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Effects of Aeration Rates and Rheological Properties of Fermentation Broth on Pullulan Fermentation

Yong Chul Shin*, Jong Kwon Han and Si Myung Byun

Department of Biological Science and Technology (KAIST), Seoul
**Department of Microbiology, College of Natural Sciences, Gyeongsang National University, Chinju*

Abstract

In polysaccharide fermentation with *Aureobasidium pullulans*, the aeration effects on the production of polysaccharide and the rheological properties of fermentation broth were studied. The increase of the aeration rates from 0.5 to 2.0 vvm at 500 rpm yielded the maximum specific production rate of polysaccharide from 0.046 to 0.093 (hr^{-1}), and the maximum specific growth rate of cells from 0.168 to 0.192 (hr^{-1}). The viscosity behavior of fermentation broths at the different aeration rates followed the power-law, $\tau = K(\gamma)^n$. The viscosity attributed by cells was about 10% of the total viscosity of fermentation broth and most of viscosity was attributed by the polysaccharide produced. The relationship between power-law parameters and the concentration of polysaccharide generally satisfied the equations with the regression coefficient greater than 0.980, $\ln K(t) = \ln(\tau)_0 - n(t)\ln(\gamma)_0$ and $K(t) = A\,P(t)^B$.

Key words: aeration, rheology, pullulan fermentation

Introduction

Biopolymers produced by microbes are rapidly emerging as important sources of novel and unique polymeric materials. One of biopolymers of great potential for industrial applications is the pullulan produced by a yeast-like fungus, *Aureobasidium pullulans*(1). The chemical structure of pullulan had been elucidated by many investigators(2-4). The pullulan consists of linear polymer of maltotriose units connected by α-(1-6) linkage. For the production of pullulan with *A. pullulans*, the effects of carbon(5-7) and nitrogen sources(8), the pH of fermentation broth(9), and rheological properties of fermentation broth(10,11) have been investigated. Because the fermentation broth of pullulan exhibits non-Newtonian characteristics and is approximated by pseudoplastic flow, the mass tranfer and mixing during the fermentation process are difficult problems. In fermentation process, the mass transfer and mixing are occurred via the aeration and agitation. Especially in aerobic fermentation process, the purpose of aeration and agitation is firstly to supply the dissolved oxygen for the microorganism. Many investigators reported that the oxygen supply by aeration and agitation plays an important role in maintenance or acceleration of the metabolic activities of microorganisms(12,13). However, in the pullulan fermentation, the effects of aeration on the cell growth and the polysaccharide production have not yet been examined.

In this paper, the aeration effects on the production of pullulan and the rheological properties of fermentation broth were studied.

Materials and Methods

Microorganism and inoculum

A. pullulans SH 8646 is a mutant obtained from *A. pullulans* IFO 4464 with the treatment of a nitrosoguanidin mutagen in our laboratory. It was maintained at 4°C on agar plates containing; Sucrose 5%: K_2HPO_4, 0.5%: NaCl, 0.1%: $MgSO_4 \cdot 7H_2O$, 0.02%: $(NH_4)_2SO_4$, 0.06%: yeast extract, 0.1%: and agar, 2% (w/v). The initial pH was adjusted to 7.5 with a concentrated HCl solution. It was maintained by transferring to a fresh medium for every week. One loop of *A. pullulans* was transferred to 500 m*l* Erlenmeyer flask containing 100 m*l* of medium and cultivated for 3 days at 27°C, 200 rpm in a shaking incubator (Lab-Line Inst.). The 3 day-old culture was used to inoculate the fresh medium at the ratio of 5% (v/v) and followed the cultivation for 24 hr in the same conditions described, which was used as an inoculum for the main cultures.

Corresponding author: Si Myung Byun, Dept. of Biol. Sci. & Technol. (KAIST) P.O.B. Cheongryang 150, Seoul, Korea

Cultivation in jar fermentor

A laboratory fermentor with a working volume of 3 *l* was used in this work. The temperature of the fermentation broth was controlled at 27 °C by circulating water with a thermostat. Agitation of the fermentation broth was provided by two four-bladed impellers. Aeration was controlled at a constant rate of air flow (vvm) by the gas regulator of the compressed air. Five % (v/v) of inoculum was used to seed 3 *l* of fermentation medium and the fermentation was carried out at 27 °C, 500 rpm. Fifty m*l* of fermentation borth was sampled and analyzed at the time intervals of 12 hr in all experiments.

Measurement of dry cell weight and polysaccharide

Four m*l* of fermentation broth was diluted to two times with distilled water and centrifuged at 10,000 × g for 30 min. The cells harvested were washed with equal volume of distilled water. The washed cells were dried to a constant weight at 105 °C. To measure the polysaccharide, the supernatant was collected and followed the addition of two volumes of ethanol. The precipitated polysaccharide was collected by centrifugation at 5,000 g for 15 min and washed with ethanol and acetone. The washed polysaccharide was dried to a constant weight in an oven at 105 °C.

Measurement of remaining sugar

After the removal of the polysaccharide from the supernatant by ethanol precipitation, the ethanol was evaporated by a rotary evaporator at 50 °C and the volume was adjusted to 3 m*l* with distilled water, which was used for the measurement of the remaining sugar. The phenol-sulfuric acid method(15) was used for measuring the remaining sugar by using the sucrose as the internal standard.

Measurement of viscosity

Brookfield-Synchrolectric Viscometer (model RVT) equipped with UL-adapter was used. For every 12 hr interval of the fermentation period, 16 m*l* of sample was put in UL-adapter container and kept at 25 °C by an external circulatory thermostat. After the dial deflection had reached a steady value at each speed of revolution such as 5, 10, 25, 50, and 100 rpm, the apparent viscosity, shear rate, and shear stress were calculated using the formulars given by the manufacturer as follows;

$$\text{Shear rate}\,(\gamma,\ \text{sec}^{-1}) = \frac{2W\ Rb^2\,Rc^2}{R^2\,(R_c^2 - R_b^2)} = 1.224\,N$$

$$\text{Shear stress}\,(\tau,\ \text{dyne/cm}^2) = \frac{M}{2Rb^2L} = 0.7829D$$

$$\text{apparent viscosity}\,(\eta_a,\ C_p) = 100\,(\tau/\gamma)$$

where, W is the angular velocity of spindle, Rb is the radius of spindle, Rc is the radius of container, R is the radius at which shear rate is being calculated, N is the revolution rate of spindle, M is the torque input by instrument, L is the effective length of spindle, D is the dial reading. To measure the viscosity of the cell-free solution, 20 m*l* of the fermentation broth was centrifuged at 10,000 × g for 30 min and the supernatant was used as a cell-free solution.

Measurement of K_La

The volumetric oxygen transfer coefficients (K_La, hr^{-1}) were determined by using the dynamic measurement method and the corrections for the probe response were carried out by the method reported by Fuchs *et al.*(16). All calculations and analysis of data were performed on IBM personal computer.

Results and Discussion

Effect of aeration rate

The importance of aeration in aerobic fermentations is well recognized and the primary objective of aeration is to supply the necessary oxygen to the microorganism in order to achieve the proper metabolic activities. The increase of oxygen supply by increasing the aeration rate is shown in Fig. 1. In our fermentation system, the oxygen transfer coefficient (K_La, hr^{-1}) increased from 55 to 175 (hr^{-1}) for the distilled water and from 25 to 165 (hr^{-1}) for the 3% pullulan solution (Pullulan PF-20, Hayashibara Biochem. Co., Japan) by changing the aeration rate from 0.5 to 2.0 vvm at 500 rpm. The pullulan concentrations higher than 3% showed the sharp decreases of K_La (data not shown). From this results, it can be expected that the oxygen transfer coefficient of fermentation broth decreases by the accumulation of polysaccharide in batch fermentation,

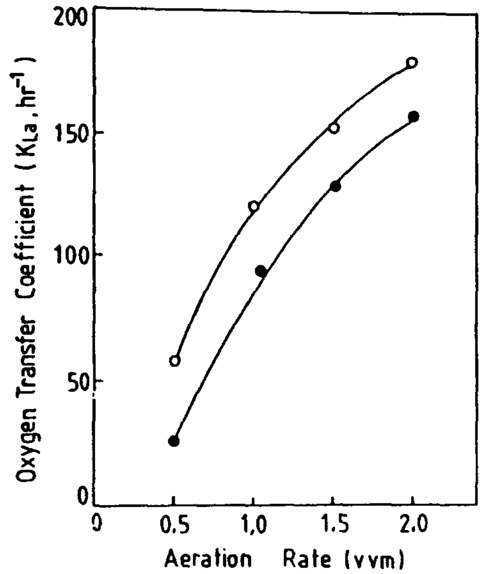

Fig. 1. Effect of air sparging on the oxygen transfer coefficient (K_La) for water (○) and 3% pullulan solution (●) in a baffled stirred fermenter

Single orifice sparger; two four-bladed impellers, diameter 60 mm in vessel of 160 mm diameter; agitation rate, 500 rpm; temperature, 27 °C

as fermentation process is proceeded.

The effects of air supply on the production of pullulan were examined at the different aeration rates, 0.5, 1.0, and 2.0 vvm. Experimental values of the dry cell weight, remaining sugar, polysaccharide concentration, and pH of fermentation broth are presented in Fig. 2. Increase of the aeration rate accelerated the pH decrease in fermentation broth. Fermentation time was shortened from 7.0 to 4.5 days by increasing the aeration rates from 0.5 to 2.0 vvm. The cell growth, substrate consumption, and polysaccharide production were increased by increasing the aeration rate. All these results indicated that the increase of the aeration rate accelerates the overall metabolic activities of cells for pullulan biosynthesis. Final kinetic and stoichiometric results were summarized in Table 1. At the different aeration rates, 0.5, 1.0, and 2.0 vvm, the final cell concentrations (X_{max}) and the biomass yields ($Y_{X/S}$) were similar 9.8-13.0 (g/*l*) and 0.22-0.26, respectively. Maximum specific growth rates (μ_{max}) were varied from 0.168 to 0.192 (hr^{-1}). From these results it can be concluded that the variations in aeration rate from 0.5 to 2.0 vvm gave a small effect on the cell growth. However, the production of polysaccharide was greatly influenced by the aeration rate. With the increases of the aeration rate from 0.5 to 2.0 vvm, the maximum specific production rates and volumetric productivities linearly increased from 0.046 to 0.093 (hr^{-1}) and from 0.145 to 0.290 (g/*l*.hr), respectively. At the later stage of fermentation process, the viscosity of fermentation broth was increased by the accumulation of polysaccharide and so the oxygen transfer rate will be inversely decreased. At this period, the higher rate of aeration supplies the more oxygen to cells. This can be contributed to the increase of polysaccharide production by accelerating the overall metabolic activities of cells. Conclusively, the increase of oxygen supply by aeration is necessary to maintain or accelerate the overall metabolic activities of cells for the polysaccharide production.

Rheological properties of fermentation broth

The fermentation broth and the cell-free solution were used for measuring the viscosity. The shear stress (dyne/cm^2) was evaluated at the range of shear rate (sec^{-1}) from 12.24 to 122.4. All the rheological data obtained from the different aeration rates were exceptionally well fitted to the power-law equation, $\tau = K(\gamma)^n$. From the plots of ln (τ) vs. ln (γ), the power-law parameters, the consistency index (K) and the non-Newtonian index(n) were determined from the intercept and slope with the regression coefficient greater than 0.980 in all experiments, indicating an excellent correlation. Results obtained at the different aeration rates are shown in Fig. 3. The power-law parameters changed in a very similar manner irrespective of the aeration rates. With the proceeding of fermentation process up to 7 days, the consistency index (K) gradually increased and the non-Newtonian index(n) decreased to show more pseudoplasticity of fermentation broth. The differences in the power-law parameters between fermentation broth and cell-free solution were very small. Thus it can be concluded that the presence of cells in the fermentation broth gives a small effect on the pseudoplasticity. When the differences of viscosities between the fermentation broth and the cell-free solution were plotted against the dry cell weight, the linear relationship was obtained with the regression coefficient

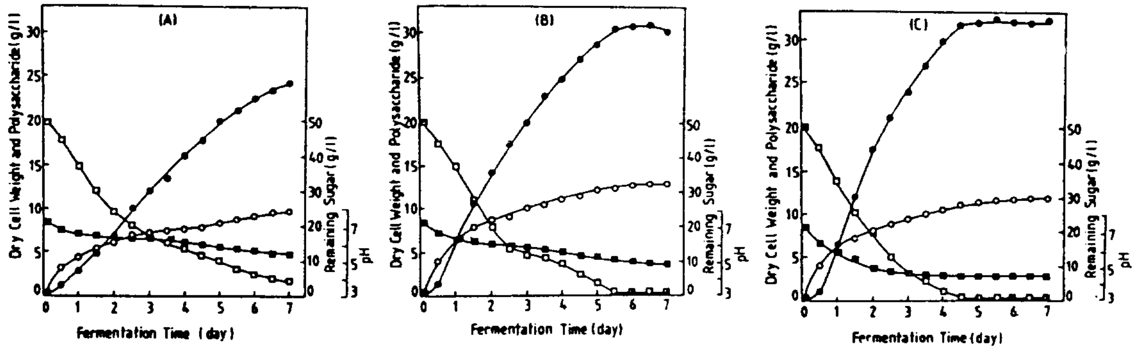

Fig. 2. Changes of the fermentation parameters in batch cultivation of *A. pullulans*
(A) aeration rate 0.5 vvm, (b) aeration rate 1.0 vvm, (C) aeration rate 2.0 vvm. Dry cell weight (○); polysaccharide (●); remaining sugar (□); and pH (■)

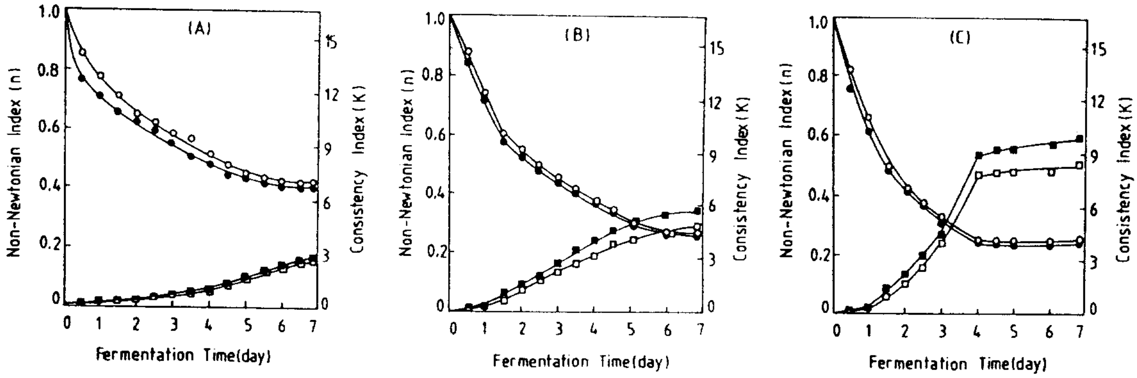

Fig. 3. Changes of the non-Newtonian index (○, ●) and the consistency index (□, ■) of the fermentation broths (closed symbols) and the cell-free broths (open symbols) under the different aeration rates
(A) 0.5 vvm, (B) 1.0 vvm, (C) 2.0 vvm

Table 1. Kinetic and stoichiometric results obtained at the different aeration rates

Aeration rate (vvm)	Fermentation time (day)	X_{max} (g/l)	P_{max} (g/l)	$Y_{x/s}$[a] (g/g)	$Y_{p/s}$[b] (g/g)	μ_{max} (hr^{-1})	q_p^{max} (hr^{-1})	Productivity (g/l, hr)
0.5	7.0	9.8	24.5	0.22	0.54	0.168	0.046	0.145
1.0	5.5	13.0	30.5	0.26	0.61	0.192	0.079	0.231
2.0	4.5	12.0	32.0	0.24	0.64	0.192	0.093	0.290

[a] biomass yield = $\Delta X/\Delta S$
[b] product yield = $\Delta P/\Delta S$

greater than 0.832; $\eta_{a,cell}$ = 0.229X-0.169, where $\eta_{a,cell}$ is the apparent viscosity contributed by cells at the shear rate 122.4(Sec^{-1}) and X is the dry cell weight (g/l). The slope of the equation, 0.229, represents the increment of the apparent viscosity by unit gram of cell per liter.

Recently, Thomson and Ollis[14] reported the relationships between the concentration of polysaccharide and the both of the power-law parameters for the microbial polysaccharides. Yet, the general applications of the these relationships were not considered to describe the behavior of the power-law parameters of pullulan fermentation. The relationship between K and n is $\ln K(t) = \ln(\tau)_0 - n(t)\ln(\gamma)_0$, where $(\tau)_0$ and $(\gamma)_0$ are the constants. As shown in Fig. 4, the data obtained at the different aeration rates satisfied the equation with the regression coefficient greater than 0.987, indicating the excellent

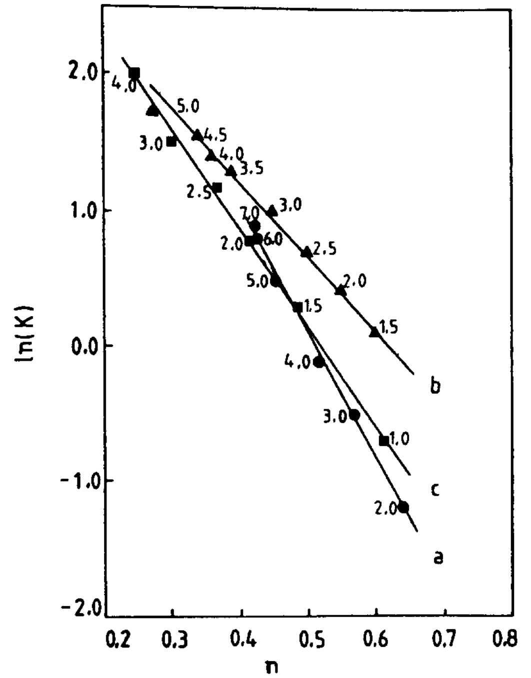

Fig. 4. Relationships of ln(K) and n for fermentation broths under the different aeration rates
(a) 0.5 vvm, (b) 1.0 vvm, (c) 2.0 vvm. Numbers around the points represent the sampling time of fermentation broth (day)

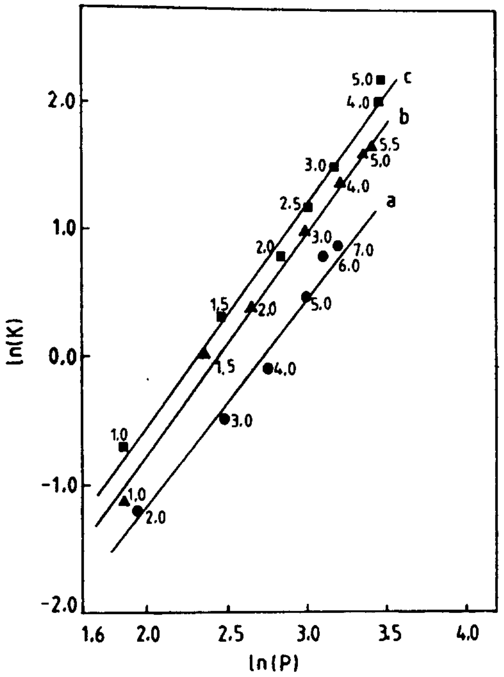

Fig. 5. Relationships of ln(K) and ln(P) for fermentation broths under the different aeration rates
(a) 0.5 vvm, (b) 1.0 vvm, (c) 2.0 vvm. Numbers around the points represent the sampling time of fermentation broth (day)

fitness.

In examining the power-law parameters as a function of polysaccharide concentration, the following equation was used, $K = A(P)^B$ i.e., $\ln(K) = \ln(A) + B \ln(P)$, where A and B are the constants and P is the concentration of polysaccharide. As shown in Fig. 5, all the data of ln(K) and ln(P) were well fitted in this equation with the regression coefficient greater than 0.986, indicating that this equation is a useful relationship of K and P, irrespective of the aeration rates. The relationship constants of the above two equations, $(\tau)_0$, $(\gamma)_0$, A, and B were calculated from the slopes and the intercepts of the Figs. 4 and 5. As shown in Table 2, the relationship constants were quite different according to the aeration rates. These differences were considered as due to the variations in the morphology of cells and the degree of the polymerization of polysaccharide obtained at the different aeration rates. However, for one set of the fermentation process, the linear relationships were observed with the high regression coefficient. From these results, it can be concluded that in the pullulan fermentation, the relationships between the power-law parameters and the concentration of the polysaccharide are well expressed as the above two equations and the equations will be generally applicable to the pullulan fermentation to specify, predict, and control the fermentation process.

Table 2. The relationship constants obtained at the different aeration rates

Aeration rate (vvm)	$\tau_0^{a)}$	$\gamma_0^{a)}$	$R^{c)}$	$A^{b)}$	$B^{b)}$	$R^{c)}$
0.5	114	10829	0.997	0.0093	5.59	0.986
1.0	22	124	0.987	0.0216	5.07	0.994
2.0	42	1261	0.997	0.0199	5.53	0.996

a) relationship constants of $\ln K(t) = \ln(\tau)_0 - n(t) \ln(\gamma)_0$.
b) relationship constants of $\ln(K) = \ln(A) + B \ln(P)$.
c) regression coefficient.

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풀루란 발효시 통기속도의 영향과 발효액의 물성에 관한 연구

신용철* · 한종권 · 변시명

한국과학기술원 생물공학과, *경상대학교 자연과학대학 미생물학과

풀루란 발효에서 500 rpm에서 통기속도를 0.5 vvm, 1.0 vvm, 2.0 vvm으로 증가시켰을 때 최대 비성장속도는 0.168(hr^{-1})에서 0.192(hr^{-1})로 증가되었으며 풀루란의 최대 비생산속도는 0.046(hr^{-1})에서 0.093(hr^{-1})으로 증가되었다. 풀루란 발효액의 물성은 power-law를 따르는 Pseudoplasticity를 나타냈으며 Power-law 변수들과 풀루란 농도 사이에는 $\ln K(t) = \ln(\tau)_o - n(t)\ln(\gamma)_o$와 $K(t) = AP(t)^B$의 상관관계를 보였다.