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Pilot-scale Optimization of Parameters Related to Dissolved Oxygen for Mass Production of Pullulan by *Aureobasidium pullulans* HP-2001

Wa Gao[1,2], Yi-Joon Kim[1,2], Chung-Han Chung[2,3], Jianhong Li[4] and Jin-Woo Lee[2,3]*

[1]*Department of Medical Bioscience, Graduate School of Donga-A University, Busan 604-714, Korea*
[2]*BK21 Bio-Silver Project of Dong-A University, Busan 604-714, Korea*
[3]*Department of Biotechnology, Dong-A University, Busan 604-714, Korea*
[4]*College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China*

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Parameters related to dissolved oxygen for the production of pullulan by *Aureobasidium pullulans* HP-2001 were optimized in 7 l and 100 l bioreactors. The optimal concentrations of glucose and yeast extract for the production of pullulan were 50.0 and 2.5 g/l, respectively, and its conversion rate from glucose was 37% at a flask scale. The optimal initial pH of the medium and temperature for cell growth were 7.5 and 30°C, whereas those for the production of pullulan were 6.0 and 25°C. The optimal agitation speed and aeration rate for cell growth were 600 rpm and 2.0 vvm in a 7 l bioreactor, whereas those for the production of pullulan were 500 rpm and 1.0 vvm. The production of pullulan with an optimized agitation speed of 500 rpm and aeration rate of 1.0 vvm was 18.13 g/l in a 7 l bioreactor. Maximal cell growth occurred without inner pressure, whereas the optimal inner pressure for the production of pullulan was 0.4 kgf/cm^2 in a 100 l bioreactor. The production of pullulan under optimized conditions in this study was 22.89 g/l in a 100 l bioreactor, which was 1.38 times higher than that without inner pressure.

Key words : *Aureobasidium pullulans*, pullulan, agitation speed, aeration rate, inner pressure

Introduction

Pullulan is a linear extracellular homopolysaccharide consisting of maltotriose and maltotetraose units interconnected by α-(1→6) and α-(1→4) linkages [1,25]. Due to its structural flexibility and enhanced solubility resulting from its regular alternation [17], pullulan provides highly viscous solutions at relatively low concentrations and can be utilized to make an oxygen-impermeable membrane [32]. Membranes formed from pullulan are suitable for coating foods and pharmaceuticals, especially when exclusion of oxygen is desirable [10]. Pullulan has a wide range of commercial and industrial applications in many fields, such as food industries, pharmaceuticals manufacturing, health care, and cosmetic industries [32].

Pullulan can be produced in large quantities by fermentation of *Aureobasidium pullulans* [20]. Although the mechanism of pullulan biosynthesis is still not fully understood, it was definitely proved that the production of pullulan is directly related to the conversion rate of substrates [14,31]. Some important physiological factors which affect production of pullulan are carbon sources, nitrogen sources , initial pH of the medium , temperature , the oxygen supply , and mineral salts [2,6,19,29]. The fungus, *Aureobasidium pullulans*, has a complex life cycle and can grow in a range of morphological forms including blastospore (yeast-like cells), hyphae, psuedohyphase, swollen cells, and chlamydospores [22,29]. High production of pullulan has been found to be correlated with high concentration of yeast-like cells in the culture [27]. The morphological form of *A. pullulans* is influenced by medium compositions and cultural conditions such as carbon and nitrogen sources, pH, temperature, and dissolved oxygen level [9]. However, there have been few reports on effect of dissolved oxygen on the production of pullulan by *A. pullulans*. Maximal production of pullulan from 100.0 g/l glucose by *A. pullulan* was reported to be 36.9 g/l, however, its conversion rate of pullulan was 36.9% and concentration of residual sugar after fermentation was relatively high, which interrupted to recover pullulan from culture broth [29]. Continuous culture of *A. pullulans* was reported to enhance production of pullulan as well as its conversion rate [30].

We had developed optimal concentrations of salts for the enhanced production of pullulan by *A. pullulans* HP-2001 in

***Corresponding author**
Tel : +82-51-200-7593, Fax : +82-51-200-7505
E-mail : jwlee@dau.ac.kr

a medium with higher concentrations for carbon and nitrogen sources [6]. In this study, effects of parameters related to the dissolved oxygen (DO) in the medium with previously optimized concentrations of salts on cell growth and production of pullulan by *A. pullulans* HP-2001 were investigated. The agitation speed and aeration rate in a 7 l bioreactor and the inner pressure in a 100 l bioreactor for cell growth and the production of pullulan by *A. pullulans* were optimized in this study.

Materials and Methods

Bacterial strain and medium

Aureobasidium pullulans HP-2001, a UV-induced mutant of *A. pullulans* ATCC 42023, was transferred monthly to fresh nutrient agar medium [29]. Cells in agar slants were incubated at 30°C for 48 hr and stored at 4°C. The medium for cell growth and the production of pullulan by A. pullulans HP-2001 contained 50 g/l glucose, 0.25 g/l yeast extract, 7.5 g/l K_2HPO_4, 1.0 g/l NaCl, 0.1 g/l $MgSO_4 \cdot 7H_2O$, and 2.4 g/l $(NH_4)_2SO_4$, which was optimized for higher concentrations of carbon sources in the previous work [6]. Glucose was autoclaved separately and added to the medium in aseptic conditions.

Production of pulluan

Starter cultures were prepared by transferring cells from a slant to 50 ml of medium in 250 ml flasks and incubated at 30°C and 200 rpm for 48 hr. Each starter culture was used as an inoculumn for 100 ml of medium in 500 ml Erlenmeyer flasks. Samples were periodically withdrawn from the cultures to examine cell growth and the production of pullulan by *A. pullulans* HP-2001.

Culture broth after 96 hr was centrifuged at 15,000 ×*g* for 15 min to remove cells. Supernatant was mixed with 2 volumes of isopropyl alcohol and incubated at 4°C for 24 hr to precipitate the crude product which was separated by centrifugation at 15,000 ×*g* for 20 min. The precipitated material was repeatedly washed with acetone and ether, dissolved in deionized water (DW) and dialyzed against DW by using dialysis tubing with a molecular weight cut off of 14,000~12,000. After dialysis for 2 to 3 days with four or five changes of DW, the dialyzed solution was lyophilized.

Batch fermentations for the production of pullulan by *A. pullulans* HP-2001 were performed in 7 l and 100 l bioreactors (Ko-Biotech Co., Korea). Working volumes of 7 l and 100 l bioreactors were 5 l and 70 l, respectively. Carbon and nitrogen sources for batch fermentations were 50.0 g/l glucose and 2.5 g/l yeast extract, respectively. Temperatures for fermentations in 7 l and 100 l bioreactors were maintained at 25°C. The agitation speed and aeration rate of a 7 l bioreactor ranged from 200 to 600 rpm and from 0.5 to 2.0 vvm during the investigation of effects of agitation speed and aeration rate on cell growth and the production of pullulan by *A. pullulans* HP-2001. The agitation speed and aeration rate of a 100 l bioreactor were 300 rpm and 1.0 vvm, respectively. Agitation was provided by three six-flat-blade impellers in 7 l and 100 l bioreactors. Inner pressure in the 100 l bioreactor ranged from 0.0 to 0.8 kgf/cm^2. Inoculumn size of batch fermentations for production of pullulan by *A. pullulans* HP-2001 was 5% (v/v).

Analytical methods

Harvested cells were washed three times with distilled water and transferred to pre-weighted aluminum dishes, dried at 90-100°C to a constant weight and then weighed [29]. The concentration of pullulan was determined colorimetrically by the phenol-sulfuric acid method [3]. A standard curve for quantitation of pullulan was prepared from authentic pullulan (Sigma-Aldrich, St. Louis, USA). Reducing sugar contents in the culture broth were determined by the dinitrosalicylic acid (DNS) method [23]. DNS reagent (1 ml) was mixed with 0.3 ml of the culture broth, and the mixture was placed in a boiling water bath for 15 min. After the mixture was cooled to room temperature, the concentration of reducing sugar was examined at 550 nm with a spectrophotometer (Unicam Co., Helios Delta, UK).

Results and Discussion

Effect of glucose on production of pullulan

The effects of glucose as a carbon source on cell growth and the production of pullulan by *A. pullulans* HP-2001 were investigated. The concentration of glucose ranged from 0.0 to 300.0 g/l. Cell growth and the production of pullulan by *A. pullulans* HP-2001 increased with increased concentrations of glucose up to 200.0 g/l and 150 g/l, respectively, as shown in Table 1. Significance of each value was analyzed by DPS software version 3.01 (DPS Co., Middlesex, UK). Total utilization yield ($Y_{x/s}+Y_{p/s}$) from various concentrations of glucose as a carbon source ranged from 0.10 to 0.55. Maximal production of pullulan by *A. pullulans*

Table 1. Effect of glucose on cell growth and the production of pullulan by *A. pullulans* HP-2001[1]

Glucose (g/l)	Final pH[2]	DCW (g/l)	Pullulan (g/l)	Reducing sugar (g/l)	Yield		
					$Y_{x/s}$	$Y_{p/s}$	$Y_{p/x}$
0.0	7.51±0.14^{a3}	0.63±0.22^{e}	0.14±0.12^{d}	0.12±0.10^{e}	-	-	0.22
25.0	6.22±0.16^{b}	5.19±0.40^{d*}	5.76±0.42^{c*}	2.24±0.81^{e}	0.21	0.23	1.11
50.0	4.82±0.13^{c}	12.15±1.05^{c*}	15.56±1.26^{b*}	5.76±0.90^{e}	0.24	0.31	1.29
100.0	4.07±0.12^{e}	13.12±1.24^{bc*}	20.12±1.82^{a*}	25.87±1.87^{d*}	0.13	0.21	1.53
150.0	3.96±0.14^{e}	14.23±1.26^{ab*}	20.84±1.72^{a*}	51.55±3.21^{c*}	0.09	0.14	1.46
200.0	4.35±0.12^{d}	16.05±1.43^{a*}	19.19±1.56^{a*}	86.65±6.42^{b*}	0.08	0.10	1.20
300.0	4.40±0.10^{d}	15.53±1.36^{a*}	16.21±1.31^{b*}	125.19±9.43^{a*}	0.05	0.05	1.04

[1]This experiment was carried out at 30°C and 200 rpm in a shaking incubator for 96 hr.
[2]Results are means of triplicate experiments.
[3]Values with different letters are significantly different at $p<0.05$.

HP-2001 after 96 hr was 20.84 g/l when the glucose concentration was 150.0 g/l, however, the highest conversion rate of pullulan from glucose was found to be 31% when the concentration of glucose was 50.0 g/l.

The optimal concentration of glucose or sucrose as a carbon source for the production of pullulan has been reported to be 50.0 or 75.0 g/l and the highest conversion rate of pullulan was about 38% [29,31]. Kinetic studies on the production of pullulan by *A. pullulan* ZQ-01 showed that the maximal production of pullulan from 50.0 g/l sucrose was 16.0 g/l [26].

Effect of yeast extract on production of pullulan

The effects of yeast extract as a nitrogen source on cell growth and the production of pullulan by *A. pullulans* HP-2001 were also investigated. The concentration of yeast extract ranged from 0.0 to 20.0 g/l. Cell growth and the production of pullulan by *A. pullulans* HP-2001 also increased with increased concentrations of yeast extract up to 7.5 g/l and 2.5 g/l, respectively, as shown in Table 2. Total utilization yield of 50.0 g/l glucose and various concentrations of yeast extract ranged from 0.11 to 0.62. The maximal production of pullulan by *A. pullulans* HP-2001 was 18.42 g/l and its conversion rate from glucose was 37% when the concentration of yeast extract was 2.5 g/l. The optimal concentration of yeast extract for cell growth was different from that for production of pullulan by *A. pullulans* HP-2001.

The optimal concentration of yeast extract for the production of pullulan by *A. pullulans* HP-2001 varied with the concentration of glucose and the depletion of nitrogen sources might be essential for higher production of pullulan [29]. The production of pullulan was affected by the nitrogen source in the medium and its yield fell when excess nitrogen sources were present. Cell growth increased with increased concentrations of yeast extract up to 5.0 g/l, however, it decreased beyond this concentration due to substrate inhibition [30]. The optimal concentration of yeast extract as a nitrogen source for the production of pullulan was reported to be 0.31 g/l based on analysis of the multivariable linear regression [34].

Table 2. Effect of yeast extract on cell growth and the production of pullulan by *A. pullulans* HP-2001[1]

Yeast extract (g/l)	Final pH	DCW (g/l)	Pullulan (g/l)	Reducing sugar (g/l)	Yield		
					$Y_{x/s}$	$Y_{p/s}$	$Y_{p/x}$
0.0	3.62±0.21^{f}	5.74±0.48^{e*}	2.98±0.32^{ef}	36.30±3.72^{a*}	0.11	0.06	0.52
1.0	3.64±0.18^{f}	11.21±0.95^{c*}	11.33±1.04^{b*}	8.14±1.46^{b*}	0.22	0.23	1.01
2.5	4.33±0.15^{e*}	12.45±1.02^{bc*}	18.42±1.42^{a*}	0.26±0.12^{c}	0.25	0.37	1.48
5.0	5.63±0.12^{d*}	13.58±1.18^{ab*}	9.22±0.81^{c*}	0.24±0.16^{c}	0.27	0.18	0.68
7.5	5.88±0.14^{cd*}	14.39±1.26^{a*}	6.05±0.43^{d*}	0.44±0.24^{c}	0.29	0.12	0.42
10.0	6.11±0.12^{bc*}	12.75±1.09^{abc*}	3.69±0.37^{e*}	0.47±0.18^{c}	0.26	0.08	0.29
15.0	6.31±0.14^{b*}	7.51±0.62^{d*}	3.55±0.33^{e*}	1.87±0.43^{c}	0.15	0.07	0.47
20.0	7.59±0.14^{a*}	3.52±0.33^{f}	2.12±0.32^{f}	5.67±0.82^{b*}	0.07	0.04	0.60

[1]The concentration of glucose as a carbon source was 50.0 g/l.

Effect of initial pH of medium on production of pullulan

The effects of initial pH of the medium on cell growth and the production of pullulan were examined. The initial pH of the medium ranged from 5.0 to 8.5. Total utilization yield of 50.0 g/l glucose and 2.5 g/l yeast extract at initial pHs from 5.0 to 8.5 ranged from 0.48 to 0.65, as shown in Table 3. The optimal initial pH for cell growth was 7.5, whereas that for the production of pullulan by *A. pullulans* HP-2001 was 6.0. The maximal production of pullulan from 50.0 g/l glucose and 2.5 g/l yeast extract was 20.58 g/l when the initial pH of the medium was 6.0. The optimal initial pH for cell growth was also different from that for the production of pullulan by *A. pullulans* HP-2001.

The optimal pHs for cell growth and the production of pullulan by *A. pullulan* ATCC 42023 have been reported to be 3.5 and 6.5, respectively [19]. The optimal initial pHs for cell growth of *Agrobacterium* sp. and *S. paucibilis* were 4.2 and 5.8, respectively, whereas those for the production of curdlan and gellan by these strains were 5.0 and 6.8, respectively [13,21]. Initial pH of the medium is one of the most critical parameters which affect cell growth and the production of pullulan [18]. Cell growth was very high at a low initial pH such as 2.0, however, the production of pullulan was low at this initial pH. Due to this phenomenon, two-staged fermentation for the production of pullulan was developed. In this process the first stage of fermentation was conducted at the very acidic pH for the best production of biomass. When the biomass concentration reached its maximum value, the second stage of fermentation was initiated by adjusting the medium pH to a higher value for promoting the synthesis of the polysaccharide [15]. Initial pH of the medium also affects the molecular weight of pullulan and the morphology of *A. pulluans* [19].

Effect of temperature on production of pullulan

The effects of temperature on cell growth and the production of pullulan were also examined. The temperature ranged from 20 to 40°C. Total utilization yield of 50.0 g/l glucose and 2.5 g/l yeast extract at initial pH of 6.0 ranged from 0.06 to 0.61, as shown in Table 4. The optimal temperature for cell growth was 30°C, whereas that for the production of pullulan by *A. pullulans* HP-2001 was 25°C. The maximal production of pullulan from 50.0 g/l glucose and

Table 3. Effect of initial pH of medium on cell growth and the production of pullulan by *A. pullulans* HP-2001[1]

Initial pH	Final pH	DCW (g/l)	Pullulan (g/l)	Reducing sugar (g/l)	Yield		
					$Y_{x/s}$	$Y_{p/s}$	$Y_{p/x}$
5.0	2.89±0.31^{e}	10.69±0.84^{b}	16.73±1.35^{b*}	4.34±0.73^{a*}	0.22	0.33	1.57
5.5	3.01±0.24^{e}	11.89±0.96^{ab}	17.50±1.50^{b*}	1.05±0.32^{b*}	0.24	0.35	1.47
6.0	3.47±0.26^{d*}	11.92±0.87^{ab}	20.58±1.72^{a*}	0.16± 0.14^{c}	0.24	0.41	1.72
6.5	4.50±0.16^{c*}	11.96±0.92^{ab}	17.44±1.43^{b*}	0.19±0.15^{c}	0.24	0.35	1.46
7.0	4.43±0.14^{c*}	13.00±1.13^{a*}	13.90±1.13^{c}	0.26±0.18^{c}	0.26	0.28	1.07
7.5	5.05±0.12^{b*}	13.30±1.24^{a*}	12.66±1.25^{c}	0.28±0.16^{c}	0.27	0.25	0.95
8.0	6.05±0.10^{a*}	11.40±1.07^{ab}	12.69±1.07^{c}	0.25±0.14^{c}	0.23	0.25	1.11
8.5	6.12±0.12^{a*}	11.77±1.13^{ab}	11.84±1.03^{c}	0.30±0.21^{c}	0.24	0.24	1.01

[1]Concentrations of glucose and yeast extract as carbon and nitrogen sources were 50.0 g/l and 2.5 g/l, respectively.

Table 4. Effect of culture temperature on cell growth and production of pullulan by *A. pullulans* HP-2001[1]

Temperature (°C)	Final pH	DCW (g/l)	Pullulan (g/l)	Reducing sugar (g/l)	Yield		
					$Y_{x/s}$	$Y_{p/s}$	$Y_{p/x}$
20	3.31±0.22^{c}	10.15±0.96^{b*}	17.75±1.41^{b*}	0.46±0.18^{c}	0.20	0.36	1.75
25	3.57±0.18^{c}	10.57±0.87^{b*}	19.94±1.58^{a*}	0.53±0.21^{c}	0.21	0.40	1.89
30	3.61±0.16^{c}	12.54±1.06^{a*}	17.70±1.49^{b*}	3.83±0.54^{bc}	0.25	0.35	1.41
35	4.21±0.15^{b*}	10.13±0.98^{b*}	1.65±0.28^{c}	4.63±0.82^{b*}	0.20	0.03	0.16
40	6.28±0.12^{a*}	2.26±0.19^{c}	0.53±0.32^{c}	45.83±4.61^{a*}	0.05	0.01	0.23

[1]Concentrations of glucose and yeast extract as carbon and nitrogen sources were 50.0 g/l and 2.5 g/l, respectively. The initial pH of medium was 6.0.

0.25 g/l yeast extract was 19.94 g/l when the temperature was 25°C. The optimal temperature for cell growth was also different from that for the production of pullulan by *A. pullulans* HP-2001 like other productions of microbial exopolysaccharides and enzymes.

The optimal initial pH and temperature for cell growth were 7.3 and 32°C, whereas those for the production of carboxymethylcelluase by *B. amyloliquefcaucne* were 6.8 and 32°C [12]. Temperature is of great significance for the production of pullulan by *A. pullulan*, and optimal temperatures for the production of pullulan have been shown to be normally between 24 and 26°C [2]. The most significant factor for the production of pullulan by *A. pullulans* was found to be temperature [11].

Effect of agitation speed on production of pullulan in a 7 l bioreactor

The effects of agitation speed on cell growth and the production of pullulan by *A. pullulans* HP-2001 were investigated in a 7 l bioreactor. The agitation speed ranged from 300 to 600 rpm and the aeration rate was 1.0 vvm. The carbon and nitrogen sources were 50.0 g/l glucose and 2.5 g/l yeast extract. The initial pH of the medium and temperature were 6.0 and 25°C. Cell growth and the production of pullulan by *A. pullulans* HP-2001 increased with increased agitation speeds, whereas reducing sugars decreased with increased agitation speed, as shown in Fig. 1. The optimal agitation speed for cell growth was 600 rpm, whereas that for the production of pullulan by *A. pullulans* HP-2001 was 500 rpm. Productions of pullulan by *A. pullulans* HP-2001 with agitation speeds of 300, 400, 500, and 600 rpm after 96 hr were 16.51, 17.12, 17.73, and 16.84 g/l, respectively. The highest conversion rate of pullulan from 50.0 g/l glucose was 34.5% in a 7 l bioreactor.

The production of pullulan by *A. pullulans* increased with oxygen availability, however, more than certain concentration of dissolved oxygen in the medium with an increase in the agitation speed resulted in a decline of the production of pullulan [16]. *A. pullulans* has a complex life cycle involving five different morphological types depending on culture conditions [33]. The increased mass transfer of oxygen changes in the percentage of yeast-like cells of *A. pullulan* in the medium, which is directly linked with the production of pullulan [24].

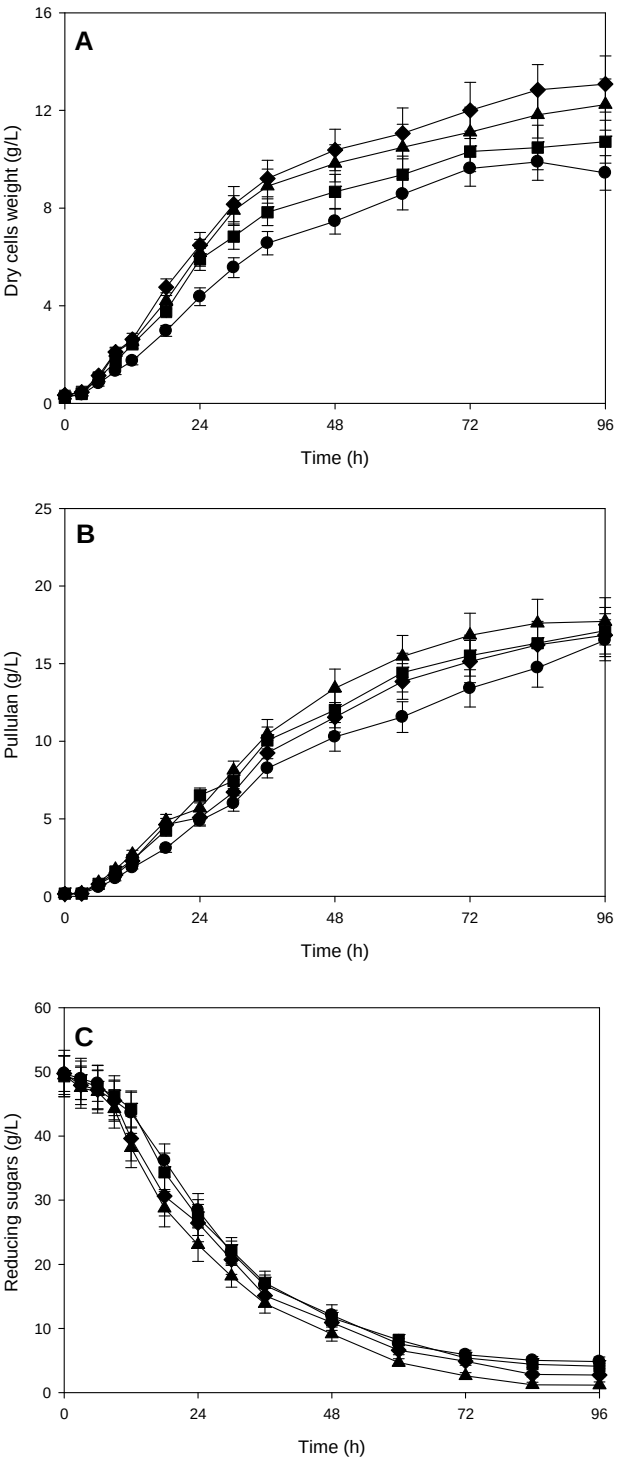

Fig. 1. Effect of agitation speed on cell growth (A), the production of pullulan by *A. pullulans* HP-2001 (B), and reducing sugar (C) in the culture medium in a 7 l bioreactor (●, 300 rpm; ■, 400 rpm; ▲, 500 rpm and ◆, 600 rpm).

Effect of aeration rate on production of pullulan in a 7 l bioreactor

The effects of aeration rate on cell growth and the production of pullulan by *A. pullulans* HP-2001 were also investigated in a 7 l bioreactor. The aeration rate ranged from 0.5 to 2.0 vvm and the agitation speed was 500 rpm. Cell growth of *A. pullulans* HP-2001 increased with increased aeration rates, however, the production of pullulan decreased with increased aeration rates, as shown in Fig. 2. The optimal aeration rate with an agitation speed of 500 rpm for cell growth was 2.0 vvm, whereas that for the production of pullulan by *A. pullulans* HP-2001 was 1.0 vvm. Productions of pullulan by *A. pullulans* HP-2001 with aeration rates of 0.5, 1.0, 1.5, and 2.0 vvm were 16.05, 18.13, 17.61, and 14.43 g/l, respectively. The highest conversion rate of pullulan from 50.0 g/l glucose was 36.3% in a 7 l bioreactor with an agitation speed of 500 rpm and an aeration rate of 1.0 vvm.

The optimal aeration rate for cell growth has been found to be 1.5 vvm with an agitation speed of 500 rpm in a 7 l bioreactor, whereas that for the production of pullulan by *A. pullulan* ATCC 42023 was 0.5 vvm [18]. The maximal production of pulluan with a conversion rate of 40% was achieved at an aeration rate of 1.0 vvm in a stirred tank reactor [28]. Generally speaking, the optimal agitation speed and aeration rate for cell growth seems to be higher than those for the production of microbial metabolites [7,12].

Effect of inner pressure on production of pullulan in a 100 l bioreactor

The effects of inner pressure on cell growth and the production of pullulan by *A. pullulans* HP-2001 were investigated in a 100 l bioreactor. The inner pressure ranged from 0.0 to 0.8 kgf/cm^2. The carbon and nitrogen sources were 50.0 g/l glucose and 2.5 g/l yeast extract. The initial pH and cultural temperature were 6.0 and 25°C. The agitation speed and aeration rate in a 100 l bioreactor were 250 rpm and 1.00 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 250 rpm is almost same as that of a 7 l bioreactor at 500 rpm.

The pH in the medium rapidly decreased until 24 hr after cultivation, then was maintained around 3.5, as shown in Fig. 3. The concentration of the dissolved oxygen (DO) in the medium dramatically decreased and then reached 0% in the middle of the log phase, at which point the production of pullulan by *A. pululans* HP-2001 started. After a limited

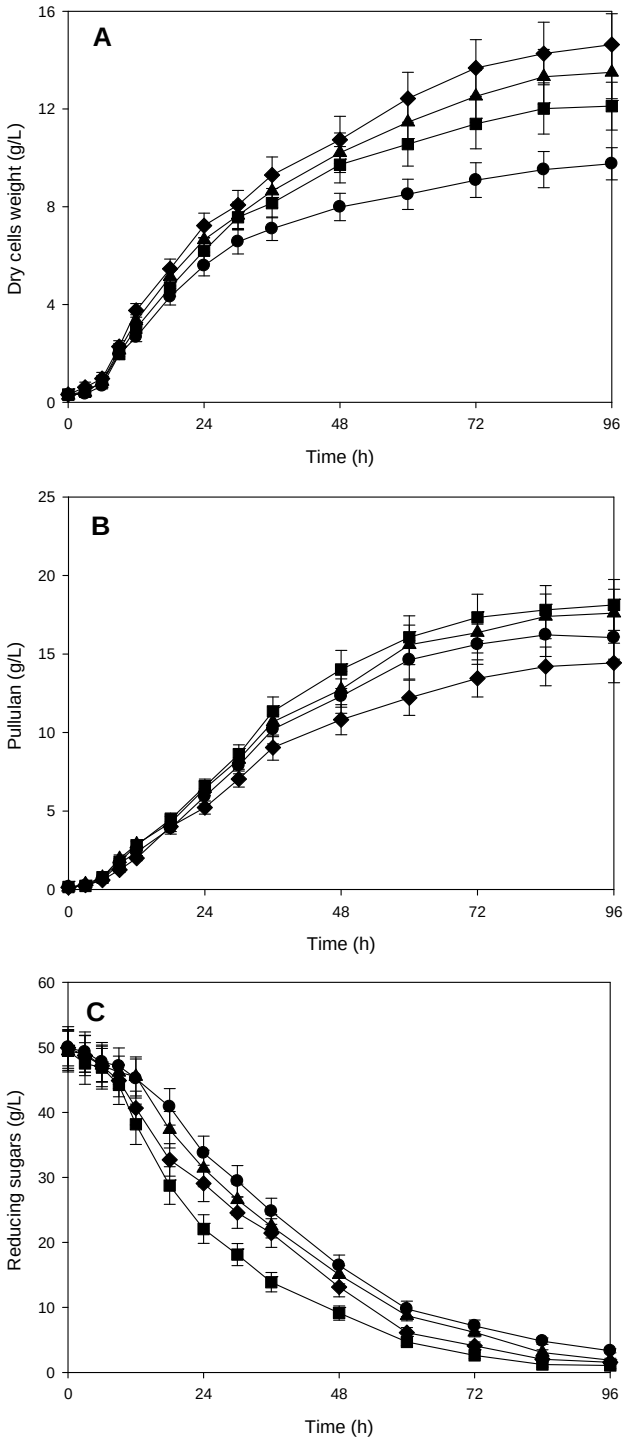

Fig. 2. Effect of aeration rate on cell growth (A), the production of pullulan by *A. pullulans* HP-2001 (B), and reducing sugar in the culture medium (C) in a 7 l bioreactor (●, 300 rpm; ■, 400 rpm; ▲, 500 rpm and ◆, 600 rpm).

time with a shortage of dissolved oxygen, it rose gradually until the final stage of cultivation. A certain period of time with a shortage of dissolved oxygen in the medium has been

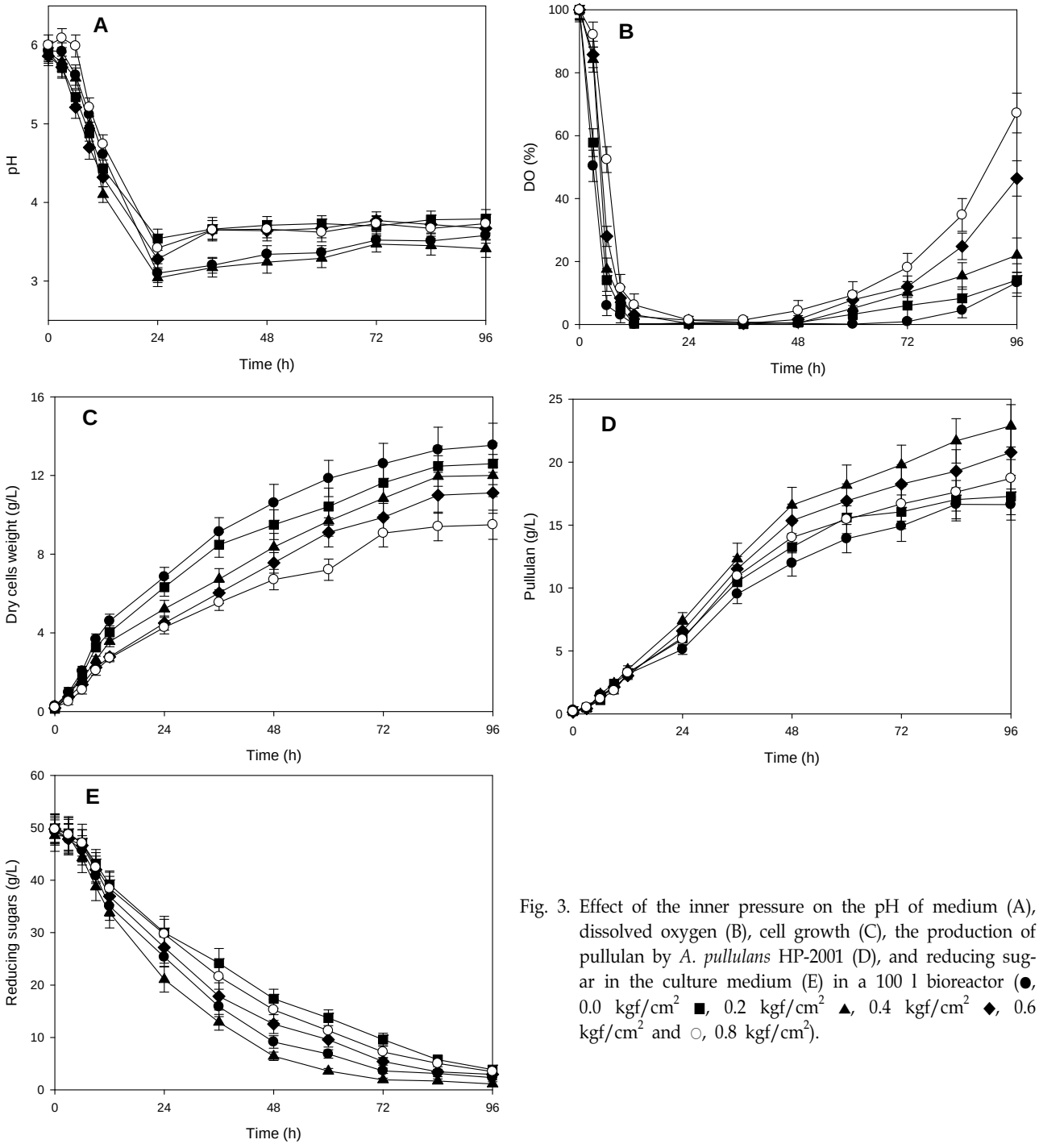

Fig. 3. Effect of the inner pressure on the pH of medium (A), dissolved oxygen (B), cell growth (C), the production of pullulan by *A. pullulans* HP-2001 (D), and reducing sugar in the culture medium (E) in a 100 l bioreactor (●, 0.0 kgf/cm^2 ■, 0.2 kgf/cm^2 ▲, 0.4 kgf/cm^2 ◆, 0.6 kgf/cm^2 and ○, 0.8 kgf/cm^2).

reported to be necessary to enhance the production of pullulan [31]. A shortage of dissolved oxygen or depletion of nitrogen sources seems to change morphological forms of *A. pullulans* to yeast-like cells, which is correlated with high production of pullulan [27]. Maximal cell growth occurred without an inner pressure in a 100 l bioreactor, however, the optimal inner pressure for the production of pullulan by *A. pullulans* HP-2001 was found to be 0.4 kgf/cm^2. Production of pullulan by *A. pullulans* HP-2001 with an inner pressure of 0.0, 0.2, 0.4, 0.6, and 0.8 kgf/cm^2 were 16.63, 18.28. 22.89, 20.78, and 18.71 g/l, respectively, as shown in Table 5. The production of pullulan with an inner pressure of 0.4 kgf/cm^2 was 1.38 times higher than that without inner pressure. Increased inner pressure in a 100 l bioreactor resulted in a higher concentration of dissolved oxygen in the medium, which might enhance the production of pullulan

Table 5. Effect of inner pressure on cell growth and production of pullulan by *A. pullulans* HP-2001 in a 100 l bioreactor

Inner presure (kgf/cm^2)	Final pH	DCW (g/l)	Pullulan (g/l)	Reducing sugar (g/l)	Yield		
					$Y_{x/s}$	$Y_{p/s}$	$Y_{p/x}$
0.0	3.58 ± 0.10^{ab}	$13.54\pm1.12^{a*}$	16.63 ± 1.24^{c}	$2.33\pm0.41^{b*}$	0.27	0.33	1.25
0.2	$3.79\pm0.12^{a*}$	$12.60\pm1.06^{ab*}$	17.78 ± 1.46^{c}	$3.86\pm0.62^{a*}$	0.25	0.36	1.41
0.4	3.41 ± 0.11^{b}	$12.00\pm1.07^{ab*}$	$22.89\pm1.68^{a*}$	1.13 ± 0.43^{c}	0.24	0.46	1.91
0.6	$3.67\pm0.14^{a*}$	11.12 ± 1.04^{bc}	$20.78\pm1.83^{ab*}$	$2.93\pm0.61^{ab*}$	0.22	0.42	1.87
0.8	$3.73\pm0.12^{a*}$	9.50 ± 0.74^{c}	18.71 ± 1.49^{bc}	$3.50\pm0.54^{a*}$	0.19	0.37	1.96

by *A. pullulans* HP-2001, as described in a previous report [29]. The production of pullulan by *A. pullulans* is tightly linked to morphological compositions of the culture [8]. The yeast-like cells are major forms involved in the production of pullulan [9].

The concentration of dissolved oxygen in the medium, which is influenced by agitation speed, aeration rate, and the inner pressure of bioreactors, can change the morphological content of *A. pullulans* [5,7]. Variations in the agitation speed and aeration rate result in a change in the concentration of dissolved oxygen in the medium, which in turn affects cell growth and the production of microbial metabolites such as lipase and β-mannanase [4,5]. It seems that a dissolved oxygen level higher than the optimal concentration for the production of pullulan by *A. pullulans* HP-2001, due to higher agitation speeds, aeration rates, and inner pressures, does not lead to the yeast-like cells.

In this study, concentrations of glucose and yeast extract as carbon and nitrogen sources, initial pH of the medium, and temperature for the production of pullulan by *A. pullulans* HP-2001 were optimized under optimized concentrations of salts in the previous work [6]. Some parameters related to the dissolved oxygen in the medium such as agitation speed, aeration rate, and inner pressure of bioreactor were also optimized. The optimal conditions for cell growth were found to be different from those for the production of pullulan by *A. pullulans* HP-2001. With the optimized conditions of agitation speed, aeration rate, and inner pressure in this study, the highest production of pullulan from 50.0 g/l glucose by *A. pullulans* HP-2001 was 20.89 g/l, which conversion rate of pullulan was 45.8 %. The production of pullulan with optimized inner pressure was 1.38 times higher than that without inner pressure.

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References

1. Catley, B. J., A. Ramsay, and C. Servis. 1986. Observations on the structure of fungal extracellular-polysaccharide, pullulan. *Carbohydr. Res*. **153**, 79-86.
2. Chi, Z. and S. Zhao. 2003. Optimization of medium and cultivation conditions for pullulan production by a new pullulan-producing yeast strain. *Enzym. Microb. Technol*. **33**, 206-211.
3. Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith. 1956. Colorimetic method for determination of sugars and related substances. *Anal. Chem*. **28**, 350-356.
4. Elibol, M. and D. Ozer. 2000. Influence of oxygen transfer on lipase production by *Rhizopus arrhizus*. *Process Biochem*. **36**, 325-329.
5. Feng, Y., Z. He, S. L. Ong, J. Hu, Z. Zhang, and W. J. Ng. 2003. Optimization of agitation, aeration, and temperature conditions for maximum β-mannase production. *Enzym. Microb. Technol*. **32**, 282-289.
6. Gao, W., Y. J. Kim, C. H. Chung, J. Li, and J. W. Lee. 2010. Optimization of mineral salts in medium for enhanced production of pullulan *by Aureobasodium pullulans* HP-2001 using orthogonal array method. *Biotechnol. Bioprocess Eng*. (in press).
7. Giavasis, I., L. M. Harvey, and B. McNeil. 2006. The effect of agitation and aeration on the synthesis and molecular weight of gellan batch cultures of *Sphingomonas paucimobilis*. *Enzym. Microb. Technol*. **38**, 101-108.
8. Guterman, H. and Y. Shabtai. 1996. A self-tuning vision system for monitoring biotechnological process. I. Application to production of pullulan by *Aureobasidium pullulans*. *Biotechnol. Bioeng*. **51**, 501-510.
9. Heald, P. and B. Kristiansen. 1985. Synthesis of polysaccharide by yeast-like forms of *Aureobasidium pullulans*. *Biotechnol. Bioeng*. **27**, 1516-1519.
10. Jelinek, M., R. Cristescu, E. Axente, T. Kocourek, J. Dybal, J. Remsa, J. Plestil, D. Mihaiescu, M. Albulescu, T. Buruiana, I. Stamatin, I. N. Mihailescu, and D. B. Chrisey. 2007. Matrix assisted pulsed laser evaporation of cinnamate-pullulan and tosylate-pullulan polysaccharide derivative thin films for pharmaceutical applications. *Appl. Surf. Sci*. **253**, 7755-7760.
11. Jiang, L. 2010. Optimization of fermentation conditions for

pullulan production by *Aureobasodium pullulans* using response surface methodology. *Carbohyr. Polym.* **79**, 414-417
12. Jo, K. I., Y. J. Lee, B. K. Kim, B. H. Lee, C. H. Chung, S. W. Nam, S. K. Kim, and J. W. Lee. 2008. Pilot-scale production of carboxymethylcellulase from rice hull by *Bacullus amyloliquefaciens* DL-3. *Biotechnol. Bioprocess Eng.* **13**, 182-188.
13. Jung, D. Y., Y. S. Cho, C. H. Chung, D. I. Jung, K. Kim, and J. W. Lee. 2001. Improved production of curdlan with concentrated cells of *Agrobacterium* sp. *Biotechnol. Bioprocess Eng.* **6**, 107-111.
14. Kondratyeva, T. F. 1981. Production of the polysaccharide pullulan by *Aureobasidium (Pullularia) pullulans*. *Uspechi Microbiol.* **16**, 175-192.
15. Lacroix, C., A. Le Duy, G. Noel, and L. Choplin. 1985. Effect of pH on the batch fermentation of pullulan from sucrose medium. *Biotechnol. Bioeng.* **27**, 202-207.
16. Lazaridou, A., T. Roukas, C. G. Biliaderis, and H. Vaikousi. 2002. Characterization of pullulan produced from beet molasses by *Aureobasidium pullulans* in a stirred tank reactor under varying agitation. *Enzym. Microb. Technol.* **31**, 122-132.
17. Leathers, T. D. 1993. Substrate regulation and specificity of amylases from *Aureobasidium* strain NRRL Y-12,974. *FEMS. Microbiol. Lett.* **110**, 217-222.
18. Lee, J. H., J. H. Kim, I. H. Zhu, X. B. Zhan, J. W. Lee, D. H. Shin, and S. K. Kim. 2001. Optimization of conditions for the production of pullulan and high molecular weight pullulan by *Aureobasidium pullulans*. *Biotechnol. Lett.* **23**, 817-820.
19. Lee, J. H., J. H. Kim, M. R. Kim, S. M. Lim, S. W. Nam, J. W. Lee, and S. K. Kim. 2002. Effect of dissolved oxygen concentration and pH on the mass production of high molecular weight pullulan by *Aureobasidium pullulans*. *J. Microbiol. Biotechnol.* **12**, 1-7.
20. Lee, J. W., W. G. Yeomans, A. L. Allen, R. A. Gross, and D. L. Kaplan. 1999. Biosynthesis of novel exopolymers by *Aureobasidium pullulans*. *Appl. Environ. Microbiol.* **65**, 5265-5271.
21. Lee, N. K., Y. B. Jo, I. H. Jin, C. W. Son, and J. W. Lee. 2009. The effect of potassium phosphate as a pH stabilizer on the production of gellan by *Spingmonas paucibilis* NK-2000. *J. Life Sci.* **19**, 1033-1038.
22. Madi, N., B. McNeil, and L. M. Harvey. 1997. Effect of exogenous calcium on morphological development and biopolymer synthesis in the fungus *Aureobassidium pullulans*. *Enzym. Microb. Technol.* **21**, 102-107.
23. Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **31**, 426-428.
24. Moscovici, M., C. Ionescu, C. Oniscu, O. Potea, P. Protopopescu, and L. D. Hanganu. 1996. Improved exopolysaccharide production in fed-batch fermentation of *Aureobasidium pullulans* with increased impeller speed. *Biotechnol. Lett.* **18**, 787-790.
25. Pollock, T. J., L. Thorne, and R. W. Armentrout. 1992. Isolation of new *Aureobasidium* strains that produce high molecular-weight pullulan with reduced pigmentation. *Appl. Environ. Microbiol.* **58**, 877-883.
26. Qian, X., Y. Zhu, J. W. Lee, and X. Zhan. 2001. Kinetic studies on production of pullulan by *Aureobasidium pullulans*. *J. Life Sci.* **11**, 116-119.
27. Ronen, M., H. Guterman, and Y. Shabtai. 2002. Monitoring and control of pullulan producing using vision sensors. *J. Biochem. Biophys. Meth.* **51**, 243-249.
28. Roukas, T. and M. Liakopoulous-Kyriakides. 1999. Production of pullulan from beet molasses by Aureobasidium pullulans in a stirred tank fermentor. *J. Food. Eng.* **40**, 89-94.
29. Seo, H. P., C. H. Chung, S. K. Kim, R. A. Gross, D. L. Kaplan, and J. W. Lee. 2004. Mass production of pullulan with optimized concentrations of carbon and nitrogen sources by *Auriebaisdium pullulans* HP-2001 in a 100L bioreactor with the inner pressure. *J. Microbiol. Biotechnol.* **14**, 237-242.
30. Seo, H. P., C. W. Son, C. H. Chung, D. I. Jung, S. K. Kim, R. A. Gross, D. L. Kaplan, and J. W. Lee. 2004. Production of high molecular weight pullulan by Aureobasisium pullulans HP-2001 with soybean pomace as a nitrogen source. *Bioresour. Technol.* **95**, 293-299.
31. Seo, H. Y., K. I. Jo, C. W. Son, J. K. Yang, C. H. Chung, Nam, S. W., S. K. Kim, and J. W. Lee. 2006. Continuous production of pullulan by *Aureobasidum pullulans* HP-2001 with feeding of high concentration of sucrose. *J. Microbiol. Biotechnol.* **16**, 374-380.
32. Singh, P. S., G. K. Saini, and J. F. Kennedy. 2008. Pullulan: microbial sources, production and applications. *Carbohydr. Polym.* **73**, 515-531.
33. Simon, L., B. Bouchet. C. Caye-Vaugien, and D. J. Gallant. 1995. Pullulan elaboration and differentiation of the resting forms in *Aureobasidium pullulans*. *Can. J. Microbiol.* **40**, 35-45.
34. Tarabasz-Szymanska, L., E. Galas, and T. Pankiewicz. 1999. Optimization of productivity of pullulan by means of multivariable linear regression analysis. *Enzym. Microb. Technol.* **24**, 276-282.

초록 : *Aureobasidium pullulans* HP-2001 균주를 사용한 풀루란의 대량 생산을 위한 파이롯트 규모에서 용존산소와 관련된 조건의 최적화

고와[1,2] · 김이준[1,2] · 정정한[2,3] · 이잔홍[4] · 이진우[2,3]*

([1]동아대학교 대학원 의생명과학과, [2]동아대학 BK21 생물자원 실버바이오사업 인력양성단, [3]동아대학교 생명공학과, [4]중국 화중농업대학교 식물과학기술대학)

Aureobasidium pullulans HP-2001 균주를 사용하여 풀루란을 대량 생산을 위하여 7 l 및 100 l 생물배양기를 사용하여 용존산소와 관련된 조건을 최적화하였다. 풀루란의 생산에 최적인 탄소원과 질소원은 각각 50.0 g/l 포도당 및 2.5 g/l 효모추출물이었으며 플라스크 규모에서의 풀루란 변환율은 37%이었다. 풀루란 생산 균주의 생장에 최적인 배지의 초기 pH 및 배양온도는 7.5 및 30°C이었으나 풀루란의 생산에 최적인 배지의 초기 pH 및 배양온도는 각각 6.0 및 25°C이었다. 7 l 생물배양기에서 *Aureobasidium pullulans* HP-2001 균주의 생육에 최적인 교반속도 및 통기량은 각각 600 rpm 및 2.0 vvm이었으나 풀루란 생산에 최적인 조건은 각각 500 rpm 및 1.0 vvm이었으며 최적 조건에서 풀루란의 생산농도는 18.13 g/l이었다. 100 l 생물배양기에서 풀루란 생산 균주의 생장에 최적인 내압은 0.0 kgf/cm^{2}이었으나, 풀루란 생산에 최적인 내압은 0.4 kgf/cm^{2}이었으며 최적 조건에서 풀루란의 생산 농도는 22.89 g/l이었다. 이는 내압이 없는 상태에 비하여 풀루란의 생산 농도가 1.38배 증가한 것이다.