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Effect of viscosity-inducing factors on oxygen transfer in production culture of bacterial cellulose

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Abstract–Bacterial cellulose (BC) production culture requires high oxygen transfer rate (representatively k_La) at a low shear force. Considering that oxygen exhaustion is observed at the latter half of the exponential growth phase where BC production actually begins, it is highly probable that the drastic reduction of k_La (oxygen volumetric transfer coefficient) is caused by the drastic increase of the soluble and insoluble viscous materials. Therefore, we examined the apparent viscosity-inducing materials generated during BC culture and investigated their effects on k_La. Using the saccharified liquid from food waste as the culture medium, we discussed the relationship between the concentration of the generated solid matters, especially BC and the viscosity, the relationship between the BC concentration and k_La, and the correlation between the viscosity and k_La. The relationship between the solid matter (BC), which is the insoluble viscosity-inducing material, and k_La showed that the BC concentration and k_La were in exponentially reciprocal proportion with the linear regression equation. In case of using agar as the soluble viscosity-inducing material, the correlation between the viscosity and k_La showed that the viscosity depending on the agar concentration was in exponentially reciprocal proportion with k_La in both tap water and the saccharified liquid medium. The results indicated that the effect of the BC concentration on k_La was not great in the saccharified liquid medium. As the agar concentration increased in tap water and the saccharified liquid medium, the viscosity was increased and k_La was decreased gradually, showing a linear relation between the logarithm of the viscosity as agar and k_La. In conclusion, the effect of the soluble viscosity on k_La was greater than that of solid matter (BC). Also, it was suggested that the soluble viscosity-inducing matters like agar were rather more effective than k_La in BC production.

Key words: Viscosity, Bacterial Cellulose (BC), Oxygen Transfer Efficiency (k_La)

INTRODUCTION

Bacterial cellulose (BC) is produced as pure cellulose without impurities such as lignin or hemicellulose, different from plant-originated cellulose [1,2], and has excellent physical properties such as high mechanical strength (high tensile strength and Young's modulus) high water retention, high crystallinity and bio-degradability because the 20-50 nm of microfibrils form three-dimensional network structure by means of hydrogen bonding [3]. As a result, BC can be used for high-quality paper, cosmetics, artificial blood vessel, artificial skin, high-performance diaphragm, vulnerary and dietary food [4-6].

The three major methods of BC production are stationary culture [7,8], agitating culture [9,10], and the culture using an internal-loop airlift reactor [11]. In general, BC is produced in the form of a thin pellicle on the surface of the culture medium by stationary culture. However, the traditional BC production by stationary culture is not efficient for mass production from the industrial point of view since the productivity is low and much labor and culture time are required [12]. In addition, even though low shear force and high oxygen transfer rate are required in the BC production by *Acetobacter*, agitating culture is not the general BC production method because of the strain instability (appearance of *Cel*$^-$ mutant, the spontaneous mutant that is not able to secrete cellulose at high shear force) in the agitating culture that is used to enhance the mass transfer efficiency [13]. The same phenomena were also observed in our previous study using *Acetobacter xylinum* KJ1. The study reported that *cel*$^-$ generation by strong agitating was related to BC hydrolysis by beta glucosidase in cell wall [14].

Thus, studies have been carried out to use an airlift type reactor with low shear force. However, in those fermenters, the BC production efficiency was decreased by the oxygen exhaustion in the latter half of the column circulation part because of long liquid circulation path, and the oxygen transfer was not good as the BC lumps are accumulated at the lower part of liquid circulation in the case of high BC concentration [15].

Since the strain for BC production, *Acetobacter xylinum*, is sensitive to shear force, it is very important to reduce shear force in producing BC. Hence, many studies have been conducted recently to reduce shear force by adding agar [16], a water-soluble polysaccharide, or acetan [17]. We also performed the study to reduce the shear force by adding agar to the up-and-down circulation type bubble column reactor newly developed for BC mass production [18].

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However, addition of agar resulted in the reduction of oxygen transfer efficiency because of the increased viscosity.

Since the productivity of a product in arbitrary aerobic culture is closely related to k_La, oxygen transfer rate plays the role of a key limiting-factor [19,20]. For this reason, k_La is usually kept constant in the scale-up of bioreactor for aerobic culture [21-23].

Therefore, we investigated the effect of viscosity to enhance the BC productivity through the increase of k_La, by quantitatively examining the effect of soluble polymer (represented by agar in this study) and insoluble BC on the viscosity and k_La, which are the main viscosity-inducing materials in BC production culture, and determining the factor that has the greatest effect on k_La.

MATERIALS AND METHODS

1. BC Production Culture

1-1. Preparation of Medium

To prepare the saccharified liquid medium, three enzymes of Termanyl 120 L, and Spirizyme plus (NCS00020) and Viscozyme (KTN02171) were used in the saccharification of food wastes. Termamyl 120 L (Novozyme), an enzyme used for starch saccharification, is α-amylase produced from *Bacillus licheniformis*, the enzyme that hydrolyzes 1,4-α-glucosidic linkage. Spirizyme Plus (Novozyme) is amyloglucosidase (glucoamylase), the enzyme that hydrolyzes 1,4-α, 1,6-α linkage in the liquified starch substrate. Viscozyme (Novozyme) is a conjugated enzyme that is composed of beta-glucanase, xylanase, cellulase and hemicellulase. Each 15 mL (1,800 U of Termanyl 120 L, 6,900 U of Spirizyme plus, and 1,815 U of Viscozyme) of the enzymes diluted with pH 5.0 sodium acetate buffer was added to 30 kg of wet food waste and saccharification was performed at 50 °C for 10 hours. The element composition of the food waste used in this study was carbon 46.81%, hydrogen 8.09% and nitrogen 2.52%. The concentration of reducing sugar in the saccharified liquid medium obtained by centrifugation (8,000 rpm, 20 min, 4 °C) following the saccharification was 117 g/L and the reducing sugar concentration was readjusted to 70 g/L by diluting with tap water for the follow experiments.

1-2. BC Culture

The preliminary culture for preculture was performed by means of stationary culture for 36 hours at 30 °C after inoculating *A. xylinum* KJ1 stored on a solid medium with a platinum loop to a 500 mL flask containing 100 mL of the saccharified liquid medium. 4% of the supernatant of the preliminary culture was used as the inoculation liquid for the preculture and the stationary culture was performed for 36 hours at 30 °C. 1,200 mL of the precultured medium was pulverized with a homogenizer (10,000 rpm, 1 min) and used as the inoculation liquid for the main culture. 1,200 mL of the precultured medium was inoculated to 30 L of the saccharified liquid medium (the reducing sugar concentration 70 g/L and the initial pH 5.25) and cultured for three days at 30 °C, the initial pH of 5.25, and the air flow rate of 1.0 vvm.

2. Measurement of k_La

k_La was measured by the gassing out method among the static methods [1]. First, the culture medium was substituted by nitrogen gas to set DO=0%. Then, k_La was obtained by measuring the increasing rate of the dissolved oxygen over time as air flowed in under a constant air flow condition. The increase of the dissolved oxygen can be expressed by integrating Eq. (1) to Eq. (2). A linear relation is obtained by plotting the logarithm of (C^*-C_L) against time and the slope of the line is $-k_La$.

$$dC_L/dt = k_La\,(C^*-C_L) \quad (1)$$

$$\ln(C^*-C_L) = -k_La\cdot t \quad (2)$$

where, C_L: dissolved oxygen concentration in the culture medium (mg/L)
dC_L/dt: rate of oxygen transfer (mg/L-min)
k_L: oxygen transfer coefficient of the liquid film (cm/hr)
a: gas-liquid interface area per unit volume (cm^2/cm^3)
C^*: dissolved oxygen concentration in equilibrium with the bubble oxygen partial pressure (mg/L)
k_La: oxygen volumetric transfer coefficient (hr^{-1})

The k_La values are calculated by the least squares method and the slope is obtained in the relation between t and $\ln(1-C^*/C)$.

3. Measurement of Viscosity

Viscosity is one of the important factors of k_La in BC production culture. Thus, we investigated the effect of viscosity on k_La and also observed the change of the culture medium viscosity during the BC culture. The viscosity was measured by means of viscometer (Visco basic plus L, Japan). 45 mL of the culture medium was taken and the viscosity of the BC culture medium was measured with TL5 spindle at 100 rpm.

RESULTS AND DISCUSSION

1. The k_La Values Depending on the Air Flow Conditions

To investigate the change of k_La values depending on the air flow conditions in the 50 L up-and-down radio circulation fermenter, the working volume of the fermenter was set to 25 L, the reducing sugar concentration in the saccharified liquid medium to 70 g/L, and the k_La measurement temperature to 30 °C, which was the temperature for the BC production. The air flow rate conditions were varied to 0.6, 1.0, 1.2 and 1.5 vvm equivalent to 15, 25, 30 and 37.5 L/min to, respectively, measure the change in k_La values depending on the air flow conditions in tap water and in the saccharified liquid medium.

The result showed that the k_La values under the air flow conditions of 0.6, 1.0, 1.2 and 1.5 vvm were 18.8, 28.2, 33.1 and 43.1 hr^{-1}, respectively, in tap water. The k_La values under the air flow conditions of 0.6, 1.0, 1.2 and 1.5 vvm were 10.4, 16.3, 20.6 and 25.6 hr^{-1}, respectively, in the saccharified liquid medium (Table 1). The k_La values gradually increased as the air flow rate was increased

Table 1. k_La values according to various aeration rates in tap water and SFW medium in 50 L up-and-down circulation bubble column bioreactor

Aeration rate	k_La (hr^{-1})	
	Tap water	SFW medium
0.6 vvm	18.8	10.4
1.0 vvm	28.2	16.3
1.2 vvm	33.1	20.6
1.5 vvm	43.1	25.6

in tap water and the saccharified liquid medium. Under the same air flow conditions, the k_La was lower in the saccharified liquid medium than in tap water (Table 1).

According to the result of Bandaiphet and Prasertsan [24], the k_La values were measured at the logarithmic phase and stationary phase in the culture producing exopolysaccharide using *Enterobacter cloacae* WD7 depending on the air flow rates, and the result showed that the k_La was increased from 2.63 hr^{-1} to 9.72 hr^{-1} at the logarithmic phase and from 2.71 hr^{-1} to 9.97 hr^{-1} at the stationary phase as the air flow rate was increased (0.5-2.0 vvm). This result was similar to the result of our study that the k_La values were increased as the air flow rate was increased.

2. Effects of Solid Material (BC) on k_La

2-1. The k_La Values Depending on the Solid Material (BC) Concentrations

It was thought that the solid material (BC) produced during the BC culture affects k_La considerably, so we investigated the effect of BC on k_La in tap water and the saccharified liquid medium. To investigate the change of k_La depending on the BC concentrations in the 50 L up-and-down radio circulation fermenter, the working volume of the fermenter was set to 25 L, the reducing sugar concentration in the saccharified liquid medium to 70 g/L, the temperature to 30 °C, the initial pH to 5.25, and the air flow condition to 1.0 vvm. The BC cultured previously in the 50 L fermenter was centrifuged at 8,000 rpm, 20 min, 4 °C. The centrifuged BC was vacuum-dried at 80 °C for 24 hours and the water content of the BC was 96%. The change of the k_La values depending on the BC concentrations was measured by varying the concentration of BC to 1.0, 2.0, 4.0, 8.0 and 16.0% corresponding to the dry BC concentrations of 0.8, 1.6, 3.2, 6.4 and 12.8 g/L.

The result showed that the k_La values were 30.4, 23.7, 22.0, 21.6, 14.4 and 10.7 hr^{-1} at the wet BC concentrations of 0, 1.0, 2.0, 4.0, 8.0 and 16.0%, respectively, in tap water. The k_La values were 14.5, 15.6, 13.4, 15.5 and 16.8 hr^{-1} at the wet BC concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0% (Table 2), respectively, in the saccharified liquid medium.

The k_La value gradually decreased as the BC concentration was increased in tap water. In the saccharified liquid medium, the wet BC concentration did not show a significant effect on the k_La value in the range of 0-4.0%, but the k_La value was increased to some extent as the wet BC concentration was increased to 8.0%. Therefore since the BC concentration obtained in the 50 L up-and -down circulation culture system was around 8% (6-7 g/L as wet), it was able to be thought that the produced BC did not largely affect to k_La in SFW medium.

2-2. Relationship between BC and Viscosity

The viscosity of saccharified liquid medium depending on the BC concentrations in tap water was measured. The result showed that the viscosity was 2.2, 2.8, 2.3, 2.7, 6.1 and 18.6 cp at the wet BC concentrations of 0, 1.0, 2.0, 4.0, 8.0 and 16.0%, respectively. On the other hand, the viscosity in the saccharified liquid medium was 2.0, 2.1, 2.4, 4.7 and 18.5 cp at the wet BC concentrations of 1.0, 2.0, 4.0, 8.0 and 16.0%, respectively (Table 2).

When the wet BC concentration was in the range of 0-4.0% in tap water, the viscosity remained similar as 2.2-2.8 cp. But, in the case of BC concentration of 8.0% and 16.0%, the viscosity was 6.1 cp and 18.6 cp, respectively, showing a drastic increase.

In saccharified liquid medium, when the wet BC concentration was in the range of 0-2.0%, the viscosity remained similar as 2.0-2.4 cp. But, in the case of 8.0% and 16.0%, the viscosity was 4.7 cp and 18.5 cp, respectively, showing a drastic increase. From the results, when the highest BC concentration (around 8%) showed in the production culture, it could be analogized that a drastic increase of viscosity occurred.

The first-order reciprocal relation $Y=-0.024\,X+1.396$ ($R^2=0.96$) was obtained between the BC concentration and $\log(k_La)$ in tap water as shown in Fig. 1.

Conclusively, the BC showed almost no effect on the k_La value in the saccharified liquid medium, although the high BC concentration induced high viscosity in both tap water and SFW medium, while it had an effect on the k_La value in tap water.

3. Effect of Viscosity (Agar) on k_La

3-1. The k_La Values Depending on the Viscosity (Agar Concentration)

Since the strain for BC production is sensitive to shear force, it is very important to reduce shear force in producing BC. Thus, agar, a water-soluble polysaccharide, is added to reduce shear force for the improvement of BC productivity at present. The up-and-down

Table 2. k_La values and viscosity according to solid (BC) concentrations in tap water and SFW medium in 50 L up-and-down circulation bubble column bioreactor

BC concentration (wet state)	Viscosity (cp)		k_La (hr^{-1})	
	Tap water	SFW medium	Tap water	SFW medium
0% (Tap water)	2.2	2.0	30.4	14.5
1.0%	2.8	2.1	23.7	15.6
2.0%	2.3	2.4	22.0	13.4
4.0%	2.7	4.7	21.6	15.5
8.0%	6.1	18.5	14.4	16.8
16.0%	18.6	-	10.7	-

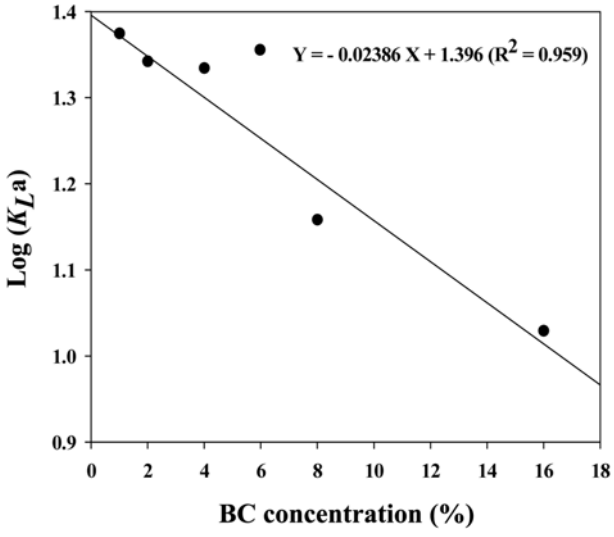

Fig. 1. Relationship between viscosity and k_La in tap water by BC concentration.

Table 3. k_La values and viscosity according to agar concentrations in tap water and SFW medium in 50 L up-and-down bubble column bioreactor

Agar concentration	Viscosity (cp)		k_La (hr^{-1})	
	Tap water	SFW medium	Tap water	SFW medium
0%	2.1	2.1	28.2	16.3
0.1%	2.3	2.7	27.5	15.5
0.2%	2.6	7.3	23.0	13.3
0.4%	7.1	25.0	17.8	9.8
0.6%	24.4	-	8.6	-
0.8%	NT	-	7.9	-

NT; not tested

circulation type bubble column bioreactor in this study requires a large amount of oxygen. Since it is highly probable that addition of agar to the BC production medium could reduce k_La and the rate of oxygen supply was limited under the constant rate of aeration, we investigated the effect of viscosity on k_La.

To investigate the change of k_La depending on the agar concentration in the 50 L up-and-down circulation fermenter, the conditions, such as the working volume of 25 L, the reducing sugar concentration of 70 g/L, and 30 °C were set. The agar concentration was varied to 0, 0.1, 0.2, 0.4, 0.6 and 0.8% and the change in the k_La values depending on the agar concentrations was measured in tap water and in the saccharified liquid medium under the air flow condition of 1.0 vvm.

The result showed that the k_La values were 28.2, 27.5, 23.0, 17.8, 8.6 and 7.9 hr^{-1} at the agar concentrations of 0, 0.1, 0.2, 0.4, 0.6 and 0.8%, respectively, in tap water. In the saccharified liquid medium, the k_La values were 16.3, 15.5, 13.3 and 9.8 hr^{-1} at the agar concentrations of 0, 0.1, 0.2 and 0.4%, respectively (Table 3).

The k_La value was gradually decreased as the agar concentration increased in tap water and the saccharified liquid medium. Under the same air flow condition, the k_La was lower in the saccharified liquid medium than in tap water, which is similar to the results obtained between BC and k_La.

3-2. Relationship between Viscosity (Agar Concentration) and k_La

The viscosity was measured in tap water and the saccharified liquid medium depending on the agar concentrations. The result showed that the viscosity was 2.1, 2.3, 2.6, 7.1 and 24.4 cp at the agar concentrations of 0, 0.1, 0.2, 0.4 and 0.6%, respectively, in tap water. The viscosity was 2.1, 2.7, 7.3 and 25.0 cp at the agar concentrations of 0, 0.1, 0.2 and 0.4%, respectively, in the saccharified liquid medium as shown in Table 3.

When the agar concentration was in the range between 0 and 0.2% in tap water, the viscosity remained similar as 2.1-2.6 cp. But, at the agar concentration of 0.6%, the viscosity increased drastically to 7.1 cp, resulting in a considerable k_La decrease from 17.8 to 8.6. When the agar concentration was 0.8%, the viscosity could not be measured for being too high beyond the maximum measurement limit.

In the saccharified liquid medium, the viscosity remained similar as 2.1-2.7 cp when the agar concentration was in the range between 0 and 0.1%. But, when the agar concentration was 0.2% and 0.4%,

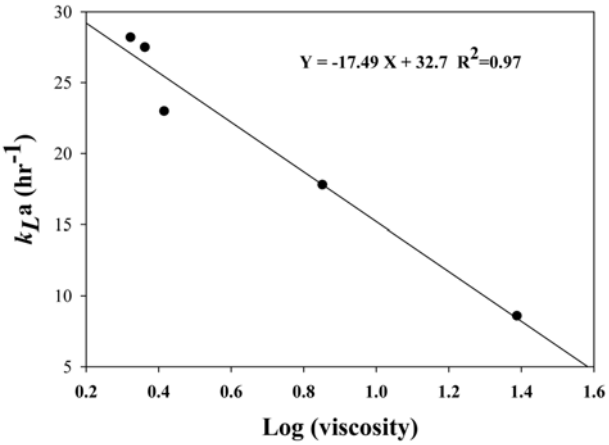

Fig. 2. Relationship between viscosity and k_La in tap water by agar concentration.

the viscosity drastically increased to 7.3 cp and 25.0 cp, respectively. The results indicated that the decreasing effect of shear force by adding agar should be limited to be less 0.4% in the SFW medium.

The k_La value was gradually decreased as the agar concentration (viscosity) increased in tap water and the saccharified liquid medium, showing a linear relation between the logarithm of the viscosity and the k_La value. The first-order reciprocal relation, $Y=-17.49X+32.7$ ($R^2=0.97$), was obtained between the log(viscosity) and k_La in tap water as shown in Fig. 2. And in the saccharified liquid medium, the relation, $Y=-5.94X+1.82$ ($R^2=0.997$), was obtained between the log(viscosity) and k_La (Fig. 3). The slope values in both equations showed that the extent of k_La decrease by agar concentration was larger in the tap water than in SFW medium (−17.49 vs −5.94), implying a larger decrease of k_La in the range of the higher viscosity. In other words, the viscosity in the SFW medium has not so great effect on k_La compared with the case of tap water.

In the study of Özbek also, the k_La value was gradually decreased from 2.65 min^{-1} to 0.083 min^{-1} as the viscosity increased (0.94-566.04 cp), depending on the glycerol solution concentration, which is similar to the result of our study [25]. In the study of Carbajal also, the k_La value was measured as the viscosity was gradually increased

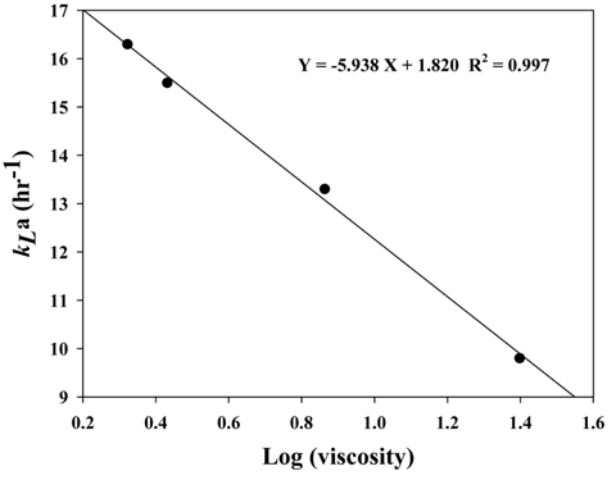

Fig. 3. Relationship between viscosity and k_La in SFW medium.

Table 4. BC concentration and viscosity changes according to agar concentration at static culture

Medium	BC concentration (g/L)	Viscosity (cp)	
		0 - Day	3 - Day
SFW	2.50	2.0	3.2
SFW+0.2% agar	3.21	2.1	3.3
SFW+0.3% agar	3.66	2.5	3.7
SFW+0.4% agar	4.58	2.7	5.4

(0.85-279 mPa·s), depending on the concentration of glycerol and glucose syrup, and the result showed that k_La value depending on the viscosity at 200 rpm was gradually decreased from 0.0243 S^{-1} to 0.00241 S^{-1} [26].

From the results of Table 2 and 3, as respects the viscosity to k_La, the agar (soluble) has a greater effect on k_La than the insoluble solid (BC). In other words, the produced BC has not a considerable effect on the oxygen transfer in the culture system using SFW medium. Therefore the soluble-inducing viscosity in the BC production culture was elucidated to be an important control factor together with k_La.

To investigate the BC productivity depending on the viscosity (agar), stationary cultures were investigated in a flask using 100 mL of the saccharified liquid medium for three days at 30 °C. The viscosity was measured at the initial stage of the culture and after the three days of culture and the result showed that the viscosity was changed from 2.0 cp to 3.2 cp in the saccharified liquid medium, from 2.1 cp to 3.3 cp in the saccharified liquid+0.2% agar medium, from 2.5 cp to 3.7 cp in the saccharified liquid+0.3% agar medium and from 2.7 cp to 5.4 cp in the saccharified liquid+0.4% agar medium (Table 4). The viscosity increase was the highest in the saccharified liquid+0.4% agar medium, and the BC productivity was also the highest in that medium as 4.58 g/L. It is assumed that the optimum agar concentration needs to be determined, considering the BC productivity in an air circulation fermenter.

In Table 3, although k_La value was decreased when the agar concentration was increased from 0 to 0.4% in the air circulation system, the BC concentration was rather higher in the static culture with high soluble viscosity of 0.4% as shown in Table 4. So far, the addition of soluble polymer like agar into the agitation culture for BC production was in order to reduce shear force. A few studies with respect to the phenomena have been known [16-18]. In the present study, it could be thought that the static cultures containing agar were almost not affected by shear force due to without agitation (Table 4). And the highest BC concentration was obtained in spite of low k_La in the agar addition of 0.4%. The result suggests that the soluble viscosity-inducing matters like agar have rather more effect on BC productivity than k_La factor, that is, the soluble viscosity is an important control factor rather than k_La.

Therefore, it was positively suggested that the BC mass production in future should be carried out in a medium containing a soluble polymer like agar by controlling two major factors of viscosity and k_La.

CONCLUSIONS

Since BC production is the culture that requires low shear force and high oxygen transfer rate, we investigated the soluble and insoluble viscosity-inducing materials in a 50 L up-and-down circulation fermenter. Among the apparent viscosity-inducing materials, the concentration of BC, which is an insoluble material, and the concentration of agar, which is soluble, were investigated to examine their effect on k_La. The relationship between the solid (BC) and k_La was investigated, and the result showed that the k_La value was gradually decreased as the BC concentration increased in tap water, but the BC concentration did not show a significant effect on k_La in the saccharified liquid medium. The relationship between viscosity (agar concentration) and k_La was investigated and the result showed that the viscosity was gradually increased and the k_La value was gradually decreased as the agar concentration increased in tap water and the saccharified liquid medium. In conclusion, the effect of viscosity caused by agar on k_La was greater than that by BC, and the BC productivity was increased as the agar concentration was increased to 0.4%. Also, it was suggested that the soluble viscosity-inducing matters like agar were rather more effective than k_La on the BC production.

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