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Effect of *Aspergillus niger* Pellets on Citric Acid Production in a Bubble Column Bioreactor

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Citrate is mainly produced from fungi and oxygen transfer has been known as one of the important factors in citric acid production. A bubble column bioreactor was used for citrate production after pellet was initially made using a stirred bioreactor for the inoculation. The relationship between the pellet size of *Aspergillus niger* and the oxygen transfer was elucidated by considering morphological characteristics of the pellet. The pellet size was determined by adjusting the impeller speed in the stirred bioreactor and the optimum diameter of the pellet was observed to be 2.2 mm under the experimental conditions. Pellet was maintained quite stable in the bubble column bioreactor and production of citric acid was significantly improved by maintaining optimal pellet conditions in the bubble column bioreactor.

Citric acid or 2-hydroxy-1,2,3-propanetricarboxylic acid has been widely used both as an additive in food and beverage industries and also as chemical feedstocks because of its non-toxic and stable nature (8). In 1917 Zahorsky obtained a patent for citric acid production using *Aspergillus niger*. The most important finding was that *Aspergillus niger* could grow well in pH values lower than 2.0. The benefit of the low pH is a decreased risk of contamination. Consequently large scale production of citric acid based on microbial cultures was started. In submerged fermentation processes, aeration is extremely critical: citric acid production is known to be increased by increased aeration (6). Today, considerable attention is directed towards the bioreactor development such as air-lift (13) and immobilized cell reactors (14) for the overproduction of citric acid. In this article, the effects of pellet size on citric acid fermentation were investigated in a stirred bioreactor and in a bubble column bioreactor for the overproduction of citric acid.

MATERIALS AND METHODS

Microorganism

The microorganism used in this study was *Aspergillus niger* KCTC 1231. The strain was maintained on agar slants containing 6% (w/v) sucrose, 0.25% ammonium nitrate, 0.1% potassium dihydrogen phosphate and 0.05% magnesium sulfate.

Medium and Preculture

The medium for the culture consisted of 10% sucrose, 0.1% NH_4NO_3, 0.1% KH_2PO_4 and 0.025 % $MgSO_4$. The medium was adjusted to pH 4.0. The preculture was carried out in a 500 ml flask containing 300 ml of medium at 30℃ for 2 days in a rotary shaker. The shaker speed was 200 rpm. The second preculture was performed in a 5 l stirred bioreactor containing 3 l of medium at 30℃ for about 43 h until the depletion of the nitrogen source. The aeration rate was 2 vvm. Impeller speeds were manipulated (with 100, 125, 150, 200, and 300 rpm) to obtain the desired size of *A. niger* pellet.

Bubble Column Bioreactor

Main cultivations were carried out in a pyrex-glass bubble column bioreactor (total volume 4.5 l). Pellets harvested in a stirred bioreactor were aseptically transferred into the bubble column bioreactor. The volume of culture broth was kept to 3.2 l at 30℃. The aeration was done in a range of 2.8~4 vvm. A galvanic type DO probe was used for monitoring the dissolved oxygen level.

Analytical Methods

The concentration of citric acid was measured by the pyridine-acetic anhydride method of Marier and Boulet (7). The concentration of ammonium ion was measured from the change in absorbance at 630 nm after the addition of Berthelot solution according to Berthelot

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reaction method (12). The viscosities of the fermentation broth were measured by means of a Brookfield synchroelectric viscometer with cylindrical spindles. Range of shear rate was 0.2~2 sec^{-1}. After harvesting the cells from the fermentation broth, dry cell weights were determined by drying washed cells at 80℃ to a constant weight. Fractional gas hold up was measured by the manometric method (2).

Data Analysis

Gas-liquid oxygen transfer coefficient, k_La was measured using the non-steady state kinetic method (9). The average pellet diameter, d_p, was calculated from the total volume of arbitrarily chosen pellets. The average volume per unit pellet, V_p, was calculated as follows:

$$V_p=\sum(3\sum\pi \cdot d_i^3 \cdot f_i/2)/\sum f_i \quad (1)$$

,where f_i is the number of pellets with diameter, d_i. Thus d_p was be calculated by the following equation.

$$d_p=(V_p/(3\pi/2))^{1/3} \quad (2)$$

Calculation of Shear Rate in a Stirred Bioreactor

Shear rate in a stirred bioreactor, which was defined as the average shear rate, Y_a (3) in this study, can be expressed as

$$Y_a=k \cdot N \quad (3)$$

,where N is the revolution rate of the impeller, k is a constant determined by reactor geometry and was given by:

$$k=\frac{112.8\ r_i^{1.8}(r_t^{0.2}-r_i^{0.2})(r_c/r_i)^{0.8}}{r_t^2-r_i^2} \quad (4)$$

,where r_i is the radius of the impeller, r_t is the radius of the fermentor and r_c is the radius of the forced vortex zone.

Contribution to the Power Input into the Bubble Column Reactor

There are two sources of power delivered to a reactor: isothermal expansion of the air as it moves up to the top of the fermentor, and the kinetic energy of the air imparted to the gas/liquid dispersion at the point of air injection (1). The contribution of kinetic energy to the total power input is known to be negligible, as compared with isothermal expansion of the air (1). Superficial air velocity, U_G, can be expressed as:

$$U_G=\frac{Q_m \cdot R \cdot T}{V_L \cdot \rho_L \cdot g} \ln\left[1+\frac{\rho_L \cdot g \cdot h_L}{P_h}\right] \quad (5)$$

,where Q_m is the molar air flow rate, R is the gas constant, T is the absolute temperature, V_L is the volume of the liquid, ρ_L is the density of the liquid, g is the gravitational acceleration, h_L is the unaerated liquid height and P_h is the reactor head-space pressure. Shear rate, γ can be calculated using the following equation reported by Kawase and Moo-Young (4):

$$\gamma=U_G/d_c \quad (6)$$

,where dc is the reactor diameter. Power input, P/V_G, can be calculated as:

$$P/V_G=\rho_L \cdot g \cdot U_G \quad (7)$$

RESULTS AND DISCUSSION

Effect of Shear Rate on the Pellet Size

Shear is an important factor in the cultivation of pellet-forming microorganisms, as in the case of *Aspergillus niger*. It was evident from the experimental observation that shear rate exerted considerable influence on the differentiation as well as the disruption of the pellets (10). With an increase of cell concentration, the disruption of the pellets became more serious. This is probably due to the fact that each pellet had a higher probability of colliding with the impeller tip of the bioreactor in the case of high concentration of cells. Since the differentiation of the pellet was also undergoing even for low shear rate, a large pellet was divided into many small pellets. Various sizes of pellets coexisted in the broth of the stirred bioreactor. The pellet size distribution obtained at the culture condition of 150 rpm and after 43 hours of cultivation in a stirred bioreactor is shown in Fig. 1. The number distribution of pellets had its maximum value between 1 and 2 mm. In order to investigate the effect of shear rate on pellet size, shear rate was changed by manipulating the impeller speed of the stirred bioreactor. As shown in Fig. 2, volume-

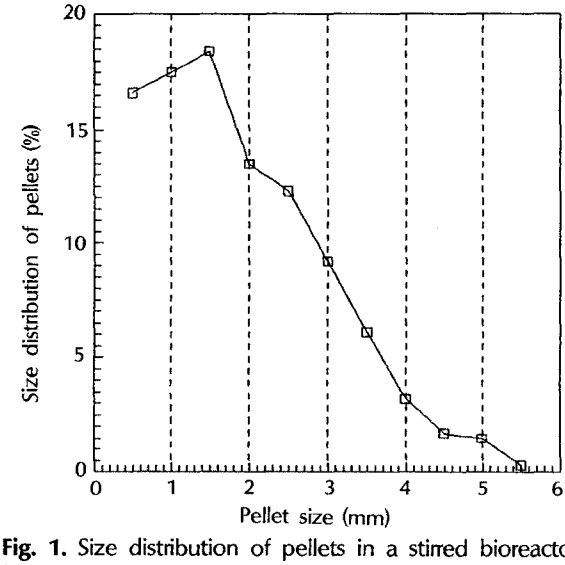

Fig. 1. Size distribution of pellets in a stirred bioreactor. (150 rpm and after 43 hours of cultivation).

averaged pellet size was decreased as the shear rate was increased in the stirred bioreactor. When the pellets harvested in the stirred bioreactor at 43 hours were aseptically transferred into the broth of the bubble column bioreactor, the average size of pellets remained almost constant as shown in Fig. 3. In the stirred bioreactor, the average size of *Aspergillus* pellet increased sharply with cell growth in the early phase and then decreased after reaching maximum. This decrease in size was considered to be caused by the deformation of the pellet by collision with an impeller. This trend was more serious at a high agitation speed. However, when the culture broth was transferred from the stirred bioreactor to the bubble column bioreactor, pellet size remained relatively constant in the bubble column bioreactor. This could be due to the relatively low shear stress in the bubble column bioreactor compared to the shear stress in the stirred bioreactor.

Morphology of *Aspergillus niger* Pellet

A photograph of a typical pellet of *Aspergillus niger* is presented in Fig. 4. There appeared to be considerable variation in the morphology of pellets as cultivation time proceeded. Morphology was influenced by the strength of shear stress as well as the growth conditions of the microorganism. Small pellets were formed from the spores during the seed culture in the flask as shown in Fig. 4(a), and then transferred in the stirred bioreactor. It was observed as shown in Fig. 4(b) that pellet size in the form of hardened growth increased until the point at which ammonium ion was exhausted in the culture broth (5). About 20~30 hours after the hardened pellet was transferred to the bubble column bioreactor, the

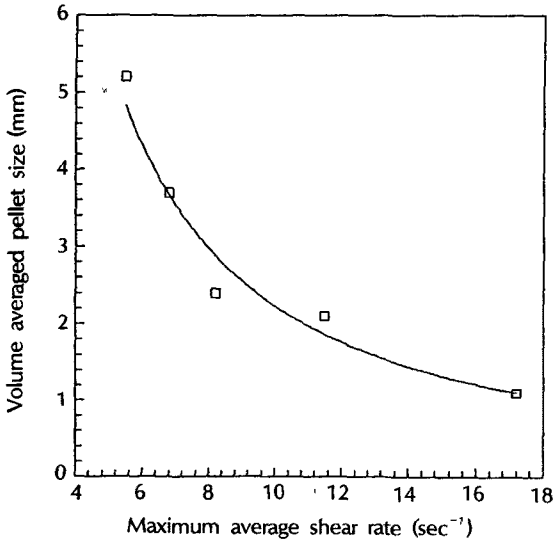

Fig. 2. Effect of maximum average shear rate on the volume averaged pellet size.

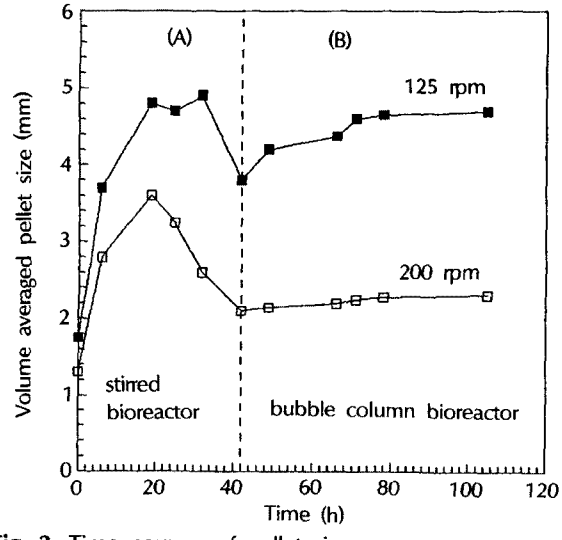

Fig. 3. Time courses of pellet size.
(A) in a stirred bioreactor (125 and 200 rpm).
(B) in a bubble column bioreactor (2.8 vvm).

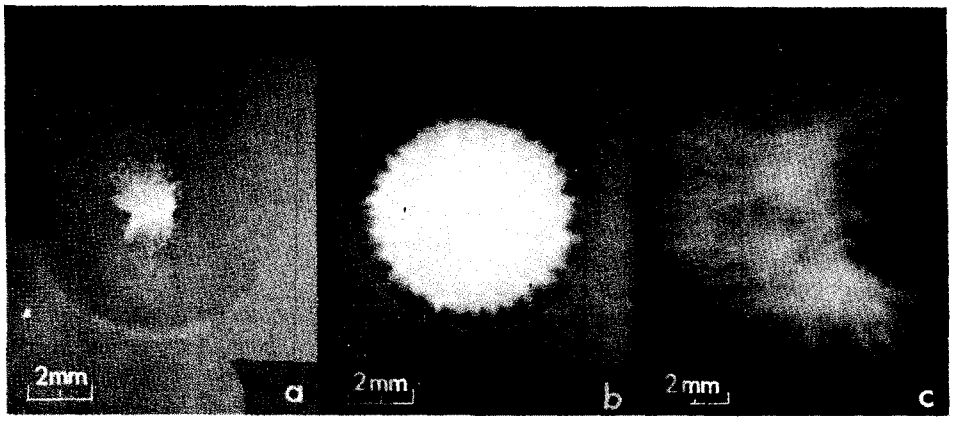

Fig. 4. Various pellet morphologies.
(a) small pellet, (b) hard-sphered pellet, (c) soft pellet.

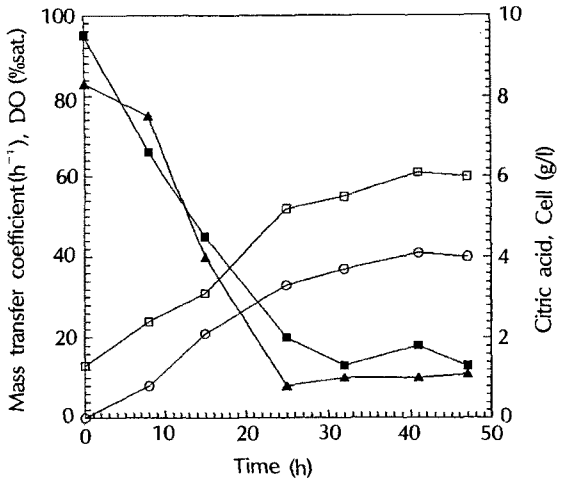

Fig. 5. Time courses of citric acid production in a bubble column bioreactor(aeration rate was 2.8 vvm).
○: citric acid concentration (g/l), □: cell concentration (g/l), ■: mass transfer coefficient (h^{-1}), ▲: DO (% saturation).

Table 1. Effects of pellet size on final dissolved oxygen level and citrate production.

Pellet size (mm)	Final DO (% sat.)	Final cell mass (g/l)	Citrate production (g/l)
1.0	10	6.1	4.05
2.2	18	8.0	5.50
3.8	32	9.2	3.04
5.1	44	13.1	1.45

morphology of the pellet changed to the soft form as shown in Fig. 4(c) with the initiation of the citric acid biosynthesis. It was reported that pellet forms were affected by the broth rheology and flow pattern in a bioreactor (11). Fig. 5 shows a typical relationship between citric acid production and oxygen transfer in the bubble column bioreactor. It was noted through experimental observation that the presence of soft pellet in the broth deformed flow patterns. Suspensions containing hard sphere pellets in the early phase showed lower viscosity of Newtonian fluid and had bubble flows, which resulted in high mass transfer as shown in Fig. 5. However, suspensions containing soft pellets in the production phase, compared with those containing hard-sphere pellets, had high viscosity of non-Newtonian and churn-turbulent flows in a bubble column bioreactor, which resulted in poor mass transfer. Consequently, oxygen transfer coefficient at 30 h decreased to about a fifth of its initial value. Table 1 shows the effect of final averaged pellet size on final DO level and citric acid production in a bubble column bioreactor. Though the final DO level increased with the pellet size, the production of citric acid was maximum at the size of 2.2 mm. It could be inferred from the result that the production of citric acid was influenced by intrapellet

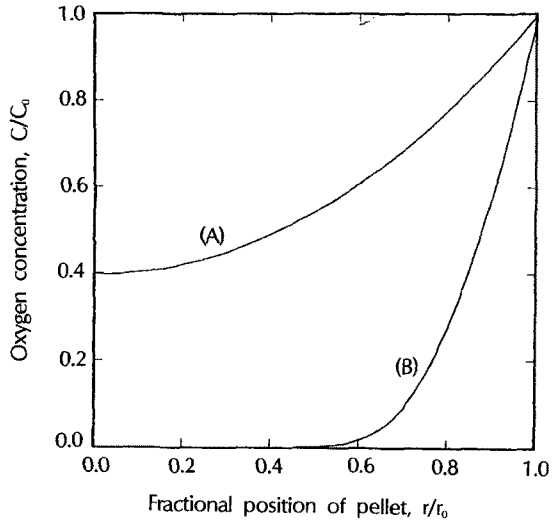

Fig. 6. Oxygen concentration profiles inside the pellet when dissolved oxygen in the broth was 40% saturation.
(A) pellet diameter ($2r_0$) was 1.0 mm, (B) pellet diameter ($2r_0$) was 4.0 mm.

mass transfer.

Effect of pellet size on the production of citric acid

Optimum pellet size exists that leads to maximum production rate of citric acid because of its aerobic metabolism. If a pellet is too small, oxygen transfer to culture broth decreases owing to high viscosity as in the case of filamentous hyphae, which results in the metabolic limitation of citric acid production. However, if a pellet is too large, the microorganisms near the pellet core becomes starved of oxygen, which results in endogeneous respiration followed by diminution of active cells. To analyze mathematically the oxygen transfer capacity of the pellet, simulation was conducted. Assuming that the pellets are spherical, and taking the material balance of oxygen on a differential volume of liquid phase around pellets at steady state assuming Monod kinetics, the following equation can be derived:

$$\frac{d^2C}{dr^2} + \frac{2dC}{rdr} = \frac{\rho}{D}\frac{Q_iC}{Ks+C} \qquad (8)$$

,where ρ is the pellet density, D is the diffusivity of oxygen, Q_i is the maximum specific oxygen uptake rate and K_s is the Monod constant.

When the dissolved oxygen concentration in the broth was 40% of saturation, oxygen profiles in the pellet with a diameter of 1 and 4 mm are shown in Fig. 6. The large pellet (diameter=4 mm) was subject to internal oxygen transfer limitation, compared to the small pellet. The fractional penetration zone of oxygen into the pellet was smaller in a large pellet than in a small pellet. Because metabolic activity of the microorganism was possible only in an outer zone where a good oxygen supply was

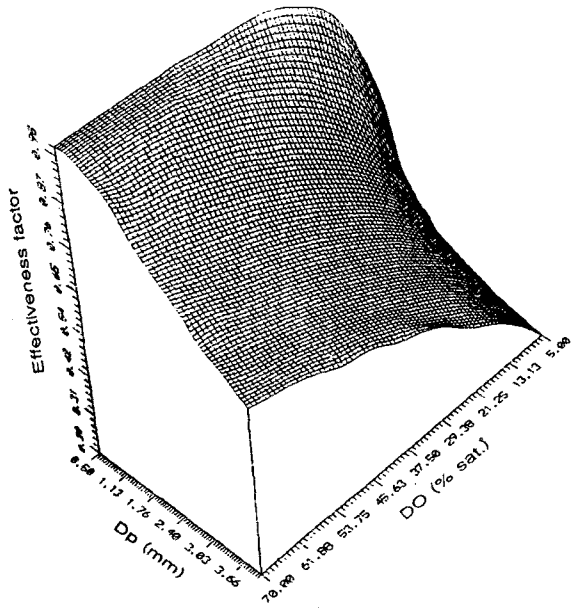

Fig. 7. Effect of pellet diameter and dissolved oxygen level (% sat.) on effectiveness factor of the pellet.

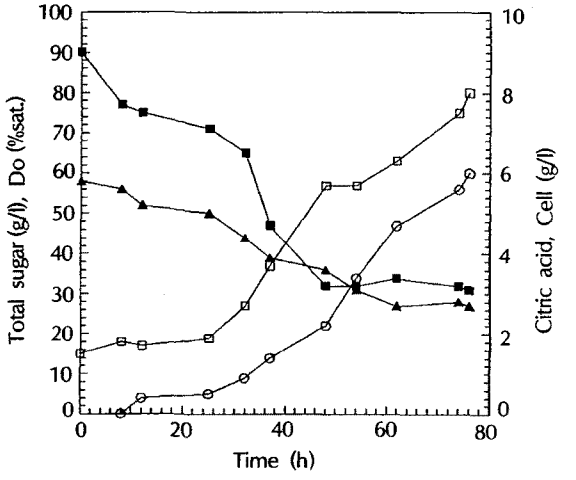

Fig. 8. Time courses of citric acid production in a bubble column bioreactor (aeration rate was 4.0 vvm).
○: citric acid concentration (g/l), □: cell concentration (g/l), ■: total sugar concentration (g/l), ▲: DO (% saturation).

available, citric acid production was therefore expected to be less effective in a large pellet. The effectiveness factor for oxygen uptake rate within the pellet was increased with the dissolved oxygen concentration in the broth although it was decreased with pellet diameter as shown in Fig. 7. Fig. 7 also implies that the effectiveness factor also increased with the increase in the air flow rate. To corroborate this result, dissolved oxygen was controlled above 30% saturation by increasing the air flow rate of 4.0 vvm in a bubble column bioreactor after inducing the pellet in a stirred bioreactor. As shown in Fig. 8, the fermentation with high value of dissolved oxygen yielded almost 10% higher product concentration than that with low dissolved oxygen value. This result was possibly due to high effectiveness factor. Because the relationship between pellet diameter and dissolved oxygen concentration had an inverse relationship with respect to the effectiveness factor for constant power input, it is important to decide the optimum diameter of pellet.

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