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# Effect of Aeration and Agitation Conditions on the Production of Glucoamylase with *Aspergillus niger* No. PFST-38

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***Aspergillus niger*** **No. PFST-38 was grown on complex media in 30L agitated fermentors at various aeration rates and stirrer speeds. We could correlate the mixing time as a function of the Reynolds' number and the apparent viscosity, as follows.** $\theta_M = 2.95\ NRe^{-0.52}$, $\theta_M = 1.88\ \eta_a^{0.57}$

**Also, the effects of the apparent viscosity ($\eta_a$), the impeller rotational speed (N), the air flow rate ($V_s$), and the mixing time ($\theta_M$) on the oxygen transfer coefficient, $K_La$ were determined experimentally, and equated as follows.** $K_La = 12.04N^{0.88}Vs^{0.71}n_a^{-0.83}$, $K_La = 30.2N^{0.88}Vs^{0.71}\theta_M^{-1.45}$

**$K_La$ increased as the agitation speed and the air flow rate increased. The rate of $K_La$ increase was dependent more on the rotational speed of impeller than on the air flow rate. The glucoamylase production increased with the increase of the agitation speed upto at 500 rpm and increased with the increase of air flow rate upto at 1.0 vvm. The values calculated from the above equation confirmed that the experimental maximum production of glucoamylase was achieved when the $K_La$ and the apparent viscosity of the broth were 260 $hr^{-1}$ and 1800 cps, respectively.**

One of the most important areas in designing and scaling-up of fermentations involves oxygen transfer from a gas phase to the liquid phase. Yet little information is available on the problem in the industrially important cases involving non-Newtonian broths. Most of the fungal enzyme broths show pseudoplastic behavior and the mass transferring rates may be critical in determining vessel productivities.

Deindoerfer and Gaden (3) examined the rate of oxygen transferring to reconstituted penicillin broths, as a function of the mycelium concentration. As the mycelial concentration increased, the apparent viscosity rose and the rate of oxygen transfer decreased by 85% of its initial value at the mycelium concentration of 13.5 g/liter.

The rate of product formation can also be affected by mixing and oxygen transferring (16). Although fungi may suffer from shear damage as the stirrer speed is raised, a higher productivity may result by the enhanced oxygen transfer rate. This has been demonstrated in the production of citric acid by *Aspergillus niger* (2) and penicillin by *Penicillium chrysogenum* (5, 14, 15).

Norwood and Metzner (9) proposed that the Newtonian mixing time versus the Reynolds' number plot might be a suitable for estimating mixing times for pseudoplastic fluids. That is,

$$\theta_M = \alpha_m \cdot N_{Re}^{\beta_m} \quad \text{(Eq. 1)}$$

Ryu and Humphrey (13) examined the effect of apparent viscosity on the mass transfer coefficient, $K_La$. They proposed the correlation for *Pen. chrysogenum* broth.

$$K_La = (P/V)^x(V_s)^y(\eta_a)^z \quad \text{(Eq. 2)}$$

For penicillin fermentations with *Pen. chrysogenum*, König *et al.* (8) as well as Gbewonyo and Wang (6) reported an increase in $K_La$ by a factor of about 4, if the culture grew in small pellets instead of as a pulpy mycelium.

We have studied the rheological properties of the culture broth of *Asp. niger* No. PFST-38 and characterized the non-Newtonian behavior by using Herschel-Bulkley equation (12). The changes in flow behavior of the broth could be monitored by the morphological parameters of the mycelia (10).

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In this paper we discuss the effect of broth viscosity on the mixing time and oxygen transfer rate of a fermentor. A model system for achieving a maximum production of glucoamylase in submerged culture of *Asp. niger* by optimizing the oxygen transfer rate and mixing was suggested.

## MATERIALS AND METHODS

### Microorganism

The microorganism used in these studies was the glucoamylase hyperproducing mutant, *Aspergillus niger* No. PFST-38 . This mutant resulted from the successive mutation of the parent strain (11). It was grown on potato-dextrose-agar (PDA) slants for 5~6 days at 28℃ and the culture was maintained lyophilized. Transfers were made once every two months.

### Preparation of Inoculum

Inoculum was prepared by washing a 5-day-old PDA plate with 10 m*l* of sterile medium, transferring medium and cells to two 500-m*l* flasks containing 100 m*l* medium and incubating the flasks at 30℃ for 48 hrs on a reciprocating shaker. The inoculum medium contained the following (g/*l*); corn meal 50.0, defatted soybean meal 10.0, and casein 5.0. The pH was adjusted to 5.0 with hydrochloric acid solution before sterilization. The substrate was sterilized at 121℃ for 30 min. The volume used for inoculation represented 5% of the media volume of the fermentor.

### Experimental Equipment

A B.E. Marubishi MSJ-U3 (30 *l*) fermentor, fitted with two turbine impellers (113 mm diameter) was used in this study. The temperature was maintained at 30℃ and the pH was monitored by using a TOA ML-120 sterilizable electrode (TOA Electronics Ltd.). A conductivity electrode system was used to detect the foam level and to add drops of an aqueous emulsion of silicone oil. Dissolved oxygen was measured as oxygen tension by using a steam sterilizable dissolved oxygen electrode (B.E. Marubishi). Gaseous oxygen in the outlet gas was measured by using an ABLE oxygen analyzer and was also continuously recorded. The impeller speed was 400 rpm unless quoted otherwise. Power consumption was calculated from torque measurements obtained by using a simple laboratory torsiometer. The medium used for the run batches is shown in Table 1. In order to liquefy the corn meal, α-amylase(0.05 g/*l*) was added before the sterilization. After sterilization, a required quantity of 2 N HCl was added to give the pH 6.0.

### Determination of Mixing Time ($\theta_M$)

The mixing time was defined as the time required to minimize concentration differences in the entire volume of liquid in the fermentor. 10 N-NaOH solution was added to the broth (0.15% v/v), and while mixing with fermentor agitator, the pH of broth was measured until the decreasing rate was 0.001/sec. The mixing time was determined as the elapsed time after the addition of NaOH solution to the end point (7).

**Table 1. Concentrations of components in the medium for the production of glucoamylase with *Asp. niger* No. PFST-38**

| Component | Concentration (g/*l*) |
|---|---|
| Corn meal | 200 |
| Defatted soybean meal | 30 |
| Corn steep liquor | 50 |
| α-Amylase | 0.05 |

### Glucoamylase Activity

Glucoamylase activity in reaction mixture that contained 0.5 m*l* of 2% soluble starch in 0.2 M sodium acetate buffer (pH 4.8) and 0.5 m*l* of enzyme solution was measured. After aerobic incubation at 60℃ for 30 min, the reaction was stopped by cooling the mixture in ice, and then the mixture was boiled in a steam bath for 10 min. The released glucose was quantified by using the hexokinase-glucose-6-phosphate dehydrogenase method (1). One unit of glucoamylase activity was defined as the amount of enzyme that liberated 1 μmol of glucose per min under the assay conditions used.

### Mycelial Weight

The amount of biomass was determined by weighing the filter cake after drying at 105℃ overnight.

### Viscosity Measurements

The viscometer used for measuring shear stress as a function of shear rate was the Haake Viscotester VT 181 viscometer (Haake Buchler Instruments, Saddle Brook, NJ) with pin-shaped(RS) rotors, as described in the previous paper (12).

### $K_La$ Measurements

The steady-state oxygen balance technique was used for the determination of $K_La$. The changes of partial pressure of oxygen and carbon dioxide were monitored by $O_2$ and $CO_2$ analyzer (Ingold 525 type) set up in the external circulation loop. The partial pressure of carbon dioxide was controlled manually within the range of 0.01~0.02 MPa by sparging part of the recycled gas through 20% (w/v) KOH solution. $K_La$ was calculated by using Eq. (3).

$$R = K_La(C_m^* - C_m), \quad \text{(Eq. 3)}$$

where R is the rate of oxygen transfer, $K_La$ is the volumetric oxygen mass transfer coefficient, $C_m^*$ is the mean dissolved oxygen concentration at gas-liquid interface, and $C_m$ is the mean dissolved oxygen concentration in bulk of liquid.

## RESULTS

### Determination of Mixing Time ($\theta_M$)

Fig. 1 shows the plot of mixing time versus the Reynolds' number. The aeration was controlled at 1.0 vvm. The Reynolds' numbers were calculated by the method of Foresti *et al.* (4). Although the change in mixing time was considerable in low NRe region, the mixing time remained reasonably constant. As can be seen from the graph, the values of $\alpha_m$ and $\beta_m$ are 2.95 and $-0.52$, respectively. Therefore, $\theta_M = 2.95\ NRe^{-0.52}$. In here, the value of $\alpha_m$ increased with the increase of pseudoplasticity and the value of $\beta_m$ shows the inhomogenecity of viscosity (7).

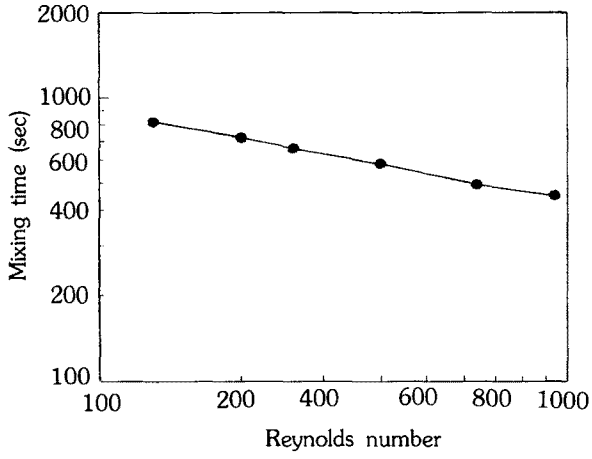


**Fig. 1. Relationship between mixing time and apparent Reynolds number of the culture broth of *Asp. niger* in a 30 *l* fermentor.**
The aeration was controlled at 1.0 vvm. The mixing time was measured during fermentation and 10 N-NaOH solution was added to the broth (0.15% v/v).

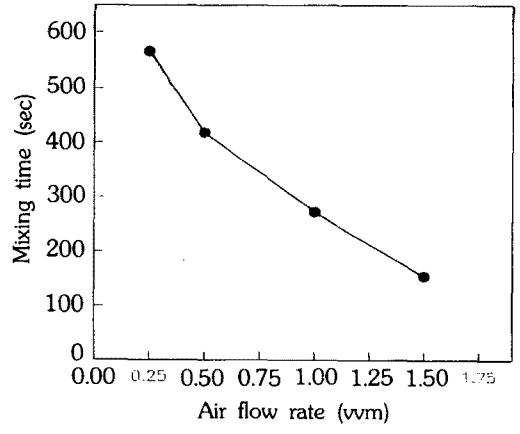


**Fig. 2. Effect of air flow rate on mixing time of the culture broth of *Asp. ngier* in a 30 *l* fermentor.**
Agitation speed: 400 rpm

Fig. 2 gives a plot of air flow rate vs. mixing time when the agitation was controlled at 400 rpm. The mixing time decreased as the air flow rate increase. This was supposed to be due to the increase of the axial drag flow as the air flow rate increases.

The mixing times are plotted as a function of fermentation time in Fig. 3. The mixing time was 35 seconds when the fermentation was started, but rapidly increased after 48 hr of fermentation. It appears that these values are closely related with the increase of the vicosity of broth. In the later stages of the fermentation when the mixing time was slightly decreased, the apparent viscosity was decreased, due to the breakage of hyphae (10). The decrease in the mixing time in later stages of the fermentation is closely related to the increase in the flow behavior index.

The mixing time against apparent viscosity of the broth made a straight line in log-log scales, and the value of $\alpha_n$ and $\beta_n$ were calculated to be 1.88 and 0.57, respectively. Therefore,

$$\theta_M = 1.88\eta_a^{0.57} \quad \text{(Eq. 4)}$$

### Determination of Volumetric Oxygen Transfer Coefficient ($K_La$)

Fig. 4 gives a plot of $K_La$ vs. fermentation time when the agitation and air flow rate was controlled at 400 rpm and 1.0 vvm, respectively. From this figure, it is apparent that $K_La$ rapidly decreased during 24~96 hr after inoculation, and then remained reasonably constant up to 168 hr.

$K_La$ was increased as the agitation speed increased, but, the increase rate decreased in higher agitation speeds. When the air flow rate was increased, , the value

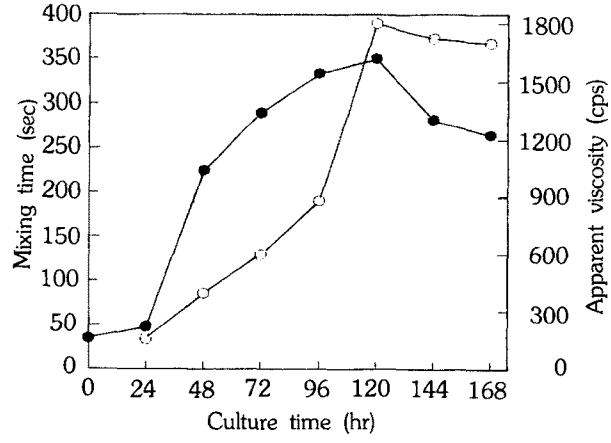


**Fig. 3. Changes in the mixing time and the broth viscosity during fermentation.**
Agitation: 400 rpm, Air flow rate: 1.0 vvm.
●—●: Mixing time, ○—○: Apparent viscosity

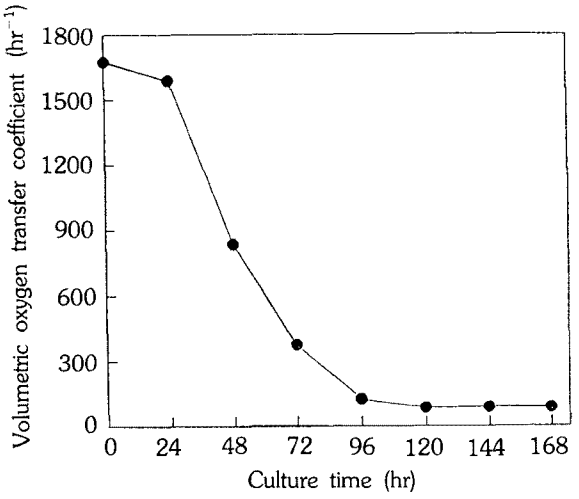


**Fig. 4. Changes in the volumetric oxygen transfer coefficient during fermentation.**
Air flow rate: 1.0 vvm, Agitation: 400 rpm.

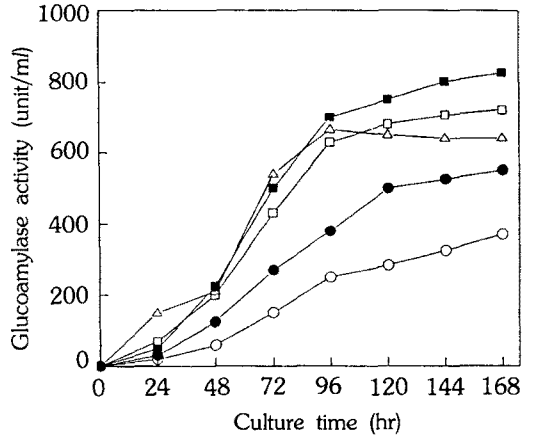


**Fig. 5. Effect of different agitation speeds on glucoamylase production.**
Air flow rate: 1.0 vvm.
○−○: 200 rpm, ●−●: 300 rpm, □−□: 400 rpm, ■−■: 500 rpm, △−△: 600 rpm

of $K_La$ was increased, but, the increasing rate was smaller than that of the rotational speed of impeller.

The agitation speed against the volumetric oxygen transfer coefficient made a straight line in log-log scales, and the exponential value of agitation speed was calculated to be 0.88 by using Eq. (2). Also, the volumetric oxygen transfer coefficient against the air flow rate and the apparent viscosity made a straight line in log-log scales, and the exponential value of air flow rate and apparent viscosity were calculated to be 0.71 and −0.83, respectively. Therefore, we could determine the experimental equation.

$$K_La = 12.04N^{0.88}V_s^{0.71}\eta_a^{-0.83} \quad \text{(Eq. 5)}$$

## Determination of the Agitation and Aeration Conditions for the Maximum Glucoamylase Productivity with *Asp. niger* No. PFST-38

Fig. 5 shows the glucoamylase activity versus the impeller rotational speed during fermentation. As can be seen from the graphs, the glucoamylase production increased with the increase in agitation speed. But, the glucoamylase production decreased when the agitation speed reached to 600 rpm at which the hyphal length started to decrease, as shown in the morphological studies with automatic image analyzer (10). A plot of the glucoamylase productivity against the air flow rate is shown in Fig. 6. The production of glucoamylase increased with the increase of air flow rate upto 1.0 vvm.

The glucoamylase productivity, the mycelium weight and the amount of total sugar against the culture time under optimum cultivation condition are plotted in Fig. 7. The mycelium weight increased rapidly upto 120 hrs

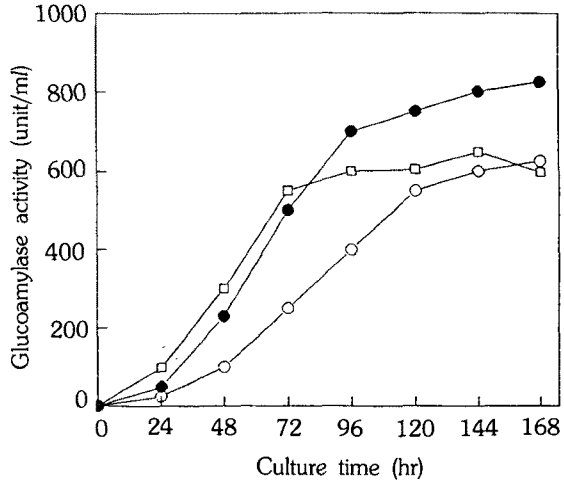


**Fig. 6. Effect of different air flow rates on glucoamylase production.**
Agitation speed: 500 rpm
○−○: 0.5 vvm, ●−●: 1.0 vvm, □−□: 1.5 vvm

of fermentation and remained almost constant afterward. The glucoamylase productivity showed similar tendency with fermentation time and it was parallel with the reduction of total sugar in the medium. When the apparent viscosity and the volumetric oxygen transfer rate is 1800 cps and 260 $hr^{-1}$, respectively, the maximal production of glucoamylase is recorded (Fig. 8). And the values calculated from Eq. (5) confirm to the experimental maximum conditions for the production of glucoamylase when the $K_La$ and the apparent viscosity of the broth is 260 $hr^{-1}$ and 1800 cps, respectively.

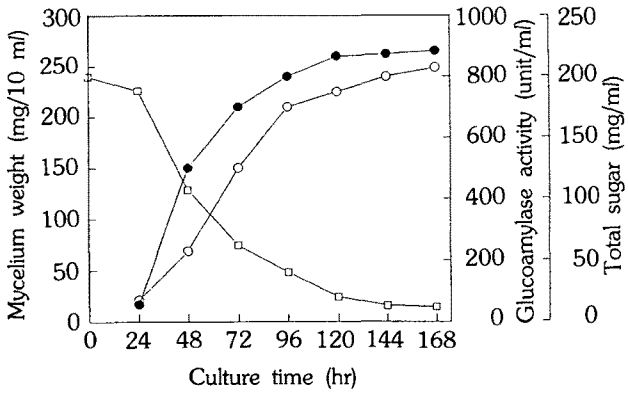


**Fig. 7. Time course curves of glucoamylase production at optimal condition of fermentation.**
Air flow: 1.0 vvm, Agitation: 500 rpm.
□−□: Total sugar, ○−○: Glucoamylase activity, ●−●: Mycelial weight

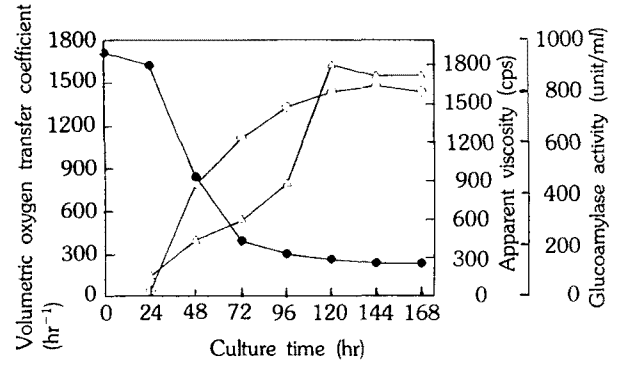


**Fig. 8. Changes in the volumetric oxygen transfer coefficient, the broth viscosity and the glucoamylase activity during fermentation.**
Air flow rate: 1.0 vvm, Agitation: 400 rpm.
●−●: Volumetric oxygen transfer coefficient, △−△: Apparent viscosity, ○−○: Glucoamylase activity

## DISCUSSIONS

Mixing time is considered to be an important criterion in fermentation processes. It is usually defined as the time required to minimize concentration differences in the entire volume of liquid in the fermentor. These concentration differences within a fermentor may be very significant and will cause regional glucose repression, which can influence yields.

The mixing times with Reynolds' number and with apparent viscosity were defined by $\theta_M = 2.95 NRe^{-0.52}$ and by $\theta_M = 1.88\eta_a^{0.57}$ for glucoamylase production with *Asp. niger* No. PFST-38, respectively.

Ryu and Humphrey (13) examined the effect of apparent viscosity on the mass transfer coefficient, $K_La$.

We also proposed the correlation for *Asp. niger* No. PFST-38 broth

$$K_La = 12.04N^{0.88}V_s^{0.71}\eta_a^{0.83} \quad \text{(Eq. 5)}$$

and if the apparent viscosity ($\eta_a$) is omitted from Eq. (4) and Eq. (5),

$$K_La = 30.2N^{0.88}V_s^{0.71}\theta_M^{1.45} \quad \text{(Eq. 6)}$$

The data show that the primary scale-up factor for submerged aerobic fermentors of conventional design is the oxygen transfer coefficient, which is defined as $K_La$ when air is employed as the source of oxygen.

## Nomenclature

P : Power (KW)
V : Liquid volume (*l*)
NRe : Reynolds number
$\eta_a$ : Apparent viscometric viscosity (cps)
N : Rotational speed (min $^{1}$)
$V_s$ : Superficial gas velocity ($m \cdot min^{-1}$)
$\alpha_n$, $\beta_n$ : Constant defined in $\theta_M = \alpha_n \eta_a^{\beta_n}$
x, y, z : Constant defined in Eq. (2)

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