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Production of bacterial cellulose by *Gluconacetobacter hansenii* using a novel bioreactor equipped with a spin filter

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Abstract–In order to improve bacterial cellulose (BC) production yield by increasing the cell density, a new fermentation system using a spin filter was developed and its performance characteristics were tested. Fermentations were carried out in a fermenter equipped with a 6 flat-blade turbine impeller and a spin filter consisting of a cylinder surrounded by stainless steel mesh and whose stainless steel bottom was attached to the agitator shaft. This new fermentation assembly was tested under different experimental conditions for BC production by *Gluconacetobacter hansenii* PJK. In periodical perfusion culture without pH control, the BC production and the total cell mass increased with the culture time to 3.07 and 5.65 g/L, respectively, at 140 h of cultivation. The BC production was also tested at adjusted pH and pH 5 was found optimum for maximum BC production. At pH 5, in periodical perfusion culture, the BC production and the total cell mass reached to 4.57 and 11.52 g/L, respectively, after 140 h of cultivation. This amount of BC production was 2.9 times higher than that obtained in a conventional jar fermenter. The productivity improved and was 0.044 g/L·h at 68 h of cultivation.

Key words: Bacterial Cellulose, *Gluconacetobacter hansenii* PJK, *Cel*$^-$ Mutants, Spin Filter

INTRODUCTION

Bacterial cellulose (BC) has various applications as new functional materials as well as diet foods because of its unique physical properties [1-3]. BC is free from hemicellulose, pectin, and lignin associated with plant cellulose [2] and therefore requires simple refining process. BC has high water retention value, tensile strength, and moldability because its diameter is only a thousandth that of plant cellulose [4,5]. These unique properties of BC, distinct from those of plant celluloses, have contributed to the development of new materials for high performance speaker diaphragms, tourniquets, diet foods, artificial skin, medical pads, make-up pads, and paint thickeners [2,4-7]. As mentioned above, BC, an eco-friendly material, is applicable to industrial and food materials and has a high potential for commercialization. BC has been produced traditionally by a static culture that has a low productivity, because a shear stress in the shaking culture converts microbial strains into non-cellulose-producing (*Cel*$^-$) mutants during cultivation, resulting in a decrease of BC production yield [8].

In our previous reports [9,10], we isolated *Gluconacetobacter hansenii* from rotten apples, which had a higher BC production yield in a shaking culture than in a static culture. It was possible to preserve the cellular activity of cellulose production without the spontaneous occurrence of *Cel*$^-$ mutants in consecutive shake-cultures by using a medium containing ethanol. However, in a strong shear stress field like an agitated culture condition, a number of cellulose-producing (*Cel*$^+$) cells were converted into *Cel*$^-$ mutants despite the ethanol addition to the medium [11]. In our other previous studies, we found an optimum culture condition for maximizing BC production in an agitated culture of a medium containing ethanol without *Cel*$^-$ mutants [12]; however, the productivity of BC is still not much higher for industrialized production.

High productivity requires high cell density in a bioreactor because productivity is proportional to the concentration of cells as well as the single cell productivity in a bioreactor. It is well known that a membrane recycling system that can increase cell density during fermentation is more suitable than any other system for improving the productivity [13-17]. Margaritis and Wilke [18] reported that cultivation of *Saccharomyces cerevisiae* ATCC 4126 in a fermenter equipped with a rotating cylinder membrane increased the cell density and ethanol up to 30 g/L and 50 g/L, respectively, and the production rate up to 27 g/L·h., which is about a 10-fold increase compared to CSTR fermentation. However, a fermentation system using a rotating cylinder membrane is not generally applied to an industrial fermentation system because it has been required to investigate systematically the rotation speed of the cylinder membrane, cell density, ionic strength, and pH of the culture broth with respect to the formation of filter cake and membrane fouling.

In this work, a special type of a bioreactor using a spin filter for the BC production has been developed which was able to maintain the separation efficacy of the membrane without filter cake. The effects of hydrodynamic and culture environmental factors were investigated. The proposed system was able to produce high cell density in the bioreactor and thus a high production yield of BC than the conventional fermentation system.

EXPERIMENTAL

1. Microorganism and Cell Culture

G. hansenii PJK (KCTC 10505BP), isolated from rotten apples and identified by using the 16S rDNA complete sequencing method

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[9], was grown on a basal medium containing glucose 10 g/L, yeast extract 10 g/L, peptone 7 g/L, acetic acid 1.5 mL/L, and succinate 0.2 g/L. The agar plates used for keeping strains were prepared by dissolving agar 15 g/L in the basal medium. The medium containing ethanol was prepared by adding 1% (v/v) ethanol to the autoclaved basal medium. The pH of the medium was adjusted to 5.0 with NaOH. Colonies of *G hansenii* were inoculated into a 50 mL medium in a 250 mL flask shaken at 200 rpm and cultured at 30 °C for 24 hours. Five percent of the culture broth collected from 4 flasks was inoculated into a 3 L medium of a 5-L jar fermenter and 1.6 L of the medium in a 2-L jar fermenter equipped with a spin filter for the cultivation at 30 °C, an agitation rate of 500 rpm, and an aeration rate of 1 vvm. The exhausted medium was removed from the fermenter equipped with a spin filter and an equal amount of fresh medium was refilled at 24, 48, 56 h and then after each 12 h up to 116 h of cultivation.

2. Bioreactor Configuration

As shown in Fig. 1(a), 1(b), fermentations were carried out in 2 L jar fermenter (Applikon Co., USA) with a 6 flat-blade turbine impeller and equipped with a spin filter, while a 5 L Jar fermenter (KoBiotech Co., Korea) was used as reference for comparison.

The spin filter was cylinder type surrounded by a stainless steel mesh (pore size: 38 μm) and the bottom consisted of stainless steel plate (Fig. 1(g)). The spin filter was attached to the agitator shaft in the jar fermenter for separation through the spin filter and BC suspension in an annulus part (Fig. 1(b)). The mixing of the culture broth and the suspension of the cells and BC was accomplished by rotation of the impeller in the down parts of the fermenter and the permeation was accomplished through the spin filter in the upper parts of the fermenter.

For selection of proper impeller type for efficient mixing of cells and BC in the jar fermenter, acrylamide and acrylic acid copolymer beads (2 mm diameter) were used as model tracer because of their easy detection with the naked eye. The various impellers that were

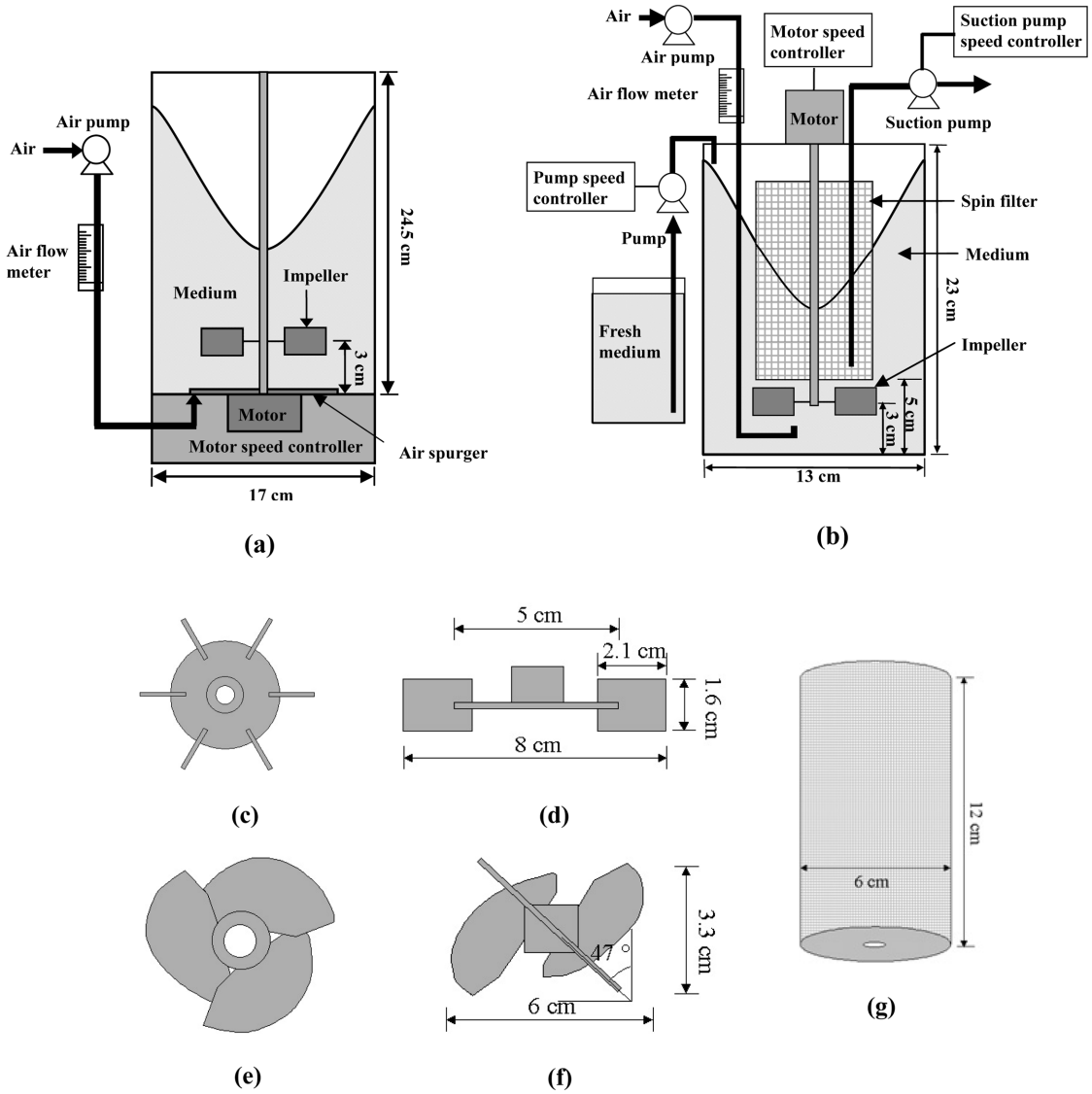

Fig. 1. A schematic diagram of (a) a 5 L jar fermenter and (b) a 2 L jar fermenter equipped with a spin filter. (c) and (d) are a top and side view of the 6 flat-blade turbine impeller, respectively. (e) and (f) are a top and side view of the 3 pitched-blade impeller, respectively. (g) is a side view of spin filter with pore size of 38 μm.

tested for suspension of these beads included a 6 flat-blade (Fig. 1(c), 1(d)) and 3 pitched-blade (Fig. 1(e), 1(f)) turbine impellers. The trace of solid particles was measured by a digital camera (Olympus Co., frame rate: 15 frames/sec).

3. Analysis of Cells and BC

BC was harvested by centrifuging the culture broth for 20 min at 3,580 g, and washed with distilled water. The process of washing and centrifugation was repeated twice. The BC dry weight, including the microbial cells, was measured after freeze-drying at −50 °C. The BC containing cells were treated with 20 mL of 0.3 M NaOH at 100 °C for 5 min in order to disrupt and dissolve the microbial cells; thereafter, the solution was filtered (pore size: 8 μm) with an aspirator to remove the dissolved materials. The filter cake was rinsed repeatedly with distilled water until the pH of the filtrate became neutral. The BC dry weight, without any microbial cells, was measured after freeze-drying at −50 °C. The dry cell weight was taken to be the difference between the weights of the dried BC containing cells and the dried BC after treatment with NaOH.

4. Detection of *Cel*$^-$ Mutants

The conversion of *Cel*$^+$ cells to *Cel*$^-$ mutants was confirmed by measuring the colony-forming unit (CFU). The culture broth was diluted with saline, spread onto an agar medium, and incubated at 30 °C until colonies formed on the agar plate. *Cel*$^-$ mutants were detected on the plates as smooth-type colonies, while *Cel*$^+$ cells formed mucous and rough-type colonies, as reported previously [10,19].

RESULTS AND DISCUSSION

1. Flow Pattern in a Fermenter Equipped with Spin Filter

It is well known that the increase in the rotation speed of the inner cylinder in an annulus reactor improves flow disturbance and affects the flow field because of the Taylor vortex caused by the centrifugal force. The height of Taylor vortex is inversely proportional to the rotating speed of a central shaft [20]. In spite of the existence of a Taylor vortex in the fermenter equipped with a spin filter, droplets of BC containing cells were settled at the bottom of the annulus because of a gravitational force. Therefore, an impeller was additionally installed in the annulus reactor just below the spin filter in the fermenter to suspend the cells and BC (Fig. 1(b)).

We investigated how effectively the impeller suspends the acrylamide and acrylic acid copolymer beads of 2 mm in diameter, which acted as a tracer instead of the cells and BC because the copolymer beads could be easily detected by naked eyes. It is well known that a 3 pitched-blade impeller installed in a conventional jar fermenter

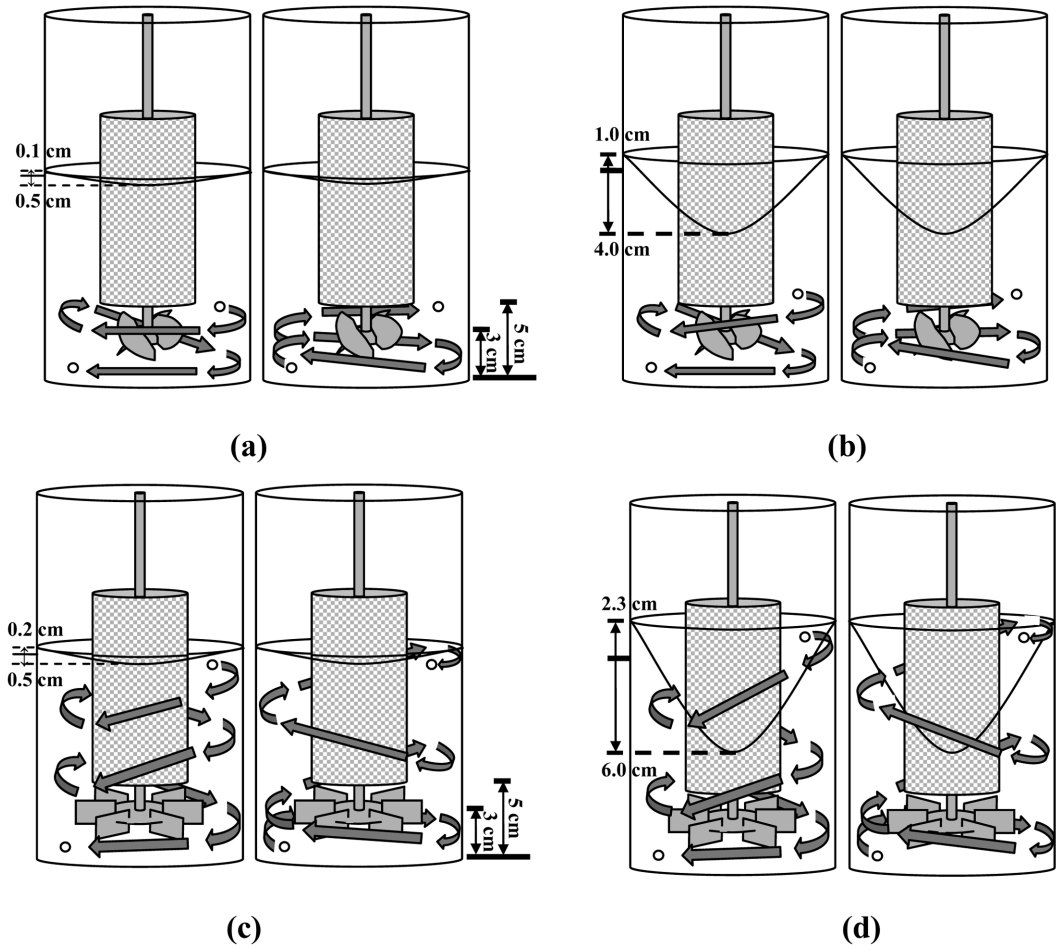

Fig. 2. The trace of copolymer bead in a fermenter equipped with a spin filter and turbine impeller according to the rotating speed of spin filter. Closed circles in each figure indicate the copolymer bead. 3 pitched-blade impeller: 100 rpm (a), 300 rpm (b), 6 flat-blade turbine impeller: 100 rpm (c), 300 rpm (d).

Table 1. Time course of BC production in batch culture and periodical perfusion culture. Cells were cultivated in a 5 L jar fermenter, with a 3 L working volume, for batch culture and 2 L jar fermenter equipped with a spin filter, with a 1.6 L working volume, for periodical perfusion culture. Aeration rate and impeller speed were maintained at 1 vvm and 500 rpm, respectively

Culture time (h)	Without pH adjustment				pH adjustment (pH 5)			
	Batch culture		Periodical perfusion culture		Batch culture		Periodical perfusion culture	
	Dry weight (g/L)		BC	Cells	BC	Cells	BC	Cells
	BC	Cells						
24	0.42	1.83	0.69	2.87	1.10	3.43	1.10	3.50
39	1.29	3.13						
43	1.63	2.97						
44					1.46	3.65		
48	1.50	2.81	1.89	3.69	1.58	3.50	2.00	4.45
51					1.60	3.34		
68			1.91	4.23			2.97	7.24
92			2.11	4.74			3.79	8.61
116			3.28	5.67			4.10	9.04
140			3.07	5.65			4.57	11.52

is suitable for suspension of solid particles and a 6 flat-blade turbine impeller is effective for mixing the fluid. However, in a fermenter equipped with a spin filter, a 3 pitched-blade impeller was not effective for suspension of solid particles, as shown in Fig. 2, which is a sketch according to the trace of solid particles measured by a digital camera. A 3 pitched-blade impeller could not effectively push the fluid forehead because the spin filter located just behind it hindered the inflow of the fluid from the backside. The copolymer particles moved only in the down part of the fermenter even at the pitched-blade impeller speed of 300 rpm. However, the fluid was pushed out in a radial direction by a 6 flat-blade turbine impeller, reached the vessel wall, and then flowed upward along the vessel wall. The copolymer could float in the upper part of the fermenter even at a flat-blade impeller speed of 100 rpm. A 6 flat-blade turbine impeller suspended solid particles more effectively than a 3 pitched impeller; thus, in this study a 6 flat-blade turbine impeller was installed below the spin filter.

2. BC Production in a Jar Fermenter Equipped with a Spin Filter

The impeller equipped in a 2-L jar fermenter without a spin filter rotating at 500 rpm for the cell cultivation for BC production effectively suppressed the occurrence of *Cel*$^-$ mutants [12]. Therefore, BC was produced at the impeller speed of 500 rpm in the jar fermenter equipped with a spin filter.

In a batch cultivation, the pH of the culture broth changed during the cultivation. The pH of the culture broth decreased at the beginning of the cultivation with gluconic acid and acetic acid produced by the catabolization of glucose and ethanol, respectively [21], and then increased with the consumption of these metabolites. However, the production of BC by *G. hansenii* PJK was not considerably affected by maintaining pH of the solution during cultivation in a conventional jar fermenter (Table 1).

In a perfusion cultivation, the filtration rate of the exhausted medium through the stainless steel membrane decreased from 73 mL/min to 28 mL/min for 140 h of cultivation when pH of the solution was not adjusted and varied with culture time (Table 2). At the beginning of cultivation, the pH decreased because of the production

Table 2. Total amount of culture broth removable through the spin filter in periodical perfusion culture

Culture time (h)	Effluent volume of culture broth (mL) for 10 min	
	Without pH adjustment	pH adjustment (pH 5)
24	728	720
48	570	600
56	590	655
68	455	652
80	425	670
92	410	660
104	300	660
116	280	660
Total	3,758	5,227

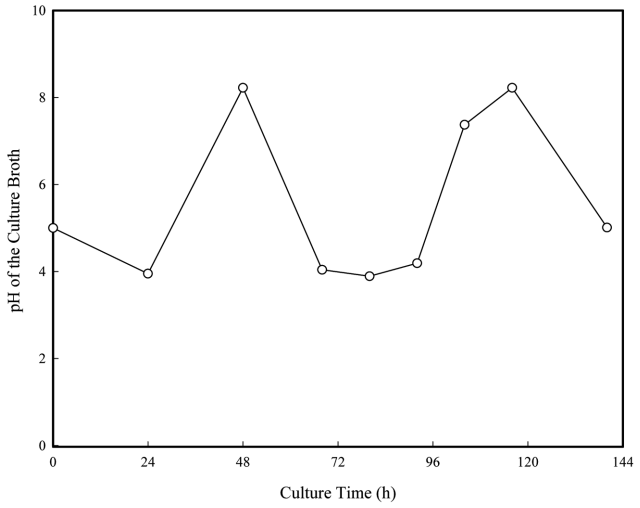

Fig. 3. The variation of pH during BC production using a periodical perfusion culture method without pH control. Cells were cultivated in a 2 L jar fermenter equipped with a spin filter, with a 1.6 L working volume, at aeration rate of 1.0 vvm and impeller speed of 500 rpm.

Table 3. Time course of the conversion of Cel^+ cells into Cel^- mutants in batch culture and periodical perfusion culture. Cells were cultivated in a 5 L jar fermenter, with a 3 L working volume, for batch culture and 2 L jar fermenter equipped with a spin filter, with a 1.6 L working volume, for periodical perfusion. Aeration rate and impeller speed were maintained at 1 vvm and 500 rpm, respectively.

Culture time (h)	Without pH adjustment				pH adjustment (pH 5)			
	Batch culture		Periodical perfusion culture		Batch culture		Periodical perfusion culture	
	CFU/mL	Cel^-/total cells	CFU/mL	Cel^-/total cells	CFU/mL	Cel^-/total cells	CFU/mL	Cel^-/total cells
24	1.65×10^7	0	7.32×10^7	0.029	2.60×10^7	0	1.75×10^7	0.006
39	1.05×10^7	0						
43	5.7×10^6	0						
44					2.40×10^7	0.004		
48	5.51×10^5	0.002	6.0×10^5	0	8.5×10^6	0.012	7.80×10^7	0.012
51					2.47×10^6	0.004		
68			1.21×10^7	0.017			3.20×10^7	0.091
92			1.30×10^6	0.023			4.10×10^7	0.451
116			4.7×10^5	0.021			3.38×10^7	0.680
140			1.07×10^6	0.290			3.32×10^7	0.810

Table 4. Time course of BC productivity in batch culture and periodical perfusion culture. Cells were cultivated in a 5 L jar fermenter, with a 3 L working volume, for batch culture and 2 L jar fermenter equipped with a spin filter, with a 1.6 L working volume, for periodical perfusion culture. Aeration rate and impeller speed were maintained at 1 vvm and 500 rpm, respectively

Culture time (h)	Productivity (g/L·h)			
	Without pH adjustment		pH adjustment (pH 5)	
	Batch culture	Periodical perfusion culture	Batch culture	Periodical perfusion culture
24	0.018	0.031	0.046	0.046
39	0.033			
43	0.038			
44			0.033	
48	0.031	0.039	0.031	0.042
51			0.031	
68		0.028		0.044
92		0.023		0.041
116		0.028		0.035
140		0.022		0.033

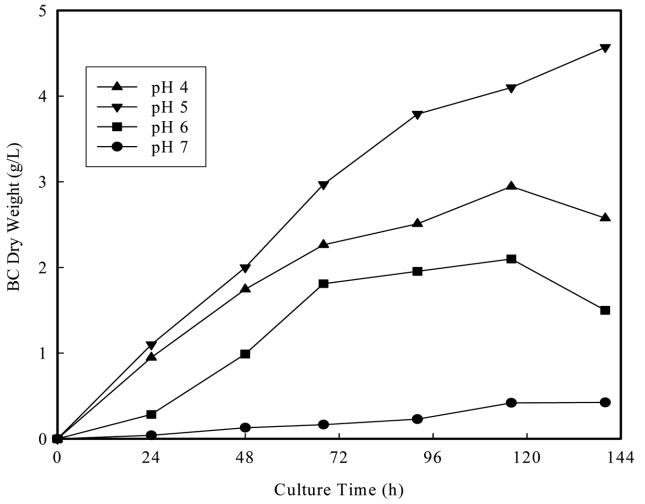

Fig. 4. Time course of BC production in a periodical perfusion culture at various pH values. Cells were cultivated in a 2 L jar fermenter equipped with a spin filter, with a 1.6 L working volume, aeration rate of 1.0 vvm and impeller speed of 500 rpm.

of gluconic acid and acetic acid, thereafter increasing to pH 8 with culture time as the microbial cells consumed these organic acids (Fig. 3). This pH pattern cycled with the renewal of the culture broth, as shown in Fig. 3. The total cell mass and the produced BC increased with culture time and reached 5.65 g/L and 3.07 g/L, respectively, at 140 h of cultivation (Table 1). Except at the beginning of cultivation, the order of magnitude of the live-cell population in a culture medium without pH control was 10^5 or 10^6 CFU/mL, which was almost equal to that in a conventional jar fermenter without a spin filter (Table 3). The live-cell population in the perfusion reactor was nearly constant during cultivation, although the total dry cell weight increased with culture time. This means that the cell growth rate was almost equal to the death rate even though the cells and BC were effectively filtered through the immersed porous stainless steel tube. This small live-cell population led to small BC production rate. As shown in Table 4, the productivity increased with culture time and reached 0.039 g/L·h at 48 h of cultivation, which was nearly the same as that obtained at 43 h of cultivation in a conventional jar fermenter (Table 4). Thereafter, the productivity decreased and reached 0.022 g/L·h at 140 h of cultivation, which may be due to the generation of Cel^- mutants with the culture time and its ratio to the total cells reached to 0.29 at 140 h of cultivation, although the live-cell concentration was nearly constant during cultivation (Table 3).

3. Increase in BC Production with pH Adjustment

The BC production was checked in a fermenter equipped with a spin filter at pH values adjusted to 4, 5, 6 and 7. Total cell densities and BC are shown in Figs. 4 and 5. The pH 5 was found to be optimum for the maximum production of BC. At pH 5, the filtration

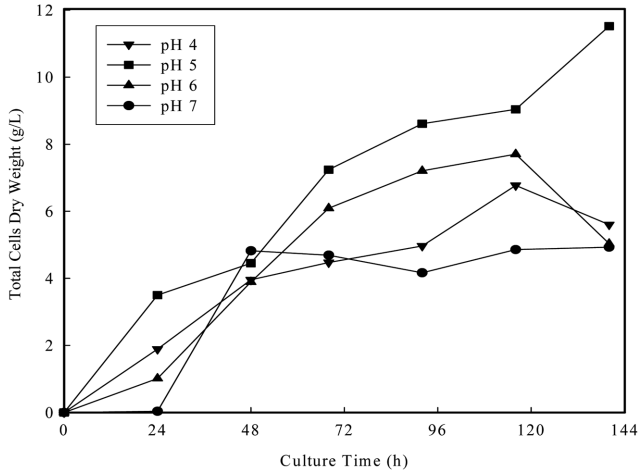

Fig. 5. The variation of total cells density during BC production using a periodical perfusion culture method at various pH values in 2-L Jar fermenter equipped with a spin filter, with a 1.6 L working volume, aeration rate of 1.0 vvm and impeller speed of 500 rpm.

rate of the exhausted medium through the stainless steel membrane was maintained constant at about 66 mL/min for 140 h of cultivation (Table 2), which was more than twice as high as that obtainable without pH adjustment. Large amount of water soluble polysaccharide produced in the perfusion reactor may prevent the fouling of the spin filter, as reported elsewhere [22,23], and the soluble polysaccharides hindered coagulation or formation of clumps of BC during cultivation. In our previous cultivation using an airlift reactor, the BC droplets attached to the edge of the draft glass tube and grew during cultivation without pH adjustment (data not shown).

The pH adjustment to 5 increased the viable cell concentration to a magnitude of 10^7 CFU/mL, which was a little higher than that obtained by pH adjustment in a conventional jar fermenter (Table 3). The high mass of viable cells produced a large amount of BC during cultivation. The cell concentration in a jar fermenter increased with culture time and reached 11.52 g dry cells/L at 140 h of culture, although the maximum cell concentration in a jar fermenter without a spin filter was 3.65 g dry cell/L at 44 h of culture (Table 1). The concentration of the BC increased and reached 4.57 g dry BC/L at 140 h of culture, which is 2.9 times greater than that obtained at 51 h of culture in a conventional jar fermenter without a spin filter. As shown in Table 4, the productivity of BC in a jar fermenter equipped with a spin filter was 0.044 g dry BC/L·h at 68 h of cultivation and was greater than 0.031 g dry BC/L h obtained at 51 h in a conventional jar fermenter. However, the productivity of BC in a jar fermenter equipped with a spin filter decreased with culture time and reached 0.033 g dry BC/L·h at 140 h even though the cell density in a jar fermenter increased with culture time.

We investigated the population of the *Cel*$^-$ mutants in a jar fermenter with the culture time because the decrease in BC production was caused by the conversion of *Cel*$^+$ cells into *Cel*$^-$ mutants in a shear stress field [8]. It is known from the literature that the conversion of *Cel*$^+$ cells of *Acetobacter xylinum* into *Cel*$^-$ mutants in the shear stress field is related to the transposon of the insertion sequence element. Compared to *Cel*$^+$ cells, *Cel*$^-$ mutants of this strain possess two or more IS1031 elements, and their DNA is rearranged within the IS elements [24]. A comparison of the protein profiles of *Cel*$^+$ cells and *Cel*$^-$ mutants of *A. xylinum* revealed that *Cel*$^-$ cells lack the ability of synthesizing two key enzymes involved in cellulose biosynthesis: phosphoglucomutase and glucose-1-phosphate uridylyltransferase. Moreover, the only difference between the *Cel*$^-$ and *Cel*$^+$ DNA fragments is the deletion of one T residue in the *Cel*$^-$ fragment [25]. As shown in Table 3, the population ratio of *Cel*$^-$ mutants to total cells obtained by measuring the CFU of cells from the culture broth increased with culture time, from 0.012 at 48 h of culture to 0.81 at 140 h. In our previous study [12], we determined that a higher rotating speed of the impeller was required at a higher BC concentration in a conventional jar fermenter to prevent the growth of *Cel*$^-$ mutants and maintain a high population ratio of *Cel*$^+$ cells to total cells. Because the high density of BC after 68 h of cultivation hindered the high stress generated at the impeller tip, the fraction of *Cel*$^-$ mutants increased with culture time, reaching 0.451 at 92 h of cultivation and 0.810 at 140 h. In order to increase the productivity at the later period of culture, it is necessary to increase the rotating speed of the impeller with the culture time.

CONCLUSIONS

We developed an innovative fermentation system using a spin filter, which was able to produce high cell density in the bioreactor and consequently improved the BC production significantly as compared to the conventional fermenter. The pH 5 was optimum for the maximum production of BC. Although, we were able to enhance BC production to 4.57 g/L at 140 h of cultivation, which was 2.9 times higher than that obtained in a conventional jar fermenter but at 92 h and onward a large number of *Cel*$^+$ cells were converted into *Cel*$^-$ mutants. If we can control this conversion, we have a fair chance of further improving the BC production, which is our next research target.

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REFERENCES

1. D. P. Delmer and Y. Amor, *Plant Cell*, **7**, 987 (1995).
2. S. Yamanaka, K. Watanabe, N. Kitamura, M. Iguchi, S. Mitsuhashi, Y. Nishi and M. Uryu, *J. Mater. Sci.*, **24**, 3141 (1989).
3. R. E. Cannon and S. M. Anderson, *Crit. Rev. Microbiol.*, **17**, 435 (1991).
4. M. E. Embuscado, J. S. Marks and J. N. BeMiller, *Food Hydrocolloids*, **8**, 419 (1994).
5. D. Klemm, D. Schumann, U. Udhard and S. Marsch, *Prog. Polym. Sci.*, **26**, 1561 (2001).
6. J. D. Fontana, A. M. De Souza, C. K. Fontana, I. L. Torriani, J. C. Moreschi, B. J. Gallotti, S. J. De Souza, G. P. Narcisco, J. A. Bichara and L. F. X. Farah, *Appl. Biochem. Biotechnol.*, **24/25**, 253 (1990).
7. E. J. Vandamme, S. De Baets, A. Vanbaelen, K. Joris and P. De Wulf, *Polym. Degrad. Stabil.*, **59**, 93 (1998).
8. M. Schramm and S. Hestrin, *J. Gen. Microbiol.*, **11**, 123 (1954).
9. J. K. Park, Y. H. Park and J. Y. Jung, *Biotechnol. Bioprocess Eng.*,

8, 83 (2003).
10. J. K. Park, J. Y. Jung and Y. H. Park, *Biotechnol. Lett.*, **25**, 2055 (2003).
11. J. K. Park, S. H. Hyun and J. Y. Jung, *Biotechnol. Bioprocess Eng.*, **9**, 383 (2004).
12. J. Y. Jung, J. K. Park and H. N. Chang, *Enzyme Microb. Technol.*, **37**, 347 (2005).
13. D. Herbert, in *Continuous culture of microorganisms*, Monograph No. 12, Society of Chemical Industries, London (1961).
14. P. L. Rogers, K. J. Lee and D. E. Tribe, *Process Biochem.*, **15**, 7 (1980).
15. M. Cheryan and M. A. Mehaia, *Process Biochem.*, **19**, 204 (1984).
16. W. G. Shim and H. Moon, *Korean J. Chem. Eng.*, **20**, 1054 (2003).
17. D. J. Kim, D. H. Ahn and D. I. Lee, *Korean J. Chem. Eng.*, **22**, 85 (2005).
18. A. Margaritis and C. R. Wilke, *Biotechnol. Bioeng.*, **20**, 727 (1978).
19. S. Valla and J. Kjosbakken, *J. Gen. Microbiol.*, **128**, 1401 (1981).
20. D. R. Gabe, *J. Appl. Electrochem.*, **13**, 3 (1983).
21. W. J. Du Toit and I. S. Pretorius, *Ann. Microbiol.*, **52**, 155 (2002).
22. Y. Chao, M. Mitarai, Y. Sugano and M. Shoda, *Biotechnol. Prog.*, **17**, 781 (2001).
23. T. Ishida, M. Mitarai, Y. Sugano and M. Shoda, *Biotechnol. Bioeng.*, **83**, 474 (2003).
24. D. H. Coucheron, *J. Bacteriol.*, **173**, 5723 (1991).
25. A. Krystynowicz, M. Koziołkiewicz, A. Wiktorowska-Jezierska, S. Bielecki, E. Klemenska, A. Masny and A. Plucienniczak, *Acta Biochim. Pol.*, **52**, 691 (2005).