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Pilot-scale Production of Carboxymethylcellulase from Rice Hull by *Bacillus amyloliquefaciens* DL-3

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Abstract Optimal conditions for pilot-scale production of the carboxymethylcellulase (CMCase) by *Bacillus amyloliquefaciens* DL-3 were investigated. The best carbon and nitrogen sources for the production of CMCase by *B. amyloliquefaciens* DL-3 were found to be rice hull and peptone and their optimal concentrations were 5.0 and 0.20% (w/v), respectively. Optimal temperature and initial pH for the production of CMCase were 37°C and 6.8. Optimal agitation speed and aeration rate for the production of CMCase were 300 rpm and 1.0 vvm in a 7 L bioreactor, which were different from those for the cell growth of *B. amyloliquefaciens* DL-3. The highest productions of CMCase by *B. amyloliquefaciens* DL-3 from 5.0% (w/v) rice hull as a carbon source under optimal conditions in a 7 or 100 L bioreactor were 220 and 367 U/mL, respectively. © KSBB

Keywords: Bacillus amyloliquefaciens, *carboxymethylcellulase (CMCase)*, *optimization*, *rice hull*

INTRODUCTION

Cellulosic biomass has an enormous potential as a renewable energy source. The production of α-amylase from different agricultural by-products such as wheat bran, sunflower meal, cotton seed meal, soybean meal, rice hull, and rice bran has been reported [1]. Corncob residues remaining after xylose via dilute acid hydrolysis was used to produce cellulases by *Trichoderma reesie* [2]. Productions of ethanol [3], organic acids [4], and other chemicals [5] from cellulosic biomass are attracting attention because of its abundance and low cost [6]. Production of ethanol from lignocellulosic materials by simultaneous saccharification and fermentation (SSF) was first reported in 1977 [7]. Production of ethanol at 16 to 19 g/L was obtained from 10% (w/v) lignocellulosic materials using a commercial cellulase (carboxymethylcellulase) and *Kluyveromyces marxianus* [8].

Rice hulls are the outer coat of rice and represent about 20% of the dry weight of harvested rice [9]. Its hydrolysate contains mainly glucose and xylose, which can be used as substrate for the production of ethanol [6]. In 2004, world rice production was about 610 million tons and the annual waste from the milling process in Korea was about 900 thousand tons of rice hulls. Due to constraints such as their low digestibility, peculiar size, low bulk density, high ash content, and abrasive characteristics [10], rice hull is mostly disposed of in land-fill sites or burned in rice fields and have become a significant problem to the ecology and environment [11].

The enzymatic hydrolysis of cellulosic biomass can be accomplished through a complex reaction of three different types of cellulases: endoglucanase (1,4-β-D-glucan-4-glucanohydrolase; carboxymethylcellulase) [12], exocellobiohydrolase (1,4-β-D-glucan glucohydrolase; avicelase) [12, 13], and β-glucosidase (β-D-glucoside glucohydrolase) [12, 14]. Wood-wastes are saccharified with an enzyme mixture of filter paperase, carboxymethylcellulase, β-glucosidase, Avicelase, xylanase, β-amylase, and glucosamylase obtained from the culture supernatant of *T. harzianum* [15].

A number of fungi and bacteria producing cellulases have been identified [11,16,17]. The production of cellulases and hemicellulases by *Aspergillus niger* using lignocellulosic biomass has been reported [6,18]. In our previous study, cellulase produced by *Bacillus amyloliquefaciens* DL-3 was reported [19]. This strain was found to utilize rice hulls and

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produced the cellulases: endoglucanase, exocellobiohydrolase, and β-glucosidase. The productivity of endoglucanase (carboxymethylcellulase) was higher than the other cellulases produced by this strain. In this study, optimal conditions for pilot-scale production of the carboxymethylcellulase (CMCase) by *B. amyloliquefaciens* DL-3 were investigated.

MATERIALS AND METHODS

Bacterial Strain and Medium

B. amyloliquefaciens DL-3 was isolated from soil and identified in a previous study [19]. It utilized rice hull as a carbon source and produced cellulases. The strain was maintained on agar medium containing 2.0% (w/v) glucose, 0.25% yeast extract, 0.5% K_2HPO_4, 0.1% NaCl, 0.02% $MgSO_4 \cdot 7H_2O$, 0.06% $(NH_4)_2SO_4$, and 1.5% (w/v) agar.

Production of Carboxymethylcellulase (CMCase)

Starter cultures were prepared by transferring cells from agar slants to 50 mL of liquid medium in 250 mL Erlenmeyer flasks. The resulting cultures were incubated for 2 days at 37°C under aerobic conditions. Each starter culture was used as an inoculum for 100 mL of medium in 500 mL Erlenmeyer flasks. Samples were periodically withdrawn from the cultures to examine cell growth and production of CMCase by *B. amyloliquefaciens* DL-3.

Batch fermentations for the production of CMCase by *B. amyloliquefaciens* DL-3 were performed in 7 and 100 L bioreactors (Ko-Biotech Co., Korea). Working volumes of the 7 and 100 L bioreactors were 5 and 70 L, respectively. Carbon and nitrogen sources for batch fermentations were 5.0% (w/v) rice hull and 0.2% (w/v) peptone, respectively. Temperatures for fermentations with 7 and 100 L bioreactors were maintained at 37°C. The agitation speed of a 7 L bioreactor was varied from 200 to 500 rpm and its aeration rate was varied from 0.25 to 1.50 vvm to investigate effects of agitation speed and aeration rate on cell growth and production of CMCase. Optimal agitation speed and aeration rate for the 100 L bioreactor were 200 rpm and 1.0 vvm, respectively. Agitation was provided by three six-flat-blade impellers in 7 and 100 L fermentors. The inner pressure in the 100 L bioreactor was 0.2 kgf/cm^2. Inoculum size for batch fermentations for production of CMCase by *B. amyloliquefaciens* DL-3 was 5% (v/v).

Analytical Methods

Cell growth was measured as the absorbance at the optical density of 600 nm, and dry cells weight was measured by directly weighing the biomass after drying to a constant weight of 100~105°C, following the collection of cells by centrifugation at 12,000 × g for 10 min. Protein concentration was determined using the Bio-Rad protein assay kit (Hucules, USA) according to the manufacturer's instructions, with bovine serum albumin as the protein standard for the calibration curve.

The activity of CMCase produced by *B. amyloliquefaciens* DL-3 was determined based on the release of reducing sugar from carboxymethylcellulose (CMC) using the 3,5-dinitrosalicylic acid (DNS) method [20]. A mixture of dialyzed culture broth, after removal of cells, and 1% (w/v) CMC dissolved in 50 mM Tris-HCl buffer (pH 7.0) was incubated at 50°C for 20 min. The reaction was stopped by addition of the DNS reagent. The treated samples were boiled for 10 min, cooled in water for color stabilization, and optical density was measured at 550 nm. The activity of CMCase was determined by using a calibration curve for glucose (Sigma-Aldrich, UK). One unit of CMCase activity was defined as the amount of enzyme that released 1 μmol of reducing sugar equivalent to glucose, per minute, under the assay conditions.

RESULTS AND DISCUSSION

Effect of Carbon and Nitrogen Sources on the Production of CMCase

The effect of carbon and nitrogen sources on cell growth and production of CMCase by *B. amyloliquefaciens* DL-3 was examined. Carbon sources tested for the production of CMCase by *B. amyloliquefaciens* DL-3 were 2.0% (w/v) glucose, fructose, maltose, sucrose, rice bran, and rice hull. Rice bran and rice hull were byproducts obtained from the rice processing industry. Nitrogen sources tested were 0.25% (w/v) malt extract, peptone, tryptone, yeast extract, ammonium chloride, and ammonium nitrate.

B. amyloliquefaciens DL-3 hydrolyzed rice hull and utilized its hydrolytic products for cell growth and production of CMCase, as previously reported [15,19]. Cellulases and hemicellulases such as CMCase, FPase, β-glucosidase, xylanase, and β-xylosidase were produced from cellulosic biomass [2,6]. Sucrose and malt extract, as well as sucrose and peptone, were found to be the best carbon and nitrogen sources for the cell growth of *B. amyloliquefaciens* DL-3 (Table 1). Rice bran and rice hull were also utilized for the cell growth of *B. amyloliquefaciens* DL-3. Glucose and ammonium sulfate, fructose and peptone, maltose and yeast extract, sucrose and yeast extract, rice bran and peptone, and rice hull and peptone, as carbon and nitrogen sources, were found to be the best carbon and nitrogen source combinations for the production of CMCase by *B. amyloliquefaciens* DL-3 (Table 2). The highest production of CMCase was 102.0 U/mL per 72 h cultivation at 37°C under aerobic conditions when the carbon and nitrogen sources were 2.0% (w/v) rice hull and 0.25% (w/v) peptone, respectively. Agro-byproducts used in this study, such as rice bran and rice hulls, were more effective than glucose, fructose, maltose, or sucrose for production of the CMCase by *B. amyloliquefaciens* DL-3. The best carbon and nitrogen sources for the production of the CMCase were not the same as those identified for the cell growth of *B. amyloliquefaciens* DL-3.

Table 1. Effect of different carbon and nitrogen sources on cell growth of *B. amyloliquefaciens* DL-3[a]

Nitrogen sources	DCW (g/L)[b]					
	Carbon sources					
	Glucose	Fructose	Maltose	Sucrose	Rice bran	Rice hull
Malt extract	2.86 ± 0.12	4.62 ± 0.44	4.70 ± 0.36	5.22 ± 0.41	1.96 ± 0.15	1.58 ± 0.21
Peptone	2.82 ± 0.22	3.34 ± 0.23	4.70 ± 0.54	5.22 ± 0.35	1.72 ± 0.22	1.50 ± 0.12
Tryptone	4.66 ± 0.35	2.96 ± 0.12	2.64 ± 0.33	2.66 ± 0.32	2.08 ± 0.16	1.70 ± 0.15
Yeast extract	2.30 ± 0.15	2.96 ± 0.32	2.46 ± 0.14	3.60 ± 0.41	1.60 ± 0.21	1.50 ± 0.22
Ammonium chloride	2.20 ± 0.24	1.40 ± 0.07	1.76 ± 0.09	2.80 ± 0.22	1.86 ± 0.13	1.60 ± 0.21
Ammonium nitrate	2.34 ± 0.33	1.78 ± 0.14	2.28 ± 0.15	2.84 ± 0.32	1.96 ± 0.24	1.58 ± 0.13

[a]Cultivated at 37°C and 200 rpm in a rotary shaking incubator for 72 h.

[b]Means of triplicate experiments.

Table 2. Effect of different carbon and nitrogen sources on the production of CMCase by *B. amyloliquefaciens* DL-3

Nitrogen sources	CMCase activity (U/mL)					
	Carbon sources					
	Glucose	Fructose	Maltose	Sucrose	Rice bran	Rice hull
Malt extract	32.9 ± 4.6	7.7 ± 2.4	3.6 ± 1.8	7.5 ± 2.3	35.8 ± 5.3	73.3 ± 9.3
Peptone	12.2 ± 2.3	86.4 ± 6.5	12.1 ± 3.2	50.9 ± 8.1	84.2 ± 6.2	102.0 ± 15.2
Tryptone	12.9 ± 2.7	14.9 ± 2.1	62.2 ± 5.6	62.5 ± 5.8	58.7 ± 6.1	71.5 ± 5.9
Yeast extract	76.4 ± 5.6	11.9 ± 2.7	86.9 ± 7.1	74.1 ± 8.1	69.5 ± 8.4	81.1 ± 9.5
Ammonium chloride	86.4 ± 6.4	8.4 ± 1.6	9.6 ± 2.5	8.7 ± 3.4	61.4 ± 7.2	73.6 ± 6.2
Ammonium nitrate	15.9 ± 3.2	12.2 ± 1.1	8.6 ± 2.0	7.4 ± 2.5	53.6 ± 5.4	61.8 ± 6.8

Table 3. Effect of different rice hull and peptone concentrations on cell growth of *B. amlyoliquefaciens* DL-3

Peptone (%)	DCW (g/L)						
	Rice hull (%)						
	0	1.0	2.0	3.0	5.0	7.5	10.0
0.00	0.32 ± 0.05	1.04 ± 0.14	1.30 ± 0.19	1.70 ± 0.23	2.10 ± 0.28	2.21 ± 0.25	1.98 ± 0.29
0.05	0.44 ± 0.04	1.02 ± 0.16	1.38 ± 0.16	1.76 ± 0.18	2.30 ± 0.32	2.32 ± 0.35	2.04 ± 0.41
0.10	0.62 ± 0.08	1.15 ± 0.13	1.40 ± 0.22	1.80 ± 0.24	2.38 ± 0.28	2. 43 ± 0.42	2.21 ± 0.39
0.15	0.60 ± 0.11	1.12 ± 0.15	1.42 ± 0.21	1.86 ± 0.27	2.36 ± 0.31	2.45 ± 0.35	2.36 ± 0.36
0.20	0.76 ± 0.06	1.10 ± 0.16	1.52 ± 0.17	1.98 ± 0.19	2.42 ± 0.36	2.52 ± 0.26	2.21 ± 0.43
0.25	0.72 ± 0.10	1.16 ± 0.09	1.48 ± 0.13	2.08 ± 0.25	2.51 ± 0.42	2.42 ± 0.35	2.18 ± 0.38
0.30	0.70 ± 0.12	1.21 ± 0.19	1.68 ± 0.21	2.10 ± 0.18	2.64 ± 0.35	2.35 ± 0.37	2.11 ± 0.37
0.50	0.68 ± 0.08	1.24 ± 0.16	1.80 ± 0.17	2.04 ± 0.27	2.36 ± 0.22	2.26 ± 0.28	2.02 ± 0.33

Effect of Different Concentrations of Rice Hull and Peptone on the Production of CMCase

The effect of different concentrations of rice hull and peptone as carbon and nitrogen sources on cell growth and production of CMCase by *B. amyloliquefaciens* DL-3 was examined. The composition of the rice hull used in this study was as follows: 47.0% fiber, 0.2% crude lipid, 2.4% crude protein, 14.1% ash, and 7.1% water. Total carbohydrate in rice hull was about 76%. Concentration of rice hull was varied from 0.0 to 10.0% (w/v), whereas the concentration of peptone was varied from 0.0 to 0.50% (w/v) to examine the effects on cell growth and production of CMCase. Cell growth of *B. amyloliquefaciens* DL-3 was enhanced with higher concentrations of rice hull as well as peptone (Table 3). The highest production of CMCase was

Table 4. Effect of different rice hull and peptone concentrations on the production of CMCase by *B. amyoliqueifaciens* DL-3

Peptone (%)	CMCase activity (U/mL)						
	Rice hull (%)						
	0	1.0	2.0	3.0	5.0	7.5	10.0
0.00	16.3 ± 4.3	26.8 ± 5.4	67.8 ± 7.2	82.1 ± 6.5	96.3 ± 13.2	73.3 ± 8.4	66.4 ± 9.3
0.05	18.5 ± 5.2	33.2 ± 4.9	65.5 ± 8.3	104.0 ± 11.4	126.6 ± 11.4	117.6 ± 13.5	87.2 ± 8.1
0.10	22.3 ± 4.3	36.3 ± 4.5	65.5 ± 6.6	105.4 ± 9.7	142.9 ± 16.3	121.4 ± 14.3	105.3 ± 21.3
0.15	20.5 ± 3.4	37.5 ± 5.3	78.8 ± 8.4	138.5 ± 10.2	157.2 ± 13.2	127.5 ± 15.3	118.1 ± 18.6
0.20	28.2 ± 3.9	46.9 ± 6.1	98.8 ± 7.3	138.5 ± 10.2	211.7 ± 19.5	131.2 ± 16.9	105.4 ± 14.3
0.25	25.6 ± 4.3	54.1 ± 5.3	81.1 ± 10.2	163.7 ± 21.4	188.7 ± 21.2	122.5 ± 15.7	97.2 ± 16.8
0.30	17.3 ± 3.6	23.3 ± 3.4	82.0 ± 9.8	166.6 ± 16.5	165.7 ± 19.3	120.6 ± 14.5	95.4 ± 18.2
0.50	14.0 ± 4.1	61.9 ± 7.2	81.5 ± 6.3	156.3 ± 17.2	149.4 ± 12.1	113.5 ± 19.3	81.2 ± 10.6

Table 5. Effect of temperature and initial pH of the medium on cell growth of *B. amylolizueifaciens* DL-3

Temperature (°C)	DCW (g/L)					
	Initial pH					
	5.8	6.8	7.3	7.8	8.3	9.3
32	2.14 ± 0.19	2.20 ± 0.16	2.96 ± 0.36	2.80 ± 0.17	2.70 ± 0.16	2.36 ± 0.32
37	2.04 ± 0.31	2.13 ± 0.27	2.30 ± 0.22	2.40 ± 0.26	2.30 ± 0.34	2.11 ± 0.16
42	1.08 ± 0.29	1.18 ± 0.16	1.24 ± 0.16	1.52 ± 0.22	1.50 ± 0.31	1.32 ± 0.29

Table 6. Effect of temperature and initial pH of the medium on the production of CMCase by *B. amyoliqueifaciens* DL-3

Temperature (°C)	CMCase activity (U/mL)					
	Initial pH					
	5.8	6.8	7.3	7.8	8.3	9.3
32	124.6 ± 17.3	204.5 ± 16.5	179.2 ± 15.6	144.0 ± 18.2	133.0 ± 16.1	104.6 ± 16.8
37	148.4 ± 11.6	211.0 ± 19.1	205.4 ± 22.3	204.8 ± 13.3	179.8 ± 21.3	142.6 ± 18.2
42	156.6 ± 23.2	199.4 ± 23.1	181.9 ± 19.6	168.8 ± 18.7	165.8 ± 19.5	140.3 ± 22.4

211.7 U/mL per 72 h cultivation at 37°C under aerobic conditions when concentrations of rice hull and peptone were 5.0 and 0.20%, respectively (Table 4), whereas the highest cell growth was obtained when concentrations of rice hull and peptone were 5.0 and 0.30%, respectively.

Effect of Temperature and Initial pH of the Medium on the Production of CMCase

The effect of temperature and initial pH of the medium on cell growth and production of CMCase was investigated. Carbon and nitrogen sources were 5.0% rice hull and 0.2% peptone. Temperatures assayed ranged from 32 to 42°C and the initial pH of the medium was varied from 5.8 to 9.3. Optimal temperature and initial pH of the medium for cell growth of *B. amyloliquefaciens* DL-3 were 32°C and 7.8, respectively (Table 5). The conditions for the optimal production of CMCase by *B. amyloliquefaciens* DL-3 were 37°C and 6.8 (Table 6). The highest production of CMCase from 5.0% rice hull and 0.20% peptone was 211.0 U/mL under these optimal conditions. Optimal temperature and initial pH of the medium for production of the CMCase by *B. amyloliquefaciens* DL-3 were also different from those conditions that produced optimal cell growth.

Effect of Agitation and Aeration on the Production of CMCase

The effect of agitation speed on cell growth and production of CMCase was investigated in a 7 L bioreactor (Ko-Biotech Co., Korea). Agitation speed was varied from 200 to 500 rpm and aeration rate was 1.0 vvm. The temperature and initial pH of medium for production of CMCase by *B. amyloliquefaciens* DL-3 were 37°C and 6.8, respectively. Higher agitation speeds, which resulted in an increased concentration of dissolved oxygen in the medium, en-

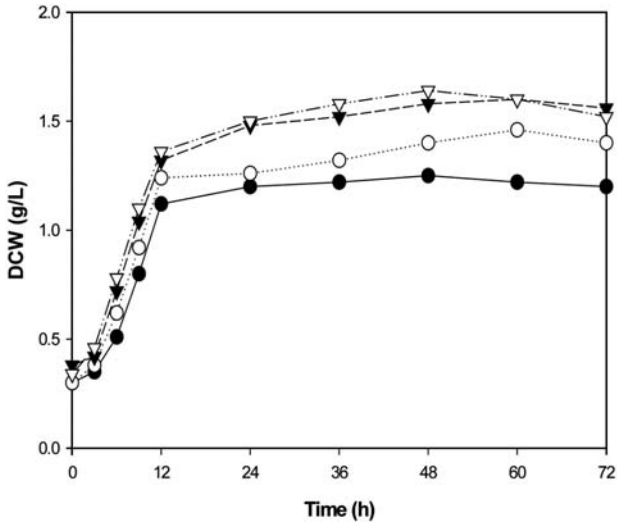

Fig. 1. Effect of agitation speed on the cell growth of *B. amyoliqueifaciens* DL-3 in a 7 L bioreactor. Aeration rate was 1.0 vvm (●, 200 rpm; ○, 300 rpm; ▼, 400 rpm; ▽, 500 rpm).

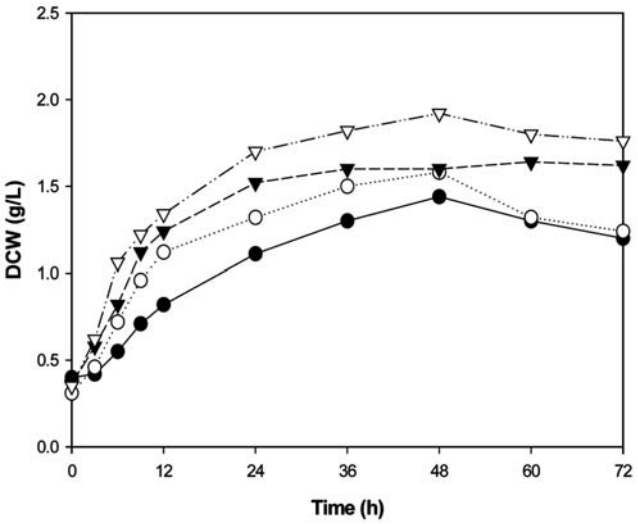

Fig. 3. Effect of aeration rate on the cell growth of *B. amyoliqueifaciens* DL-3 in a 7L bioreactor. Agitation speed was 400 rpm (●, 0.25 vvm; ○, 0.5 vvm; ▼, 1.0 vvm; ▽, 1.5 vvm).

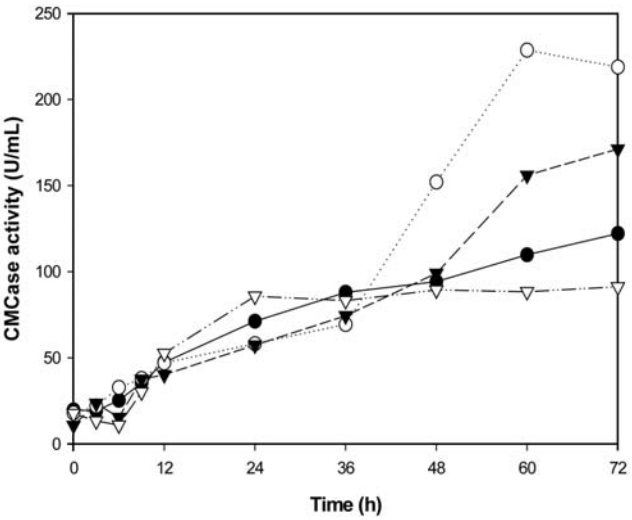

Fig. 2. Effect of agitation speed on the production of CMCase by *B. amyoliqueifaciens* DL-3 in a 7 L bioreactor (●, 200 rpm; ○, 300 rpm; ▼, 400 rpm; ▽, 500 rpm).

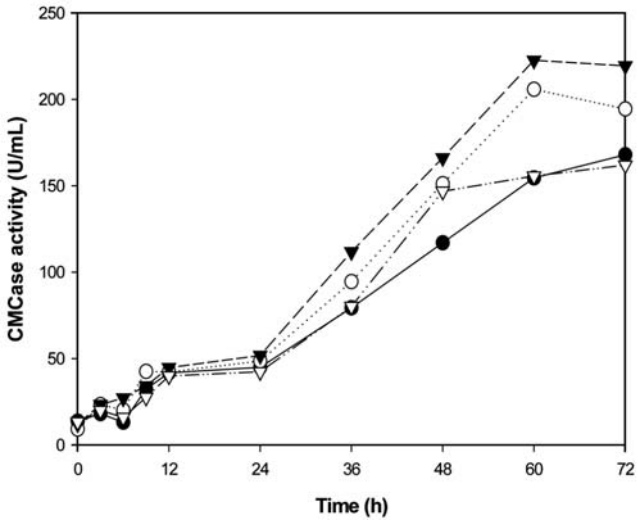

Fig. 4. Effect of aeration rate on the production of CMCase by *B. amyoliqueifaciens* DL-3 in a 7 L bioreactor (●, 0.25 vvm; ○, 0.5 vvm; ▼, 1.0 vvm; ▽, 1.5 vvm).

hanced cell growth of *B. amyloliquefaciens* DL-3 (Fig. 1). Optimal agitation speed for the production of CMCase by *B. amyloliquefaciens* DL-3 was lower than that determined for cell growth. The highest production of CMCase (219.4 U/mL) was observed at an agitation speed of 300 rpm (Fig. 2).

The effect of aeration rate on cell growth and production of CMCase was also investigated. Aeration rate was varied from 0.25 to 1.50 vvm and the agitation speed was 300 rpm. Higher aeration rates, as well as higher agitation speeds, also enhanced cell growth of *B. amyloliquefaciens* DL-3 (Fig. 3). The optimal aeration rate for cell growth was 1.5 vvm, whereas that for production of CMCase was 1.0 vvm (Fig. 4). The highest production of CMCase by *B. amyloliquefaciens* DL-3 in a 7 L bioreactor was 220.2 U/mL when the agitation speed and aeration rate were 300 rpm and 1.0 vvm, respectively.

Cell growth and production of cellulases by *T. reesei* were shown to be affected by the dissolved oxygen concentration. Production of enzymes by *T. reesei* dropped at higher agitation rates [21]. It seemed that high concentrations of dissolved oxygen promoted cell growth of *B. amyloliquefaciens* DL-3 while inhibiting the production of CMCase.

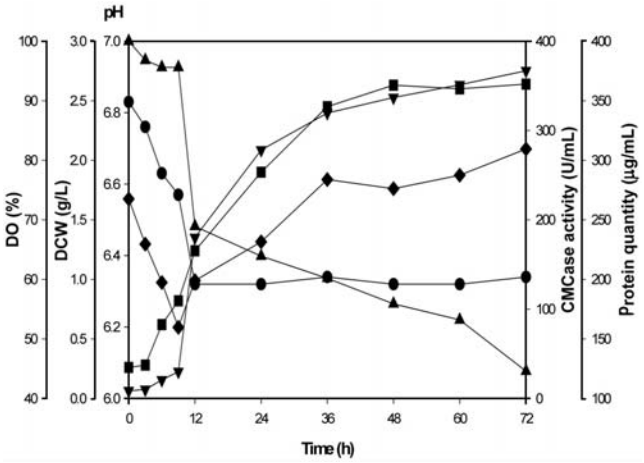

Fig. 5. Growth curve and production of CMCase by *B. amyloliquefaciens* DL-3 in a culture medium containing 5.0% (w/v) rice hull as a carbon source and 0.2% (w/v) peptone as a nitrogen source in a 100 L bioreactor (●, pH; ■, DCW; ▲, DO; ▼, CMCase activity; ◆, protein quantity).

Pilot-Scale Production of CMCase in a 100 L Bioreactor

Batch cultures for the production of CMCase by *B. amyloliquefaciens* DL-3 were performed in a 100 L bioreactor with an inner pressure of 0.2 kgf/cm^2 (Fig. 5). Carbon and nitrogen sources for the production of CMCase were 5.0% rice hull and 0.20% peptone. Temperature and initial pH of the medium were 37°C and 6.8. Agitation speed and aeration rate of the 100 L bioreactor were 200 rpm and 1.0 vvm. The radius of the impeller in a 100 L bioreactor was bigger than that in a 7 L bioreactor. The angular velocity of a 100 L bioreactor at 200 rpm is almost same as that of a 7 L bioreactor at 300 rpm.

The pH in the medium gradually decreased until 9 h of cultivation, then pH was maintained at approximately 6.3 thereafter. Cell growth of *B. amyloliquefaciens* DL-3 rapidly increased until 36 h. Production of the CMCase by *B. amyloliquefaciens* DL-3 started after a dramatic decrease in the concentration of dissolved oxygen at 9 h. The highest production of CMCase from 5.0% rice hull and 0.20% peptone as carbon and nitrogen sources in a 100 L bioreactor was 367.3 U/mL. Production of CMCase seemed to parallel the cell growth of *B. amyloliquefaciens* DL-3. The production of cellulases by *T. reesei* surged as soon as growth was limited by the hydrolysis of avicell that was used as a carbon source [21].

Various carbon sources have been used for the production of cellulases, however their cost are too high for commercial applications [22,23]. Rice hulls acquired from the rice processing industry are produced in large amounts in Korea, as well as other rice producing countries [9]. Rice hulls are a complex lignocellulosic biomass with about 15% lignin, 36% cellulose, 12% hemicellulose, and 18% ash [10]. It has no significant use, but contributes to serious environmental problems. Production of the carboxymethylcellulases by *Paecilomyces* sp. in medium containing 5% (w/v) rice hull has been reported previously [24], but the enzyme productivity was too low to be applied to a large scale of the production of cellulase. Other microorganisms have been reported to produce carboxymethylcellulases [25,26], but their substrates were not inexpensive enough for commercialization.

CONCLUSION

Production of ethanol from rice hull via dilute acid pretreatment, enzymatic saccharification, and fermentation has been reported [10]. An enzymatic approach is preferred, but at least three classes of enzymes are needed: endoglucanase, exoglucanase, and β-glucosidase [27]. The enzymatic saccharification of rice hull was performed by commercial cellulases, in which the major cellulase was CMCase [9]. CMCase is a key enzyme that functions during the decomposition of plant root-hair walls during symbiosis of bacteria with plants [25]. Exogenously added cellulases resulted in increased ethanol yield in the simultaneous saccharification and fermentation (SSF) process [28], but the hydrolysis of lignocellulosic biomass by cellulases in the SSF process must be made cost efficient for the commercial production of ethanol [29]. In this study, *B. amyloliquefaciens* DL-3 produced 367.3 U/mL of CMCase from 5% (w/v) rice hull as a substrate after 72 h of submerged fermentation in a 100 L bioreactor. The productivity and production scale of the CMCase by *B. amyloliquefaciens* DL-3 in this study are higher than any other reported production by *Trichoderma*, *Aspergillus*, *Pseudomonas*, *Streptomyces*, or *Bacillus* species [14,26,30]. The production of CMCase as demonstrated in this study can be used for the hydrolysis of lignocellulosic biomass for cost efficient commercial production of ethanol.

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