, the static culture in the HS medium [11] was used as a control culture and the other cultures used the saccharogenic liquid as a medium. The static cultures employed a 30 L fermentor installed with stainless containers (ϕ 8 cm × H 4 cm), which were arranged by 4 floor with space of height of 5 cm. Air was supplied through air diffuser of the reactor bottom and side pipe of the air supply (Fig. 1). The shaking culture was conducted using a 10 L jar fermentor (Hanil Science Industrial Co., Korea) with working volume of 5 L, and the air circulation culture was conducted using the 10 L spherical air circulation reactor with working volume of 5 L (Fig. 2). The temperature, pH, and the culturing time were set at 30°C, 5.25, and 5 days, respectively, in all types of culture. The shaking speed in the stirred culture was set at 150 rpm, and the aeration rate was set at 3 L/min.

BC purification from the culture broth was performed according to the method described by Son *et al.* [10]. Cell-free BC was then lyophilized and kept for the analysis of physical properties.

Analysis of Physical Properties of BC

Morphological Observation

In order to compare the textural structures of BC depending the culturing condition, SEM (scanning electron microscope) (S-2400, Hitachi Co., Japan) images were obtained.

Degree of Polymerization

The weight average degree of polymerization (DPw) was evaluated by the size-exclusion chromatography method (SEC) [12] with a high-performance gel permeation chromatography (GPC) system (HLC-8020, Thosoh Co., Tokyo). Bacterial cellulose was nitrated according to the methods described by Alexander and Mitchell [13]. The cellulose nitrate was dissolved in tetrahydrofuran (Aldrich), which was also used as the eluent, and polystyrenes were used as the molecular weight standards for the calibration curve.

Relative Crystallinity Index

X-ray diffraction patterns of the cellulose samples were recorded on an X-ray diffractometer (Rigaku Riken Diffractometer, Japan) using the reflective method. The radiation was Ni-filtered CuK_{α} of wavelength 1.54 nm. The X-ray unit operated at 40 kV and 27 mA. Angular scanning was varied 10~40° at 0.02° per second, and data were collected using a 2-step scan mode with angular intervals of 0.02°. The relative crystallinity index was estimated by Segal's method [14], using the following equation:

$$CrI = 1 - h_{am} / h_{cr} = 1 - h_{am} (h_{tot} - h_{am})$$

where h_{cr} is the peak intensity corresponding to the (002) plane at $2\Theta = 22.5°$ for cellulose I , h_{am} is the peak intensity of amorphous fraction at $\Theta = 18°$ for cellulose I , and h_{tot} is total height.

Tensile Strength and Young's Modulus

A suspension containing disintegrated bacterial cellulose (BC) was cast in a plastic petri dish and completely dried at 50°C overnight. The tensile strength of the sheet was measured with an ASTM Standard D 638 [15]. The sheet was cut into ribbons (5 × 30 mm) for measurement of tensile strength. The thickness of the ribbons was 100 μm, and the elongation rate was 1.0 mm/min. Young's modulus was determined from the ratio of the stress exerted on the sample to that exerted on the deformed sample, as measured by a tensile tester (SGA-100, Shin Gang Co. Ltd., Korea) [16].

RESULTS AND DISCUSSION

Productivity of BC Depending upon the Culturing Condition

As shown in Fig. 3, the productivity of BC depending upon the respective culturing conditions was as follows: in the case of the static culture in the HS medium, it was 0.43 g-cellulose/g-R.S; and in the respective cases of the static culture, the shaking culture, and the air circulation culture in the saccharogenic liquid medium (SFW), it was 0.46, 0.33, and 0.43 g-cellulose/g-R.S, respectively. It is thought that, in the shaking culture, the *Cel*$^-$ mutant is generated to a high degree in order to lower the productivity of BC [17,18]. However, the static culture and the air circulation culture using SFW brought forth the result, which was very similar to the result of the static culture of the complex HS medium. This is probably caused by the low shear stress of the spherical air circulation bioreactor, in which the culture medium showed a hydrodynamic-like internal-loop airlift type with rising and mixing of fluid by the driving force of the input of air by the central and lower parts. Therefore, the positive results from the cultivation method showed that low-cost production of BC might be possible using SFW.

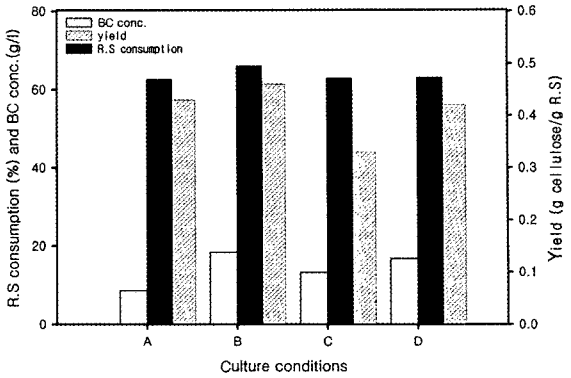

Fig. 3. Comparison of the productivity of bacterial celluloses produced under different culture conditions. A: HS + static culture, B: SFW + static culture, C: SFW + jar fermentor, D: SFW + air circulation culture.

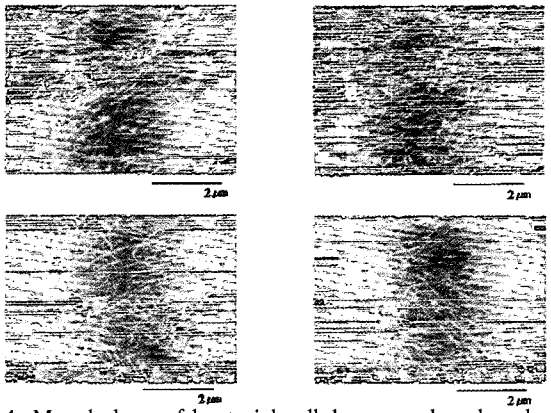

Fig. 4. Morphology of bacterial celluloses produced under different culture conditions. A: HS medium + static culture, B: Saccharification broth (SFW) + static culture, C: SFW medium + shaking culture, D: SFW medium + air circulation culture.

Morphological Observation of BC

Forms of BC, as observed through SEM, had a similar structure irrespective of the culturing condition, as shown in Fig. 4. It was also observed that BC had a typical form in which the width of the BC fibrils was less than approximately 0.1 μm, with a dense mesh structure. In general, due to the structural characteristics of BC as an ultra-fine and highly-pure fiber network, it has unique properties, including high mechanical strength, high wa-

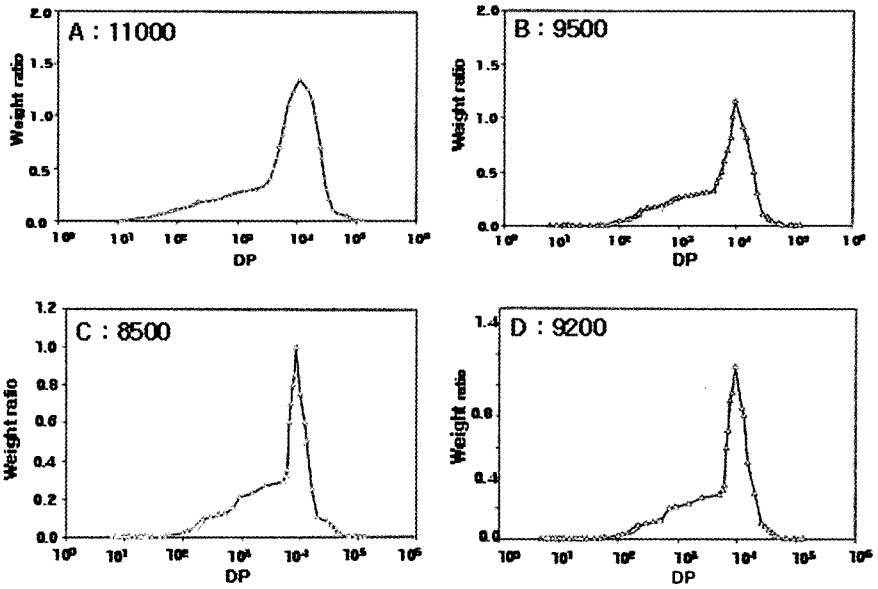

Fig. 5. Degree of polymerization (DP) of bacterial celluloses produced under different culture conditions. A: HS medium + static culture, B: SFW medium + static culture, C: SFW medium + shaking culture, D: SFW medium + air circulation culture.

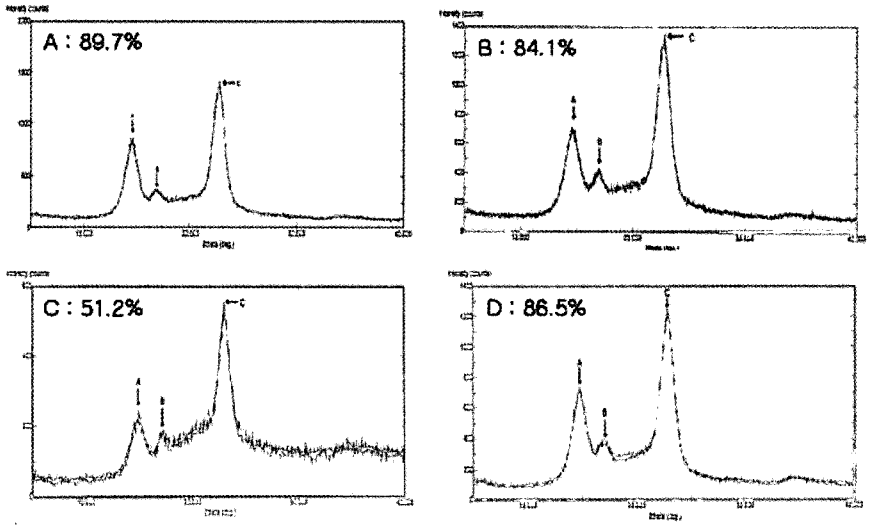

Fig. 6. Crystallinity of bacterial celluloses produced under different culture conditions. A: HS medium + static culture, B: SFW medium + static culture, C: SFW medium + shaking culture, D: SFW medium + air circulation culture.

ter absorption capacity, and high crystallinity.

Degree of Polymerization of BC

The degrees of polymerization of BC under different culturing conditions measured by the GPC method have been presented in Fig. 5. The degrees of polymerization of BC from the static culture in the HS medium and the SFW medium were 11000 and 9500, respectively. Those produced by the shaking culture and the air circulation culture using the SFW medium were 8500 and 9200, respectively. The degree of polymerization of BC was observed to be the lowest in the shaking culture. In general, the strain referred to as *A. xylinum* produces BC together with acetan and/or xylan, a water-soluble polysaccharide [19]. This water-soluble polysaccharide interferes with the hydrogen bonding between microfibrils to reduce the length of the microfibrils, and thereby reduce the degree of polymerization. This water-soluble polysaccharide, in particular, is produced at increased levels by shearing stress during the shaking culture. Therefore, in the shaking culture, where hydrogen bonding between microfibrils is less common, the degree of polymerization is lowered, differing from the case in other culturing

conditions [20].

Degree of Crystallinity of BC

In measuring the degree of crystallinity of BC as produced under different culturing conditions by the Segal's method using X-ray diffraction, respective relative degrees of crystallinity of BC showed peaks A, B, and C regardless of the culturing condition, as in the case of the common I-type crystal of natural cellulose. As shown in Fig. 6, the relative degrees of crystallinity of BC produced by static culture in the HS medium and SFW medium were 89.7 and 84.1%, respectively. Those of BC produced by the shaking culture and the air circulation culture using SFW medium were 51.2 and 86.5%, respectively. Only the degree of crystallinity of BC produced by the shaking culture using the saccharogenic liquid medium (SFW) was significantly reduced, differing from other methods. It is thought that as greatly affected by shearing stress during shaking culture, hydrogen bonding between fibrils is reduced so that the degree of polymerization and the degree of crystallinity are both reduced [20].

Young's Modulus and Tensile Strength of BC

Young's modulus and tensile strength, both known standards which show the mechanical properties of fiber, were measured using a universal tensile tester. Under respective culturing conditions, Young's modulus was 4.15 and 5.0 GPa for the static culture in the HS medium and the SFW medium, respectively. Young's modulus for the shaking culture and the air circulation culture in the SFW medium was 4.0 and 4.6 GPa, respectively. The tensile strength under respective culturing conditions was 124 MPa for the static culture in the HS medium and 200 MPa for the SFW medium. The tensile strengths for the shaking culture and the air circulation culture in the SFW medium were 80 and 184 MPa, respectively. Generally, Young's modulus and the tensile strength, both important physical properties of BC, are affected by the degree of polymerization of BC. It is thought that, during the shaking culture, shearing stress caused an increase in the production of water-soluble saccharide, so that the degree of polymerization was reduced. Further, the length of the microfibrils was reduced, and thereby, Young's modulus and tensile strength were also considerably lowered. Watanabe *et al.* [21] proposed that there was a positive correlation between the crystallinity of bacterial cellulose and Young's modulus of its sheet. Nishi *et al.* [22] also suggested that, because of its high Young's modulus, a sheet made from bacterial cellulose is expected to be suitable for making acoustic transducer diaphragms.

CONCLUSIONS

The saccharogenic liquid (SFW) obtained by the enzymatic saccharification of food wastes was used as a medium for production of BC. The physical properties, such as polymerization, crystallinity, Young's modulus, and tensile strength, of BCs produced by three culture methods: the static cultures using HS as a reference medium (A) or the SFW medium (B), the shaking culture (C) or the air circulation culture (D) using the SFW medium, were investigated. The degrees of polymerization of BCs produced under the different culture conditions (A~D) showed 11000, 9500, 8500, and 9200, respectively. Young's modulus was 4.15, 5.0, 4.0, and 4.6 GPa, respectively. Tensile strength was 124, 200, 80, and 184 MPa, respectively. All of the BC had a form of cellulose I representing pure cellulose. In the case of the shaking culture, the degree of crystallinity was 51.2%, the lowest degree. Under the other culturing conditions, the trend should remain in the range of 89.7~84%. When the saccharogenic liquid prepared from food wastes was used for the air circulation culture of BC, the productivity of BC was relatively high, and the physical properties of the BC were very similar to those of BC produced in the conventional commercial medium (HS). Therefore, it is thought that the results of this study will be of great help in the development of the technology for producing high volumes of BC at a low cost.

Acknowledgement This subject is supported by Ministry of Environment of the Republic of Korea as "The Eco-technopia 21 project".

REFERENCES

[1] Klemm, D., D. Schumann, U. Udhard, and S. Marsch (2001) Bacterial synthesized cellulose-artificial blood vessels for microsurgery. *Prog. Polym. Sci.* 26: 1561-1603.
[2] Shibazaki, H., S. Kuga, F. Onabe, and M. Usuda (1993) Bacterial cellulose membrane as separation medium. *J. Appl. Poly. Sci.* 50: 965-969.
[3] Matsuoka, M., T. Tsuchida, K. Matushita, O. Adachi, and F. Yoshinaga (1996) A synthetic medium for bacterial cellulose production by *Acetobactr xylinum* subsp. *sucrofermentans. Biosci. Biotechnol. Biochem.* 60: 575-579.
[4] Naritomi, T., T. Kouda, H. Yan, and F. Yoshinaga (1998) Effect of Lactate on bacterial cellulose production form fructose in continuous culture. *J. Ferment. Bioeng.* 85: 89-95.
[5] Chao, Y., T. Ishida, Y. Sugano, and M. Shoda (2000) Bacterial cellulose production by *Acetobacter xylinum* in a 50-L internal-loop airlift reactor. *Biotechnol. Bioeng.* 68: 345-352.
[6] Kim, K. C., S. S. Yoo, Y. A. Oh, and S. J. Kim (2003) Isolation and characteristics of *Trichoderma harzianum* FJ1 producing cellulases and xylanase. *J. Microbiol. Biotechnol.* 13: 1-8.
[7] Mandel, M. and D. Sternberg (1976) Recent advances in cellulase technology. *J. Ferment. Technol.* 54: 267-286.
[8] Yoo, S. S., K. C. Kim, Y. A. Oh, S. Y. Chung, and S. J. Kim (2002) The high production of cellulolytic enzymes using cellulosic wastes by a fungus, strain FJ1. *Kor. J. Microbiol. Biotechnol.* 30: 172-176.

[9] Kim, K. C., S. W. Kim, M. J. Kim, and S. J. Kim (2005) Saccharification of foodwastes using cellulolytic and amylolytic enzymes from *Trichoderma harzianum* FJ1 and its kinetics. *Biotechnol. Bioprocess Eng.* 10: 52-59.

[10] Son, C. J., S. Y. Chung, J. E. Lee, and S. J. Kim (2002) Isolation and cultivation characteristics of *Acetobacter xylinum* KJ1 producing bacterial cellulose in shaking cultures. *J. Microbiol. Biotechnol.* 12: 722-728.

[11] Hestrin, S. and M. Schramm (1954) Synthesis of cellulose by *Acetobacter xylinum*. 1. Micromethod for the determination of celluloses. *Biochem. J.* 56: 163-166.

[12] Kuga, S., N. Muton, A. Isogai, M. Usuda, and R. M. Brown, Jr. (1989) Cellulose: Structural and function aspects. pp. 81-86. In: J. K. Kennedy, G. O. Phillips, and P. A. Williams (eds.). Ellis Horwood, Chichester, UK.

[13] Alexander, W. J. and R. L. Mitchell (1949) Rapid measurement of cellulose viscosity by nitration methods. *Anal. Chem.* 21: 1497-1500.

[14] Segal, L., J. Creely, A. Martin, and C. Conrad (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffreactometer. *Text. Res. J.* 29: 786-794.

[15] Annual Book of ASTM Standards, section 8, Plastics, ed. by ASTM, Pennsylvania (1993) Vol. 8.

[16] *Biochemistry Experimental Book*. ed., pp. 258-259. Teaching Material Editing Committee of Korean Journal of Biochemistry, Tamgudang, Korea. 1994.

[17] Park, J. K., S. H. Hyun, and J. Y. Jung (2004) Conversion of *G. hansenii* PJK into non-cellulose-producing mutants acciording to the culture condition. *Biotechnol. Bioprocess Eng.* 9: 383-388.

[18] Jung, J. Y., J. K. Park, and H. N. Chang (2005) Bacterial cellulose production by *Gluconaacetobacter hansenii* in an agitated culture without living non-cellulose producing cells. *Enzyme Microb. Technol.* 37: 347-354.

[19] Shoda, M. and Y. Sugano (2005) Recent advances in bacterial cellulose production. *Biotechnol. Bioprocess Eng.* 10: 1-8.

[20] Yamamoto, H., F. Horii, and A. Hirai (1996) *In situ* crystallization of bacterial cellulose 2. Influences of different polymeric additives on the formation of celluloses I α and I β at the early stage of incubation. *Cellulose* 3: 229-242.

[21] Watanabe, K., Y. Hori, M. Tabuchi, Y. Morinaga, F. Yoshinaga, F. Horii, J. Sigiyama, and T. Okano (1994) Structural features of bacterial cellulose vary depending on the culture conditions. *Proceedings of '94 Cellulose R&D, 1st Annual Meeting of the Cellulose Society of Japan.* pp. 45-50.

[22] Nishi, Y., M. Uryu, S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, and S. Mitsuhashi (1990) The structure and mechanical properties of sheets prepared from bacterial cellulose. *J. Mater. Sci.* 25: 2997-3001.

[Received July 18, 2005; accepted November 3, 2005]