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Pilot-scale production of bacterial cellulose by a spherical type bubble column bioreactor using saccharified food wastes

Hyo-Jeong Song*, Hongxian Li, Jin-Ho Seo*, Myong-Jun Kim**, and Seong-Jun Kim**†**

*Department of Urban Engineering, Chonnam National University, Daejeon 305-764, Korea
**Department of Civil, Earth and Environmental Engineering, Chonnam National University, Gwangju 500-757, Korea
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Abstract−Bacterial cellulose (BC) was produced by *Acetobacter xylinum* KJ1 in a modified airlift-type bubble column bioreactor, which had a low shear stress and high oxygen transfer rate (k_La). Saccharified food wastes (SFW) were used as the BC production medium due to its low cost. An aeration rate of 1.2 vvm (6 L/min) was tentatively determined as the optimal aeration condition in a 10 L spherical type bubble column bioreactor, by analysis of the oxygen transfer coefficient. When 0.4% agar was added, the BC production reached 5.8 g/L, compared with 5.0 g/L in the culture without the addition of agar. The BC productivity was improved by 10% in the addition of 0.4% agar into the SFW medium. Then, by conversion of a linear velocity of 0.93 cm/sec, from the relationship between the linear velocity and oxygen transfer rate, 1.0 vvm (30 L/min) was determined as an optimal aeration condition in a 50 L spherical type bubble column reactor. Using SFW medium, with the addition of 0.4% agar, and air supplied of 1.0 vvm, 5.6 g/L BC was produced in the 50 L spherical type bubble column bioreactor after 3 days of cultivation, which was similar to that produced in the 10 L bioreactor. In conclusion, the addition of agar, a viscous polysaccharide, into SFW medium is effective for the production of BC, and this scale-up method is very useful for the mass production in a 50 L spherical type bubble column bioreactor by decreasing the shear stress and increasing the k_La.

Key words: Scale-up, Bacterial Cellulose, Saccharified Food Wastes, Spherical Type Bubble Column Bioreactor

INTRODUCTION

Plants produce cellulose as impure compounds of lignin, pectin and hemicellulose, while its pure form is produced by bacterium [1,2]. The diameter of bacterial cellulose (BC) microfibrils are about 20-50 nm, which form a three-dimensional structural matrix due to hydrogen bonding.

Moreover, BC has unique physical properties, including high mechanical strength (high tensile strength and Young's modulus), a high moisturizing nature, high crystallinity and biodegradability. Because of these unique properties, BC can be used as high performance speaker diaphragms, high-grade paper, make up pads, artificial skin and diet-food [3-5]. The most important property of BC is its excellent biodegradability. BC is a proper high-molecular material with respect to global environmental preservation, as it is entirely biodegradable in soil within one month. For that reason, once low-cost mass production of BC has become possible, this eco-friendly high-molecular material will have infinite feasibility and diversity for development, so much research has been undertaken to this end [6]. However, the various functionalities of BC are still not sufficiently understood, as a culture technology for the mass production of BC has not been established. Usually, BC is produced as the pellicle type on the surface of the culture under static culture conditions. However, the traditional static culture method cannot be applied to mass production, as it requires a long culture period and is labor intensive, resulting in low productivity, and therefore is not effective for industrial production [7]. Also, the production of BC by the *Acetobacter* strain requires low shear stress and a high oxygen transfer rate. The culture then has to be agitated to increase the mass transfer rate, resulting in the production of a non-cellulose mutant, with the subsequent decrease in the BC yield [8]. Conversely, in an air-lift bioreactor, a BC lump will accumulate at the culture circulation site, so this method is also not efficient. A complex medium has also been used as a commercial BC production medium; therefore, the high cost is a major defect. In this research, saccharified food wastes were used as the BC production medium, due to its low cost for mass production, with a spherical type bubble column bioreactor (modified airlift-type reactor), having low shear and high oxygen transfer rates, which was designed for the cultivation of BC with an improved yield and reduced *cel*$^-$ mutant bacteria, a non-cellulose producing strain, in a shaking culture. The optimum conditions for the production of BC were then examined by scaling-up from a 10 L to a 50 L spherical type bubble column bioreactor, via analysis of the changes in the k_La, according to the aeration rates, and the relationship between the viscosity, BC productivity and k_La.

MATERIALS AND METHODS

1. Cellulolytic Enzyme Production for Saccharification of Food Wastes

Trichoderma inhamatum KSJ1, a cellulolytic enzyme producer, which was originally isolated from rotten wood by Kim et al. [9], was pre-cultured in 100 mL of YMEB (yeast extract 4 g/L, malt extract 10 g/L, glucose 4 g/L) medium for 3 days at 30 °C, with agitation at 120 rpm. The production of the cellulolytic enzyme was performed in a 10 L jar fermentor (BioG, Hanil R&G Co., Korea),

†To whom correspondence should be addressed.
E-mail: seongjun@jnu.ac.kr

with 5 L of Mandel's medium [10] in which Avicel and CMC as the carbon sources were substituted with 1% rice straw and paper wastes. 2% of the pre-incubated culture was inoculated and cultivated for 4 days at 30 °C, with agitation and aeration rates of 200 rpm and 0.6 vvm (volume of air added to liquid volume per minute), respectively. The pH was uncontrolled during the fermentation, and the culture broth was used as the saccharifying enzyme. Yeast extract and malt extract were purchased from Merck, Germany, and CSL was purchased from Sigma, Germany. D-glucose and agar were purchased from Junsei Co., Japan.

2. Enzymatic Saccharification of Food Wastes

Enzymatic hydrolysis was performed in a 30 L fermentor, with a 15 L working volume, by reaction of 1.5 L of cellulolytic enzyme culture and 3.5 kg of wet state crushed food wastes, with saccharification reaction conditions of 50 °C, agitation rate at 150 rpm and reaction time of 10 hours [9]. Food wastes were obtained from a student cafeteria in Chonnam National University. Compositions of the food wastes used in this experiment were 2.18% nitrogen, 44.3% carbon and 6.81% hydrogen, by an elementary analysis by elemental analyzer (Vario EL, Germany) at the Daegu Center of the Korea Basic Science Institute. After enzymatic saccharification, reduced sugar concentration of the saccharogenic liquid was 100 g/L.

3. BC Producer and BC Production Medium

Acetobacter xylinum KJ1, which was isolated from rotten grapes by Son [11], was used as a BC producer. The supernatant of the saccharified food wastes was used as the BC production medium, with HS (Hestrin & Schramm) complex [12] and CSL-Fru (Corn steep liquor-fructose) media [7] used as the control; the HS medium was used during the early research on the production of BC, with CSL-Fru medium, recently developed at the Shoda laboratory at the Tokyo Institute of Technology, Japan, used as the optimized complex medium.

4. BC Culture Methods

4-1. 500 mL Flask Static Culture

Acetobacter xylinum KJ1 was inoculated by using a loop, and then static cultured at 30 °C for 36 hours in a 500 mL flask, containing 100 mL of HS medium, saccharified food wastes and CSL-Fru medium, with 4% of the preculture then inoculated into the same medium. The BC was produced in a flask containing the same medium, using a static culture, by inoculating 4% of the culture broth that had been homogenized at 10,000 rpm for 1 min. The BC production conditions were adjusted to 30 °C and an initial pH of 5.25, with an incubation period of 3 days.

4-2. 10 L Spherical Type Bubble Column Bioreactor

The 10 L spherical type bubble column bioreactor used in this research was constructed of glass. Two-hundred milliliters of precultured solution, cultivated by using the static culture method in a 500 mL flask, was homogenized at 10,000 rpm for 1 min to crush the BC, and was then inoculated into the 10 L spherical type bubble column bioreactor for the production of BC. 5 L of HS, saccharified food wastes or CSL-Fru medium was used for the production of BC, with a culture controlled at an aeration rate and temperature of 1.2 vvm (6 L/min) and 30 °C, respectively, and cultivated for 3 days.

4-3. 50 L Spherical Type Bubble Column Bioreactor

A modified airlift-type bubble column bioreactor of 50 L total volume with working volume of 30 L was designed and constructed for the large scaled BC production as shown in Fig. 1. The vessel size of the bioreactor is follows: an upper spherical diameter of 460 mm, lower cylinder diameter of 121 mm, and vessel height of 605 mm. The bioreactor showed rheological characteristics minimizing the shear stress and increasing the oxygen transfer rate.

Six-hundred milliliters of statically cultured preculture was smashed by using a homogenizer (Nissei A-7, Japan), at 10,000 rpm for 1 min, and then inoculated into 30 L of saccharified food wastes for the production of BC. The cultivation conditions were the same as in the 10 L spherical type bubble column bioreactor.

5. Measurement of Oxygen Transfer Coefficient (k_La) and Mixing Time

The oxygen transfer coefficient was measured by static gassing out method [13] using distilled water and saccharified food wastes medium in 10 L and 50 L spherical type bubble column bioreactors. Initially, the gas in each fluid was substituted with nitrogen until the DO (dissolved oxygen) concentration was confirmed as zero, with aeration then initiated and the change in the DO monitored over time. The k_La values were calculated by using following equation.

$$k_La\ (hr^{-1}) = [\ln(C_{l1} - C_{l2})]/(t_2 - t_1) \times 60$$

C_{l1} : DO concentration of culture liquid according to time at 30 °C (mg/L)
C_{l2} : Saturated DO concentration at 30 °C (mg/L)
t_1 : Initial time when DO measurement
t_2 : Time when DO concentration increased to 100%

The mixing time was determined by measuring 95% of pH change time using 4 N H_2SO_4 as a tracer.

6. Analysis of the Optimal Aeration Rate

The 10 L spherical type bubble column bioreactor was assumed to have a cylindrical shape, with an optimum aeration condition of 1.2 vvm and linear velocity. From a scale-up analysis using the same linear velocity value as in the 50 L spherical type bubble column bioreactor, the optimum aeration condition was tentatively assumed to be 0.6 vvm when a working volume of 30 L was considered. However, oxygen defect occurs due to this relatively low oxygen transfer rate; therefore, the scale-up was performed with the same k_La values for the two reactors. Therefore, the relationship between linear velocity to the k_La value was examined in 10 (working volume of 5 L) and 50 L (working volume of 30 L) reactors using distilled water and SFW medium. The k_La value at 1.2 vvm was 12.1 hr^{-1} in the 10 L spherical type bubble column bioreactor. From the relationship between the linear velocity and k_La in 50 L, the linear velocity obtained with a k_La of 12.1 h^{-1} was calculated. The linear velocity (assuming a cylindrical shape) at a constant k_La was converted using the following equation:

$$v_s = \frac{Q}{\pi D^2/4}$$

7. Measurement of BC Concentration and Yield

BC culture was initially filtered by using a 50 μm sieve and centrifuged at 7,000 g for 20 min. Then, 0.1 M NaOH was added, the culture incubated at 80 °C for 20 min to lyse the bacteria and then washed several times with distilled water, with the dry BC weight measured after vacuum drying at 80 °C for 8 hours. The productivity of BC was calculated as the ratio of the BC concentration to that

of the reducing sugar consumed.

8. Measurement of Residual Reducing Sugar Concentration

The concentration of reduced sugar, supplied as the carbon source, in the medium was measured by the DNS [14] method. Three milliliter of DNS solution was added to 1 mL of culture broth, incubated at 100 °C for 5 min and diluted with 20 mL of distilled water. The optical density was analyzed at 540 nm with a UV-Spectrophotometer (TU1800PC, Human Co., Korea). A calibration curve was constructed with 2% glucose (Junsei Co, Japan) as the standard material.

9. Measurement of Viscosity

The viscosity of each culture with respect to incubation time was measured with a viscometer (Visco basic plus L, Fungilab S. A., Spain), with 45 mL of the culture broth employing a TL5 spindle at 100 rpm.

RESULTS AND DISCUSSION

1. Construction of 50 L Spherical Type Bubble Column Bioreactor

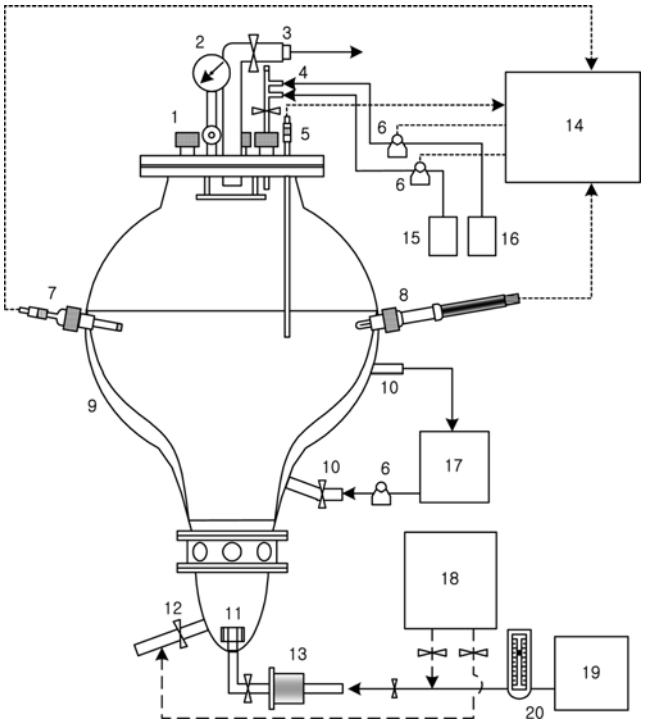

Fig. 1. Apparatus of 50 L spherical type bubble column bioreactor.

1. Nozzle for inoculation
2. Steam manometer
3. Air outflow
4. Nozzle for injection of acid and alkali inlet
5. Thermometer
6. A transfer pump
7. DO electrode
8. pH electrode
9. Water jacket
10. Water jacket inflow/outflow
11. Air inlet
12. Sampling nozzle
13. Air filter
14. Control box
15. Acid storehouse
16. Alkali storehouse
17. A constant-temperature water bath
18. Steam boiler
19. Compressor
20. Air flow meter

The productivity of BC in the 10 L spherical type bubble column bioreactor in this research was similar to that of our previous report [7]. Therefore, the mass production of BC was considered successful. Therefore, a 50 L spherical type bubble column bioreactor was constructed using the same basic configuration as for the 10 L spherical type bubble column bioreactor.

The 50 L spherical type bubble column bioreactor (Fig. 1), a modified airlift-type reactor, was constructed geometrically identical with a 10 L spherical type bubble column bioreactor. In an airlift reactor composed only in cylindrical shape, a BC clump will be accumulated at the culture circulation site; therefore, the airlift reactor was considered inefficient in BC production. However, a spherical type bubble column bioreactor used in this research, which was composed of a round cylindrical shape in the bottom and the upper part, was composed of a spherical main reactor, which has the characteristics of low shear and high oxygen transfer rates. Accordingly, the spherical type bubble column bioreactor will complement generation of *cel*$^-$ mutant, a non-cellulose producing strain, at agitated culture; as a result, the spherical type bubble column bioreactor was considered to be efficient in BC production.

In the 50 L spherical type bubble column bioreactor, a water jacket was attached to the main reactor to control the internal temperature of the reactor, and pH and DO sensors were also attached for pH control and DO monitoring. Also, axenic air was then supplied through an air filter, with sterilization performed by the steam boiler of the reactor.

For the design of the air filter to be used for the aeration, several factors had to be assumed. ① The permissible contamination generation frequency was 1/1,000 (once every thousand times); ② An aeration rate of 60 L/min (two times aeration volume per the working volume of the reactor per minute, 2 vvm); ③ Maximum incubation time of 5 days (120 hours); ④ By using glass fiber as filter material, the optimum aeration linear velocity and K (a constant of Richards's equation) were supposed as 0.15 m/sec and K 1.535 cm^{-1}, respectively. The size of the cylindrical air filter by the relation equation between bacteria removal efficiency and thickness of the filter suggested by Richards [15] was calculated as a thickness of 11.9 and diameter of 9.21 cm.

2. k_La and Mixing Time According to Aeration Rates

2-1. k_La and Mixing Time of 10 L Spherical Type Bubble Column Bioreactor

The k_La measured in 5 L of distilled water and saccharified food wastes at aeration rates of 0.6 vvm (3 L/min), 1.2 vvm (6 L/min) and 1.8 vvm (9 L/min) were 22.5, 29.3 and 39.6 hr^{-1}, respectively, and in 5 L of distilled water and saccharified food wastes. The k_La values were 22.5, 29.3 and 39.6 hr^{-1}, respectively, and 8.9, 12.1 and 17.3 hr^{-1}, respectively, as shown in Table 1. k_La values were higher at in the lower viscosity distilled water than the higher viscosity saccharified food wastes, and increased with increasing aeration rate.

A spherical type bubble column culture requires much oxygen, however, and could possibly have been rate limited by the oxygen supplied at 0.6 vvm, due to the low k_La under this condition. Also, the culture broth possibly outflowed at 1.8 vvm because of strong aeration. Therefore, the optimum aeration condition in the 10 L spherical type bubble column bioreactor was determined to be 1.2 vvm.

Under aeration conditions of 0.6, 1.2 and 1.8 vvm, using 4 N H_2SO_4 as a tracer, the mixing times in distilled water and saccharified food

Table 1. Oxygen transfer coefficient (k_La) and mixing time according to the aeration rates in distilled water and saccharified food wastes media

	Aeration rate	k_La (hr^{-1})		Mixing time (sec)	
		DW	SFW	DW	SFW
Spherical type	0.6 vvm	22.5±2.93	8.9±1.31	26±3.72	36±4.31
10 L bubble column	1.2 vvm	29.3±3.25	12.1±0.81	22±1.60	20±2.08
	1.8 vvm	39.6±1.76	17.3±2.49	10±1.02	14±2.38
Spherical type	0.6 vvm	16.0±0.92	6.0±0.53		
50 L bubble column	1.2 vvm	48.3±4.12	15.0±2.03		
	1.6 vvm	55.8±3.56	19.2±1.42		

*DW (Distilled Water), SFW (Saccharified Food Wastes)
*All the values are averages of triple experiments.

wastes were 26, 22 and 10 sec, respectively, and 36, 20 and 14 sec, respectively. Therefore, the mixing time was decreased with increasing aeration rate. The mixing times in saccharified food wastes were longer than those in distilled water at 0.6 and 1.8 vvm, but similar at 1.2 vvm; therefore, 1.2 vvm was tentatively considered as the optimum aeration condition.

2-2. k_La of 50 L Spherical Type Bubble Column Bioreactor

The k_La determined in tap water and saccharified food wastes in the 50 L spherical type bubble column bioreactor at aeration rates of 0.6 vvm (18 L/min), 1.2 vvm (36 L/min) and 1.6 vvm (48 L/min) were 16.0, 48.3 and 55.8 hr^{-1} and 6.0, 15.0 and 19.2 hr^{-1}, respectively. The k_La value was greatly increased when the aeration rate was shifted from 0.6 to 1.2 vvm. Oxygen defect may have occurred at a k_La of 6.0 hr^{-1} with an aeration rate of 0.6 vvm, so the productivity of BC would not be improved under this condition. Therefore, 1.2 vvm was provisionally determined as an appropriate aeration rate in the 50 L spherical type bubble column bioreactor, as in the 10 L spherical type bubble column bioreactor.

3. Scale-up Analysis of Aeration Rate

3-1. Scale-up to Constant Linear Velocity

The maximum BC productivity was achieved at an aeration rate of 1.2 vvm in the 10 L spherical type bubble column bioreactor; therefore, 1.2 vvm was converted to linear velocity, v_s, with the scaled-up aeration condition determined by analyzing the relation between the linear velocity and oxygen transfer efficient, k_La.

3-2. Linear Velocity of 10 L Spherical Type Bubble Column Bioreactor

When the shape of the reactor was cylindrical with a working volume of 5 L assumed, the diameter of the reaction tank was calculated as 14.9 cm. The linear velocity was calculated to be 0.57 cm/sec by dividing aeration rate, 1.2 vvm (6 L/min) by the cross sectional area.

4. Aeration Rate of 50 L Spherical Type Bubble Column Bioreactor

When the diameter, height and linear velocity of the reactor were 25.9 and 57 cm, and 0.57 cm/sec, respectively, then the aeration rate was calculated to be 18 L/min. The scaled-up reactor, with a 30 L working volume, was operated at an aeration rate of 0.6 vvm. Under this condition, oxygen deficiency occurred due to the low oxygen transfer rate; therefore, the scale-up was performed taking into account the k_La value.

5. k_La in Large Scale Bioreactors

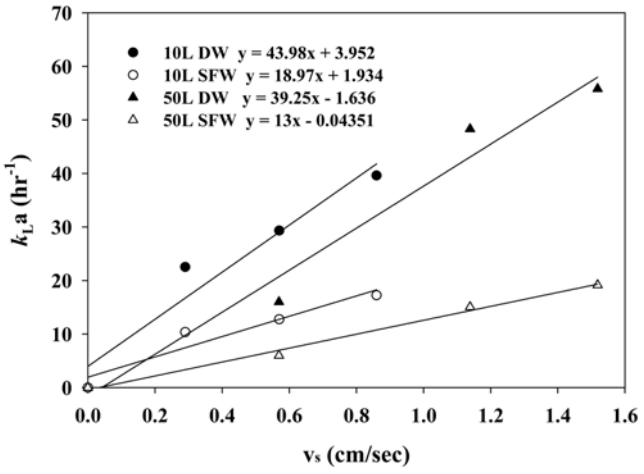

Fig. 2. Relationship between linear velocity and k_La in 10 L and 50 L spherical type bubble column reactors. DW (Distilled Water), SFW (Saccharified Food Wastes).

The relationship between the linear velocity and k_La was investigated by using DW and SFW media in both the 10 and 50 L reactors. The k_La value was 12.1 hr^{-1} at 1.2 vvm in the 10 L spherical type bubble column bioreactor, as shown in Table 1. The relationship of the linear velocity to the k_La value in the SFW medium is shown in Fig. 2. Linear curve fitting to various linear velocities was employed to ease scale-up calculation in the range of tentative optimum aeration rate. The linear velocity calculated for the 50 L reactor by using the same k_La value, 12.1 hr^{-1}, was 0.93 cm/sec.

As mentioned above, if the linear velocity (0.93 cm/sec) was converted to the aeration rate for the same k_La value, an aeration rate of approximately 30 L/min was obtained.

6. Influence of k_La and Viscosity on BC Production

In this research, 5.7 g/L of BC was produced in 500 mL flask level. Generally, in static culture of the BC production, BC was formed in thin pellicles at the surface of the culture liquid, so static culture is not an efficient method because of low productivity and long culture period for applying to mass production in industry. Therefore, the BC productivity in the static culture was used as control when productivities in the 10 L and 50 L spherical type bubble column bioreactor were compared.

A BC producer is sensitive to shear stress; therefore, decreasing

the shear rate during the production of BC is a key factor. Consequently, much research has been performed with the addition of polysaccharides, such as agar and acetan, to decrease the shear rate and improve the BC productivity. Therefore, in this research, agar was added in the SFW medium to decrease shear rate, and influence of the agar concentration was examined. As a result, maximum BC productivity was shown when 0.4% of agar was added to SFW medium in flask level.

In this study, 5.0 g/L of BC was obtained by using saccharified food wastes, without any other additives, in a 10 L spherical type bubble column bioreactor, but 5.8 g/L of BC was produced by the addition of 0.4% agar. As a result, the BC productivity was improved by 10% on the addition of agar (Fig. 3).

No significant difference was seen in the BC productivity between the saccharified food wastes (5.0 g/L) and CSL-Fru media (5.6 g/L), even though they showed similar k_La values. The BC productivity in HS medium was only 0.87 g/L, indicating a high k_La value, 27.0 hr^{-1}, and low viscosity. Also, on the addition of agar to the saccharified food wastes medium (5.8 g/L), even the k_La value decreased to 10.7 hr^{-1}, but the viscosity and BC productivity were increased (Table 2). It could be supposed that the BC productivity was increased due to the decreasing conversion rate of the *cel*$^-$ mutant by decreasing the shear stress and the prevention of BC coagulation due to the increased viscosity on the addition of a polysaccharides, such as agar [16,17]. As a result, it was concluded that the influence of the viscosity was higher than that of the k_La on the BC productivity.

7. BC Productivity of 50 L Spherical Type Bubble Column Bioreactor

The changes in the reducing sugar concentration and viscosity when air was supplied to the 50 L spherical type bubble column bioreactor are shown in Fig. 3. The viscosity and BC productivity (5.8 g/L) increased on the addition of agar to the saccharified food wastes medium in the 10 L spherical type bubble column bioreactor. As a result, as in the 50 L spherical type bubble column bioreactor, 5.6 g/L of BC was produced on the addition of 0.4% agar to the saccharified food wastes medium at aeration rate of 1.0 vvm, as shown in Fig. 4. Hence, the viscosity change was the same as proportionate to the increase in the BC productivity. On the other hand, because the viscosity change caused by the supernatant of the culture broth did not show a significant difference (data not shown), we concluded that viscosity increase of the BC culture broth was attributed to the increase of apparent viscosity by the produced BC.

From research conducted by the Shoda laboratory, Tokyo Insti-

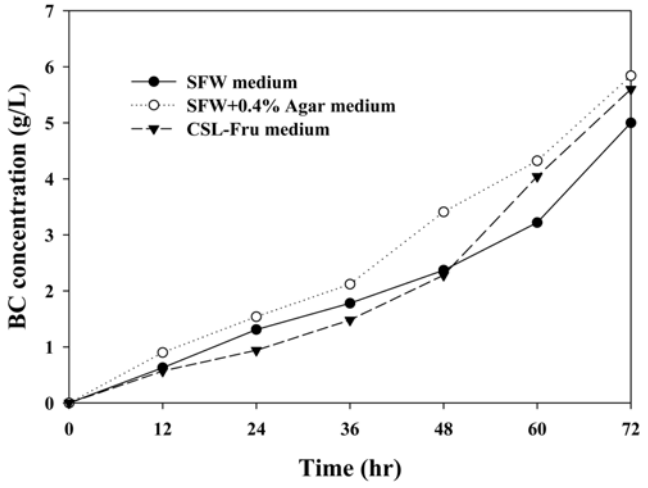

Fig. 3. BC productivity according to cultivation medium in the 10 L spherical type bubble column bioreactor.

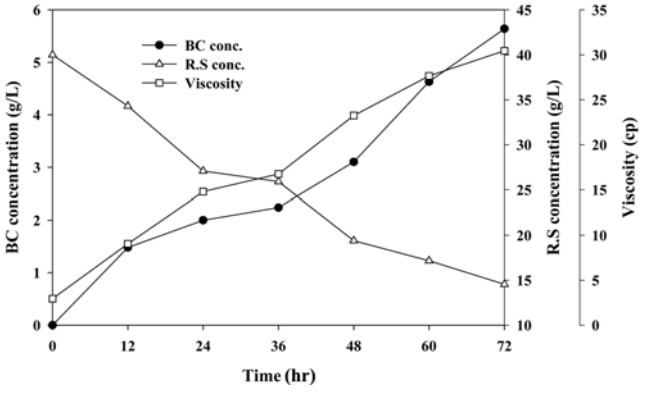

Fig. 4. Variations of viscosity, BC and reducing sugar concentration when cultured in SFW medium containing 0.4% agar in the modified 50 L spherical type bubble column bioreactor.

Table 2. Comparison of the BC productivity and yield according to the cultivation conditions

Cultivation condition		BC productivity (g/L)	BC Yield (g-cellulose/g-R.S consumed)
500 mL flask static	HS medium	2.8±0.22	0.11
	SFW medium	5.7±0.82	0.28
	CSL-Fru medium	5.6±0.71	-
10 L spherical type bubble column bioreactor	HS medium	0.87±0.24	0.04
	SFW medium	5.0±0.63	0.19
	CSL-Fru medium	5.6±0.13	-
	SFW+0.4%Agar medium	5.8±0.86	0.21
10 L spherical type bubble column bioreactor	SFW+0.4%Agar medium	5.6±0.47	0.22

*SFW (Saccharified Food Wastes), R.S (Reducing Sugar)
*All the values are averages of triple experiments.

tute of Technology, Japan, the production of 5.1 g/L of BC was reported by using an optimum complex medium of Corn steep liquor-fructose (CSL-Fru) on the addition of agar to a 67 hour culture in a 50 L Internal-Loop airlift reactor [18]. This result was similar to the BC productivity found in our experiment. However, they encountered difficulty in the production of a high productivity of BC due to the accumulation of a solid BC lump in the cyclic subspace, even though the mass production of BC was attempted with an airlift reactor.

A comparable BC productivity, 5.6 g/L, was obtained in our 50 L reactor compared with the 10 L spherical type bubble column reactor (5.8 g/L); therefore, a spherical type bubble column culture was found to be an effective cultivation method for the mass production of BC.

Accordingly, the addition of a polysaccharide with a high viscosity would be effective for BC production. Therefore, cultivation using a spherical type bubble column bioreactor, as in this research, would be an effective method for the mass production of BC.

CONCLUSIONS

A method for the mass production of BC, using a spherical type bubble column bioreactor modified from an airlift-type reactor BC production system, was developed in this research. A 50 L scaled-up spherical type bubble column bioreactor was constructed to improve the BC productivity, without blockade of a solid BC lump. The optimum aeration rate was determined a 1.2 vvm (6 L/min) by measuring the k_La in a 10 L spherical type bubble column bioreactor, and using the same k_La value, the optimum aeration rate in the 50 L spherical type bubble column bioreactor was determined to be 1.0 vvm (30 L/min). 5.6 g/L of BC was produced using saccharified food wastes on the addition of 0.4% agar to the 50 L scaled-up reactor, which was comparable to the BC productivity, 5.8 g/L, obtained in the 10 L spherical type bubble column bioreactor. Therefore, this spherical type bubble column culture method was considered effective for the production of BC.

In conclusion, the use of low cost food wastes as the medium, with the addition of high viscosity polysaccharides, would be very effective for the mass production of BC. Also, the 50 L spherical type bubble column cultivation, with decreased shear stress and an increased oxygen transfer rate, was considered effective for the mass production of BC.

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