Korean J. Chem. Eng., **28**(12), 2306-2311 (2011)
DOI: 10.1007/s11814-011-0111-8

Determination of the stoichiometry and critical oxygen tension in the production culture of bacterial cellulose using saccharified food wastes

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(*Received 8 December 2010 • accepted 24 April 2011*)

Abstract–The stoichiometry of the entire reaction in a 50 L scaled-up production culture of bacterial cellulose (BC), using saccharified food wastes (SFW), was analyzed in this study. The stoichiometric analysis was carried out using the chemical formula, yield, degrees of reduction of the major components, and the respiratory quotient (RQ). Based on the stoichiometric analysis, the amounts of substrate, oxygen supply and BC production *etc.*, were able to be predicted. In addition, the amount of energy generated in the culture was predicted based on the oxygen consumption via the stoichiometric analysis. The stoichiometry of BC production using SFW in a 50 L large scale reactor will be useful as a standard for mass production of the culture. The stoichiometric analysis can also help the designers of reactors decide on the boiler capacity and oxygen supply for a large scale bioreactor system. The OUR (oxygen uptake rate) of *Acetobacter xylinum* KJ1 in a 12 hour-age cultivation was 0.21 mg DO/L·min, from which the critical DO concentration was suggested to be maintained above 3.10 ppm to prevent oxygen limitation during the BC production culture. The results indicated that pure oxygen should be supplied during the exponential phase, where DO depletion was observed. An ascertainment experiment, with the addition of pure oxygen into the culture system, showed BC production of 7.37 g/L, which was considerable productivity.

Key words: Stoichiometry, OUR, Critical DO, Energy Generation, Bacterial Cellulose, Food Wastes

INTRODUCTION

Plants produce cellulose as impure compounds of lignin, pectin and hemicellulose, while its pure form is produced by bacteria [1]. Conversely, the bacterial cellulose (BC) produced by *Acetobacter xylinum* is a pure cellulose aggregate that does not include any impurities. The diameter of BC microfibrils is about 20-50 nm, which form a three-dimensional structural matrix via hydrogen bonding. Therefore, BC has unique physical properties, including high mechanical strength (high tensile strength and Young's modulus), a high moisturizing nature, high crystallinity and good biodegradability [2]. Because of these unique properties, BC can be used for the manufacture of high-grade paper, make up pads, artificial blood vessels, artificial skin, high performance speaker diaphragms, wound dressings and as a diet-food, *etc.* [3-5].

Three major methods exist for the production of BC: static culture, agitation culture and an internal-loop airlift reactor. Usually, BC is produced as the pellicle type on the culture surface under static culture conditions. However, the traditional static culture method cannot be effectively applied to industrial mass production as it requires a long culture period and intensive labor, resulting in low productivity [6]. In general, almost all BC producers, such as *Acetobacter,* require low shear stress and high oxygen transfer efficiency. However, strong agitation of the culture to increase the oxygen transfer rate also increases the generation of non-cellulose mutants (*cel*$^-$), which decreases the BC yield [7,8]. The same phenomena were also observed in our previous study using *Acetobacter xylinum* KJ1. Our study reported that *cel*$^-$ generation by strong agitating was related to BC hydrolysis by beta glucosidase in cell wall [8].

On the other hand, in Japan, a BC culture using an optimized complex medium, named corn steep liquor-fructose (CSL-Fru) medium, showed productivity of 3.8 g/L with an air supply, but 7.93 g/L on supplementation with pure oxygen gas in a 50 L airlift reactor [9]. However, the production method led to oxygen deficiency in the broth due to stagnation of a BC lump in the low part of the circulation column, indicating this method may be unsuitable for a scaled-up production. A newly modified 50 L bubble column bioreactor, with a low shear stress, even with a considerable oxygen transfer rate (k_La), was developed and used for the production of BC in our previous study [10].

As the *Acetobacter* strain requires oxygen as an essential substrate, the k_La is a restricting factor in the aerobic liquid fermentation using this strain. Increasing the agitation rate is the generally used method for improving oxygen transfer [11]. The formation of high turbulent flow and small bubbles caused by high shear stress can efficiently improve oxygen transfer into the liquid phase. There are also other methods known to improve oxygen transfer, such as increasing the air flow rate or the partial pressure of oxygen [11].

In our previous research [10], SFW medium was used as the BC production medium for the low cost mass production of BC, with large scale production performed using a 50 L modified bubble col-

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umn bioreactor. The aim of this research was to quantitatively evaluate the bioconversion process for the production of BC in the 50 L scale bioreactor using a stoichiometric analysis via the chemical formula yield, and degree of reduction of the major components. Also, analyzing the critical DO concentration in the BC culture can be used to increase the BC production yield by determining the optimum point for pure oxygen supplementation during the phase of oxygen depletion.

MATERIALS AND METHODS

1. Hydrolytic Enzyme Production for the Saccharification of Food Wastes

Trichoderma inhamatum KSJ1, a hydrolytic enzyme producer, was isolated from rotten wood by Kim et al. [12], and pre-cultured in 100 mL of YMEB (Yeast extract 4 g/L, malt extract 10 g/L, glucose 4 g/L) medium for 2 days at 30 °C, with agitation of 120 rpm. The culture for BC production was performed in a 30 L jar fermenter, with 20 L of modified Mandel's medium (rice straw 10 g, paper wastes 10 g, bacto peptone 1.0 g, urea 0.3 g, $CaCl_2$ 0.3 g, KH_2PO 2.0 g, $(NH_4)_2SO_4$ 1.4 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, $FeSO_4 \cdot 7H_2O$ 5.0 mg, $MnSO_4 \cdot H_2O$ 1.6 mg, $ZnSO_4 \cdot 7H_2O$ 1.4 mg and $CoCl_2$ 2.0 mg in 1.0 L of distilled water). 3% of the pre-incubated culture was inoculated and cultivated for 3.5 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, according to the method described in Kim et al. [12]. The amylase and FPase activities of the hydrolytic enzymes obtained in the culture were 3.12 and 0.25 U/mL, respectively. The culture broth was used as the hydrolytic enzymes solution for the saccharifying of food wastes.

2. Enzymatic Saccharification of Food Wastes

The food wastes used in this study were obtained from a student cafeteria at Chonnam National University. The elementary composition of the food wastes was 45.03% carbon, 7.42% hydrogen, 34.57% oxygen, 3.62% nitrogen and 1.95% sulfur, by using an elemental analyzer (EA-1110, Thermo Quest, Italia). 21.3 kg of wet state food wastes (moisture content: 77.5%) were crushed twice using a grinder (WP650A, Wonpool, Korea), and then mixed with 8.7 L (27.1 U as amylase) of hydrolytic enzymes in a 35 L bioreactor. The mixture was then saccharified at 50 °C, with agitation at 150 rpm for 20 hours. The resultant mixture was then centrifuged at 8,000 rpm and 4 °C for 20 min. The concentrations of reducing sugar and total nitrogen (T-N) in the saccharified liquid after centrifugation were 106.1 g/L and 1.77 g/L, respectively. The supernatant was used as the saccharified food wastes (SFW) medium in the BC cultures.

3. Preparation of Hydrolyzed Yeast as a Nitrogen Source

The protease producer for the hydrolysis of yeast, *Bacillus subtilis* (KCCM 40443), was purchased from the Korea culture center of microorganisms, incubated in a nutrient medium (beef extract 3 g/L, bacto peptone 5 g/L, pH 7.0) stocked, with glycerol at a 1 : 1 ratio, and then stored in a −70 °C deep-freezer prior to further use. *B. subtilis* was pre-cultured in 100 mL of SFW medium and incubated at 30 °C for 1.5 days, with agitation at 180 rpm. Then, 200 mL of the pre-cultured *B. subtilis* solution was inoculated into 7 L of SFW medium, with a reducing sugar concentration of 70 g/L, in a 10 L jar fermenter (BioG; Hanil R&D Co., Korea). The culture was performed at 30 °C for 1.5 days, with agitation and aeration at 350 rpm and 0.6 vvm, respectively. As a nitrogen source, yeast ground with a homogenizer (ANM-150, Japan) for 20 min was dried for 24 hours at 80 °C, and the protease of the *B. subtilis* culture was added to 875 g of the ground yeast, and then hydrolyzed at 30 °C for 24 hours, with agitation at 350 rpm. The hydrolyzed yeast was centrifuged at 8,000 rpm for 20 min, and the supernatant was added to the BC producing medium as a nitrogen source. The T-N concentration in the hydrolyzed yeast was 7.82 g/L.

4. Cultivation and Purification of BC

After inoculating the *Acetobactor xylinum* KJ1 into 100 mL of SFW (reducing sugar concentration of 70 g/L, pH 5.25) in a 500 mL flask, the static culture was conducted at 30 °C for 36 hours. The cultured solution was homogenized for 1 min using a homogenizer (Nissei, A-7, Japan) at 10,000 rpm. 4% of the homogenized solution was inoculated into 100 mL of SFW medium (pH 5.25) in 500 mL flask and then statically pre-cultured at 30 °C for 36 hours. 1 L of the pre-cultured KJ1 solution was inoculated into 25 L of SFW medium (reducing sugar concentration of 70 g/L, agar 0.4%, pH 5.25) in a 50 L modified bubble column bioreactor [10], and cultured for 3 days at 30 °C, with aeration at 1.0 vvm (25 L/min).

For purification of the BC from the culture, the BC culture was firstly filtered using a 50 μm sieve and centrifuged at 8,000 rpm for 20 min. Then, the BC was mixed with 0.1 M NaOH and incubated at 80 °C for 20 min to lyse the bacteria contained in the BC, and then washed 3 times with distilled water. Finally, the amount of BC was weighed after vacuum drying at 80 °C for 8 hours.

5. Measurement of Reducing Sugar Concentration

The concentration of reducing sugars contained as carbohydrate in the medium was measured by using the DNS method [13]. 3 mL of DNS solution was added to 1 mL of culture broth and incubated at 100 °C for 5 min, and then diluted with 20 mL of distilled water. The concentration was determined by measuring the optical density at 540 nm with a UV-Spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd., Korea). A calibration curve was prepared with glucose (Junsei Co., Japan) as a standard reducing sugar.

6. Measurement of the Dry Cell Concentration

To calculate the Q_{O2} (specific oxygen uptake rate) of the BC producing strain, the dry cell weight (DCW) was measured. The cell concentration was determined by measuring the optical density of the solution at 600 nm (OD_{600}) [14]. The OD_{600} of the broth from a 12 hour cultivation was measured after treatment for 2 h with 2% cellulase (Celluclast; Novo Nordisk A/S, Denmark), at pH 5.0 and 50 °C. The DCW of the culture broth was determined from the following experimental correlation between the OD_{600} and DCW (g dry cell·L^{-1}):

$$\text{DCW (g/L)} = \text{OD (600 nm)} \times 0.33 \quad (1)$$

7. Measurement of O_2 Concentration in the Exhaust Gas

The oxygen concentration in the exhaust gas was measured over incubation time by using an oxygen meter (XP-3180, NEW COSMOS, Japan) during the production of BC.

RESULTS AND DISCUSSION

1. Elemental Analysis of the Major Components in the Culture Broth

After the production of BC in the 50 L modified bubble column

Table 1. The formulas of the major components, and the related values of the parameters used in the stoichiometric analysis

	Elemental compositions				Formula	Degree of reduction (γ)	Yield (P/S)
	C (%)	H (%)	O (%)	N (%)			
SFW medium	41.68	6.73	40.39	1.65	$CH_{1.94}O_{0.73}N_{0.03}$	4.38	-
A. xylinum KJ1	59.16	4.92	27.49	4.54	$CH_{1.00}O_{0.35}N_{0.07}$	4.10	-
Bacterial cellulose	44.09	3.97	46.51	1.92	$CH_{1.08}O_{0.79}$	3.50	0.12
Culture remainder	38.96	6.47	40.86	1.56	$CH_{1.99}O_{0.8}N_{0.03}$	4.32	0.45
RQ(Respiratory Quotient, $(CO_2/O_2)=1$; $C_6H_{12}O_6$(Glucose)+$6O_2 \rightarrow 6CO_2+6H_2O$							

bioreactor, the elemental compositions of the SFW medium, *A. xylinum* KJ1, BC and integrated components of the culture-remainder as products were analyzed with an element analyzer, after drying at 80 °C. According to the results of the elemental analysis, the chemical formulas of the SFW medium, *A. xylinum* KJ1, BC, and culture remainder were expressed by using four elements: C, H, O and N, but with the exclusion of S, as shown in Table 1. The chemical formula of the BC was expressed using three elements: C, H and O, because the unit chain of BC was composed of a β-1,4-glucosidic linkage with β-D-glucose, although nitrogen was present in the elemental composition of the BC, which may have originated from the incomplete purification during the washing step.

2. Determination of Stoichiometric Coefficients

The bioconversion formula for the production of BC using the SFW medium can be expressed as shown in Eq. (2). The SFW medium was used as the substrate, and the products are expressed as two components, BC (product 1) and integrated components of the culture-remainder in the supernatant (product 2).

$$\begin{aligned} &CH_{1.94}O_{0.73}N_{0.03}\ (\text{Substrate, SFW})+a\ O_2 \\ &\quad \rightarrow b\ CH_{1.00}O_{0.35}N_{0.07}\ (\text{cell})+c\ CH_{1.08}O_{0.79}\ (\text{Product 1, BC}) \\ &\quad +d\ CH_{1.99}O_{0.79}N_{0.03}\ (\text{Product 2, culture-remainder}) \\ &\quad +e\ H_2O+f\ CO_2 \end{aligned} \tag{2}$$

Various parameters related to the stoichiometric analysis are described in Table 1. The degree of reduction (Y) is defined as the equivalent numbers of available electrons per 1 g-atom of carbon and of several major elements, as follow: C=4, H=1, N=−3, O=−2. The degrees of reductions of the SFW medium, cell, BC and culture remainder were calculated to be 4.38, 4.10, 3.50 and 4.32, respectively. The respiratory quotient (RQ) was assumed to be 1 in the BC production culture, because food wastes, with carbohydrates as the main component, were used as the substrate.

In the bioconversion process for the production of BC, water (H, O) was present in excess; therefore, based on Eq. (2), the mass balance equations for each of the components, N, e^- and RQ, were as shown in Eq. (3)-(5).

$$\text{N: } 0.03=b\times 0.07+d\times 0.03 \tag{3}$$

$$\begin{aligned} &e^-\text{: } Y_{substrate}-4a=bY_{biomass}+cY_{product\text{-}1}+dY_{product\text{-}2} \\ &\quad \Rightarrow 4.38-a\times 4=b\times 4.10+c\times 3.50+d\times 4.32 \end{aligned} \tag{4}$$

The degrees of reduction of H_2O and CO_2 are 0 both

$$\text{RQ: } f/a=1 \tag{5}$$

In Eq. (2), the values of c and d are expressed as the yields of BC and culture remainder, respectively.

$$Y_{P1/S}=[CH_{1.08}O_{0.79}]/[CH_{1.94}O_{0.73}N_{0.03}]=c \tag{6}$$

$$Y_{P2/S}=[CH_{1.99}O_{0.8}N_{0.03}]/[CH_{1.94}O_{0.73}N_{0.03}]=d \tag{7}$$

From these serial equations, the stoichiometric coefficients can be consecutively determined.

The reducing sugar concentrations in the SFW medium before and after autoclaving were 70.0 and 53.2 g/L, respectively. The decrease after autoclaving was caused by the increase in the medium volume due to the direct pressure steam sterilization. From the reducing sugar concentrations before and after autoclaving, for the initial medium volume of 25 L, that after autoclaving was calculated to be 32.9 L (=70 g/L×25 L/53.2 g/L). Also, the increase in the amount of medium was ascertained via a preliminary sterilization operation using tap water only.

During the initial BC cultivation phase, as the reducing sugar concentration, volume and molecular weight of the SFW medium were 53.2 g/L, 32.9 L and 26.05, respectively, the amount of substrate was calculated to be 67.2 mol (=53.2 g/L×32.9 L×1 mol/26.05 g). In a three-day cultivation, the BC concentration, volume of the culture remainder and the molecular weight of BC were 6.35 g/L, 32.0 L and 26.26, respectively. As a result, the total amount of BC produced was calculated to be 7.74 mol (=6.35 g/L×32.0 L×1 mol/26.26 g). The reducing sugar concentration, volume and molecular weight of the culture remainder were 25.4 g/L, 32.0 L and 27.06, respectively. From these results, the amount of the culture remainder was calculated to be 30.0 mol (=25.4 g/L×32.0 L×1 mol/27.06 g).

The value of c was the BC yield, which was expressed by the ratio of BC, as $CH_{1.08}O_{0.79}$, to SFW medium, as $CH_{1.94}O_{0.73}N_{0.03}$, which was calculated to be 0.12 (=7.74 mol/67.2 mol). As the value of d is the yield of the culture remainder in the SFW medium, d was calculated to be 0.45 based on 30.0 mol/67.2 mol.

When c=0.12 and d=0.45 were substituted into Eqs. (3)-(5), the values of the stoichiometric coefficients were: a=f=0.26 (O_2, CO_2), b=0.24 (cell as *A. xylinum*), c=0.12 (BC) and d=0.45 (culture remainder).

When the values of a, b, c, d and f were substituted into the oxygen mass balance, Eq. (8), the value of e was calculated to be 0.20.

$$\text{O: } 0.73+2a=0.35b+0.79c+0.79d+e+2f \tag{8}$$

Finally, the stoichiometric equation for the BC production process using the SFW medium was determined, as shown in Eq. (9).

$$\begin{aligned} &CH_{1.94}O_{0.73}N_{0.03}\ (\text{SFW})+0.26\ O_2 \rightarrow 0.24\ CH_{1.00}O_{0.35}N_{0.07}\ (\text{cell}) \\ &\quad +0.12\ CH_{1.08}O_{0.79}\ (\text{BC})+0.45\ CH_{1.99}O_{0.79}N_{0.03}\ (\text{culture-remainder}) \\ &\quad +0.20\ H_2O+0.26\ CO_2 \end{aligned} \tag{9}$$

From the stoichiometric analysis, 1 mol of the SFW medium required 0.26 mol of oxygen to produce 0.12 mol BC, and also produced

0.24 mol of cell and 0.45 mol of culture remainder, with the evolution of 0.26 mol CO_2. In other words, in the case of using 100 kg of dry-food waste, with the assumption of a saccharification ratio of 50%, 10 kg of oxygen would be required, and produced 9.0 and 5.9 kg of cell and BC, respectively.

3. Estimation of Energy Generation

The energy balance equation for aerobic growth is shown in Eq. (10), where Q_o corresponds to 26.95 kcal as the amount of energy originating from available electron g-equivalent. Based on the oxygen consumption from the stoichiometric analysis, the amount of heat generated was calculated. When O_2 is reduced to H_2O on obtaining electrons in the redox reaction, 1 mol of O_2 corresponds to 4 electron equivalents, as shown in the half-reaction of Eq. (11). The total heat generated based on the oxygen consumption was calculated to be 1,884 kcal (=$Q_0$4a=26.95 kcal/available electron g-equivalent×(4×67.2 mol×0.26)). In other words, total heat of 1,884 kcal was generated in the BC production process using 25 L of the SWF medium in the 50 L bioreactor, suggesting a guide for the design capacity of heat transfer equipment for the scale-up system for mass production.

$$Q_o cY_b + Q_o dY_{p1} + Q_o dY_{p2} = Q_o Y_s - Q_o 4a \quad (10)$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (11)$$

4. Measurement of OUR and Q_{O2}

The dissolved oxygen content was found to be depleted during the exponential phase of the BC production cultivation, leading to a decrease in the BC productivity. Therefore, to determine if the supplementation of pure oxygen to the culture was required, the critical DO concentration not inhibiting the metabolism of *A. xylinum* KJ was investigated by measuring the OUR (oxygen uptake rate) and Q_{O2} (specific oxygen uptake rate).

To measure the OUR and Q_{O2}, a BC culture was performed by inoculating *A. xylinum* KJ into 30 L of SFW medium (reducing sugar concentration of 70 g/L, T-N concentration of 2.5 g/L, agar of 0.4%, initial pH 5.25) at 30 °C, with aeration at 1.0 vvm (30 L/min) in a 50 L modified bubble column bioreactor. During the cultivation of BC, air was supplied for 18 hours, with pure oxygen also partially added. When the total aeration rate was 30 L/min, pure oxygen was added to the culture at rates of 5, 15, 10, 5 and 2 L/min from 18 to 22, 22 to 28, 28 to 50, 50 to 58 and 58 to 72 hours, respectively, with residue aeration performed using only air, without pure oxygen. The DO concentration in the culture was maintained above 2.13 ppm for the initial 36 hours until the exponential phase, and then around 1.12±0.23 ppm from 36 hours to the end of the culture, as shown in Fig. 1. The oxygen concentration in the outlet gas was also maintained between 21 and 50%.

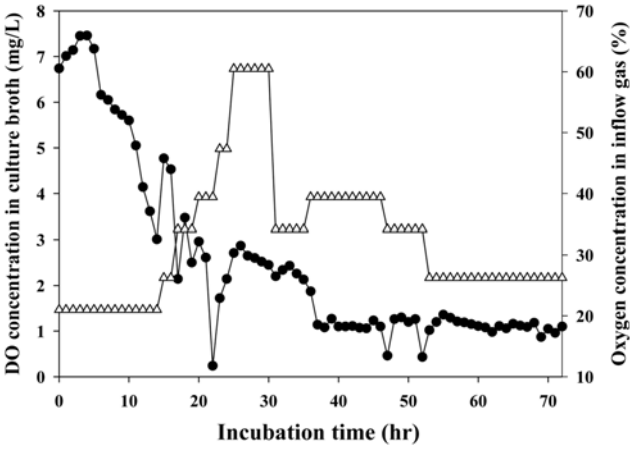

Fig. 1. The changes of the oxygen concentration in the inflow gas and the DO concentration in the culture broth according to the BC incubation time in the 50 L modified bubble column bioreactor (●: DO concentration (mg/L), △: Oxygen concentration in inflow gas (%)).

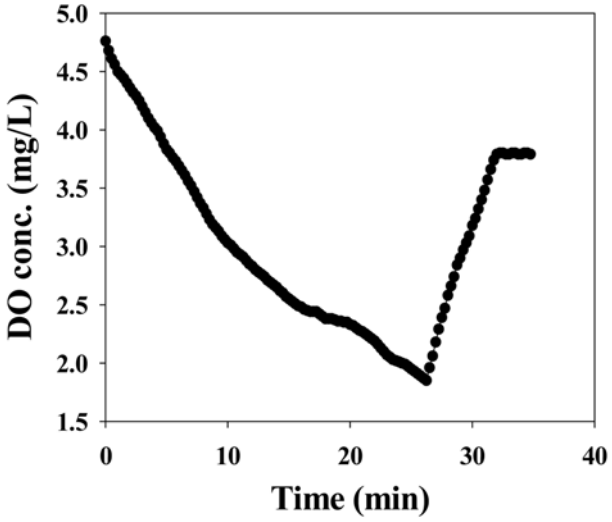

Fig. 2. The changes of the DO concentration during the OUR measurement, using the dynamic method, in the 50 L modified bubble column bioreactor.

The OUR was determined by measuring the rate of change of the DO concentration after 12 hours (exponential phase) of cultivation (dC_L/dt), as shown in Fig. 2 [15]. The monitoring data between 9.5 and 13 min time elapsed for the change of the DO concentration were employed for calculating the OUR and Q_{O2}, using Eqs. (12) to (14).

$$OUR = -\frac{dC_L}{dt} = -\frac{(4.9-4.15)(mgDO/L)}{(9.5-13)(min)} = 0.21\ mgDO/L\cdot min \quad (12)$$

$$OUR = Q_{O2}\cdot X \quad (13)$$

$$X = DCW = OD\ (600\ nm) * 0.33 = 2.53 * 0.33 = 0.84\ g\text{-dry cell/L}$$

$$Q_{O2} = \frac{OUR}{X} = \frac{0.21\ mgDO/L\cdot min}{0.84\ gdrycell/L} = 0.25\ mgDO/gcell\cdot min \quad (14)$$

As a result, the calculated OUR was 0.21 mg DO/L·min, with a specific respiratory rate for the bacterium, Q_{O2} of 0.25 mg-DO/g·cell·min, as obtained in Eq. (13). The curve for the changes in the DO concentration over time was fitted using a quadric polynomial: $C_L = -30.21 + 1.958\,t - 0.02809\,t^2$, and was differentiated to $dC_L/dt = 1.958 - 0.05618\,t$. The linear relationship of C_L and ($dC_L/dt + x\cdot Q_{O2}$) gave the k_La value, with a slope of $-1/k_La$, as shown in Fig. 3. The calculated value of k_La was 8.49 hr^{-1}.

5. Suggestion of the Critical DO Concentration

In the procedures for the oxygen transfer to cells from the oxy-

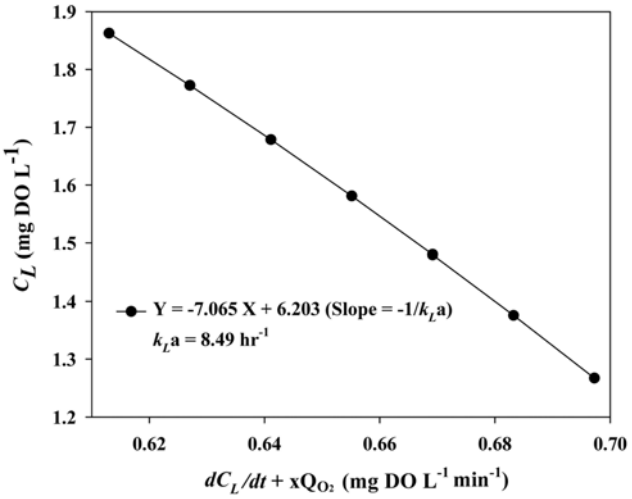

Fig. 3. Determination of the k_La value, using the dynamic method, in the 50 L modified bubble column bioreactor.

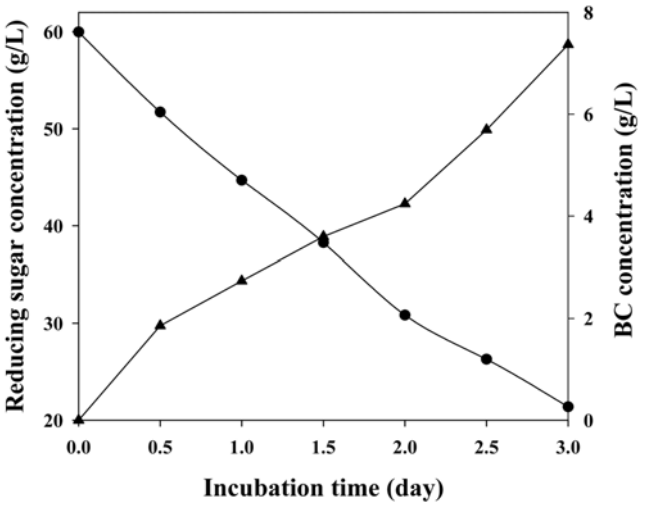

Fig. 4. The changes of the dry weight of BC and reducing sugar concentrations, according to the incubation time, when air and pure oxygen were supplemented into the 50 L modified bubble column bioreactor (●: Reducing sugar concentration, ▲: Dry weight of BC).

gen in air, the oxygen transfer rate to cells from air bubbles is generally limited by the oxygen transfer through the liquid-film surrounding the bubbles. Therefore, the oxygen uptake rate (OUR) to cells from the gas phase is almost equal to the oxygen transfer rate (dC_L/dt) to the liquid from the gas phase, as described in Eq. (15).

$$OTR = \frac{dC_L}{dt} = k_L a(C^* - C_L) = OUR \quad (15)$$

where, dC_L/dt is the oxygen transfer rate (mg O_2/L-h), k_L the oxygen transfer rate coefficient (cm/h), a the gas-liquid interfacial area (cm^2/cm^3), k_La the volumetric oxygen transfer coefficient (h^{-1}), C^* the saturated DO concentration (mg/L), and C_L the DO concentration in the culture liquid (mg/L). To calculate the saturation DO concentration in the culture, the linear relationship between the salinity (X) and DO concentration (Y), described as $Y=7.507-0.3665\ X$ ($R^2=0.999$) at 30 °C, was used [16]. When the total amount of organic and inorganic matters in the SFW medium was converted into salinity of 8%, the saturated DO concentration of the SFW medium at 30 °C was 4.58 ppm (Eq. (16)).

$$C^*(8\%\ \text{of salinity}) = (7.507 - 0.3665 \times 8)\ \text{ppm} = 4.58\ \text{ppm} \quad (16)$$

From Eq. (15), since the OUR, k_La, and C^* values were 0.21 mg DO/L·min, 8.49/hr and 4.58 ppm, respectively, C_L was calculated to be 3.10 ppm. In conclusion, the DO concentration in the culture liquid should be maintained above 3.10 ppm during the production of BC, suggesting that the supplementation of pure oxygen is necessary during the exponential phase of the active growth period.

6. Ascertainment of the Productivity of BC

To ascertain the productivity of BC under the above calculated and predicted conditions, a BC production culture was performed by adding pure oxygen, with aeration at 1.0 vvm (30 L/min) for 3 days in the 50 L modified bubble column bioreactor. The result showed a BC productivity of 7.37±0.3 g/L (Fig. 4).

From the report of Chao et al. [9], when BC was produced using an optimized complex medium, Corn steep liquor-fructose (CSL-Fru), in a 50 L Internal-Loop Airlift Reactor, with a 67 hours cultivation, 3.8 g/L BC was obtained, which increased to 7.93 g/L on the addition of pure oxygen [9]. In this study, the productivity of BC on the addition of pure oxygen was comparable to that using the optimal complex medium, CSL-Fru medium, developed in Japan, even though the SFW originating from food wastes was used as the medium in our study.

CONCLUSION

The stoichiometric equation for the BC production process using SFW medium in a 50 L modified bubble column bioreactor was determined as follows:

$$CH_{1.94}O_{0.73}N_{0.03}\ (\text{SFW}) + 0.26\ O_2 \rightarrow 0.24\ CH_{1.00}O_{0.35}N_{0.07}\ (\text{cell}) + 0.12\ CH_{1.08}O_{0.79}\ (\text{BC}) + 0.45\ CH_{1.99}O_{0.79}N_{0.03}\ (\text{culture-remainder}) + 0.20\ H_2O + 0.26\ CO_2$$

Based on the stoichiometric analysis, the amounts of substrate, oxygen supply, and BC production can be predicted, and the total heat generated can also be predicted based on the oxygen consumption. Therefore, the stoichiometric equation could be used as an operational guide for mass production. In addition, the critical DO concentration without inhibition of the metabolism for the production of BC was determined to be above 3.10 ppm from analyzing the OUR of the cells. Therefore, it was suggested that supplementation with pure oxygen during the exponential phase of a BC production culture will be required. The experiment for ascertaining the productivity of BC under the above predicted conditions indicated the production of 7.37 g/L BC in the 50 L bioreactor with the supplementation of pure oxygen.

ACKNOWLEDGEMENTS

This work was supported by a Korea Science and Engineering Foundation (KOSEF) grant, funded by the Korean government (MEST) (No. 2010-0277).

NOMENCLATURE

C^* : the saturated DO concentration [mg/L]
C_L : the DO concentration in the culture liquid [mg/L]
DCW : dry cell weight [g-dry cell/L]
e^- : electron [dimensionless]
k_La : volumetric oxygen transfer coefficient [h^{-1}]
OD : optical density [dimensionless]
OTR : oxygen transfer rate [mg O_2/L·h]
OUR : oxygen uptake rate [mg DO/L·min]
Q_o : heat evolution per g-electron equivalent [kcal/g-electron]
Q_{O2} : specific oxygen uptake rate [mg DO/g cell·min]
RQ : respiratory quotient [dimensionless]
X : cell concentration [g-dry cell/L]
$Y_{P1/S}$: yield of BC [mol product 1/mol substrate]
$Y_{P2/S}$: yield of the culture remainder [mol product 2/mol substrate]
γ_{p1} : degree of reduction of BC [equiv available electrons/g atom carbon]
γ_{p2} : degree of reduction of the culture remainder [equiv available electrons/g atom carbon]
γ_s : degree of reduction of the substrate [equiv available electrons/g atom carbon]

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