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Rheological Properties of Mycelial Broth in Submerged Culture of *Aspergillus niger* No. PFST-38

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The flow behavior of the mycelial broth of glucoamylase hyperproducer *Asp. niger* No. PFST-38 for the production of glucoamylase were studied. The mycelial broth followed Bingham-pseudoplastic flow model described by Herschel-Bulkley equation. The yield stress increased with the increase in mycelial concentration. The dependency of the consistency index and the flow behavior index on the mycelial concentration could be expressed by a linear relationship. The consistency index increased proportionally with the mycelial concentration while the flow behavior index decreased with the increase in mycelial concentration. The flow property of the broth was related to the morphological data obtain in the previous study. The changes in apparent viscosity of the broth could be expressed as a function of the hyphal thickness as shown below.

$$\eta_a = 1.51 \cdot L_d^{5.62}$$

The rheological behavior of a fermentation broth is of considerable importance in describing the transport phenomena in a fermentor. The rheological studies were undertaken in order to obtain quantitative information about the technological aspects of the transport of mass and heat and of momentum in fermentation liquids in a large scale equipment (2, 5, 6). Several reports described that, in the cultivation of filamentous molds in a air-lift fermentor, oxygen transfer and mixing are rather inefficient mainly due to the low shear in fermentor and high apparent viscosity of the culture broth (11, 1).

The fermentation liquid is actually a suspension of microorganisms which have, in the case of mycelial broths, a high ratio of length to diameter. From the existing suspension theories it is suspected that such suspensions will show, even when the solid content is fairly low, marked non-Newtonian characteristics. An important feature of the batch fermentation process is the change of rheological behavior during fermentation due to the changes in mycelial concentration and morphological characteristics of the mycelia.

There are reasonable amounts of data which demonstrate the non-Newtonian behavior of the fungal fermentation broths. Results from an early experiment of Wittler *et al.* (15), demonstrated that *Penicillium chrysogenum* broth behavior could be described by a pseudo-plastic model. König *et al.* (4) showed that penicillin production is possible by using *Pen. chrysogenum* in air lift tower loop reactors, when the high viscosity of the filamentous mold is reduced by using pellet suspensions. Kim *et al.* (3) reported that the filamentous mycelial suspensions showed a marked deviation from Newtonian behavior and that the experimental data obtained were correlated by a pseudo-plastic model. Mitard and Riba(7) have examined the shear diagrams for *Aspergillus niger* pellet suspensions by using a helical ribbon impeller system.

The present paper deals with the rheological behavior of *Asp. niger* mycelial broths in a submerged production of glucoamylase. The rheological model applicable for the description of the flow behavior of the fermentation broth was examined, and the effects of changing mycelial concentration on the rheological behavior of the broth were determined. The rheological behavior of the broth was related to the morphological data of the mycelia, which was determined in the previous study (10).

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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 (9). It was grown on potato-dextrose-agar(PDA) slants for 5~6 days at 28℃ and the culture was maintained lyophilized.

Preparation of Inoculum

Inoculum was prepared by washing a 5-day-old PDA plate with 10 m*l* of sterile medium, transferring the 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.

Growth Medium and Cultivation

A B.E. Marubishi MSJ-U3 (30 *l*) fermentor was used in these studies. The fermentor was operated at a 18 *l*. The medium composition consisted of 200 g of corn meal, 30 g of defatted soybean meal, 50 g of corn steep liquor, and 5 m*l* of bacterial α-amylase. The pH was adjusted to 6.0 with hydrochloric acid solution before sterilization. The temperature was maintained at 30℃. The aeration rate was maintained at 1 vvm. The impeller speed was 500 rpm unless quoted otherwise. In order to investigate the influence of the mycelial concentration on the model parameters, the mycelium suspensions were reconstituted in its filtrate to give wide ranged concentrations from 4 to 20 g/*l* by thorough mixing for about 1 hr. The amount of biomass during fermentation was determined by weighing the filter cake after drying it at 105℃ overnight.

Measurement of Rheological Properties

The rheological measurements were made on both the model fluids and the fermentation broths. 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. The dimensions of the rotors used are shown in Table 1. The total length of the stainless steel impeller was 220 mm with 40 mm i.d. The impeller was a six-pin rotor. The containers used to hold the samples were 1-*l* glass beakers(height=13.5 cm; diameter=7.3 cm).

Table 1. Rotor dimensions (RS type)

Height(mm)	220
Radius(mm)	40
Height/Radius	5.5
Number of pins	6
Pin thickness(mm)	2
Pin length(mm)	20

Silicone fluid (Toshiba Co.) was used as the Newtonian calibration liquid. The density and the viscosity of the liquid at 30℃ were found to be 952.3 Kg/m^3 and 0.352 Pa·s, respectively. A 0.4% (by wt.) aqueous solution of xanthan gum was used as the non-Newtonian calibration liquid. The flow curve of this fluid was determined at 30℃ by using the standard measuring system, MV III of Haake Viscotester VT 181, and the relationship between torque(M) and rotational speed(N) was obtained as follows; $M=2.53\times10^{-3}\ N^{0.25}$. This relationship was used for the calculation of the shear stress of samples at different shear rate.

Analysis of Rheological Parameters

The Haake Viscotester VT 181 viscometer was used to determine the shear stresses (τ) and apparent viscosities at different shear rates ($\dot{\gamma}$). The relationships were interpreted in terms of the different rheological model: Power law model; $\tau=K\cdot\dot{\gamma}^n$ (13), Bingham plastic model; $\tau=\tau_o+\eta\dot{\gamma}$ (13) and Herschel-Bulkley model; $\tau=\tau_o+K\cdot\dot{\gamma}^n$ (8).

Morphological Measurements

For image analysis, a Magiscan MD image analyzer (Joyce Leobl Ltd., UK) attached to a Nikon Optiphot microscope was used, as described in the previous study (10). Samples were taken from the fermentation broth, which was used for the measurement of the rheological properties.

RESULTS

Rheological Model Applicable to the Mycelial Broth of *Asp. niger*

Figure 1-3 show the shear stress (τ) versus shear rate ($\dot{\gamma}$) plots made by different rheological equations expressing the flow behavior of fermented broths. Where the power law applies, a plot of log τ against log $\dot{\gamma}$ should give a straight line, A closer inspection of Fig. 1 shows that the experimental results in the low shear rate range agree poorly with power law. According to the Bingham plastic equation, a plot of τ against $\dot{\gamma}$ is shown in Fig. 2. It also shows a large deviation at the low shear rate range. As can be seen from Fig. 3, the Herschel-Bulkley equation is in excellent agreement with experiment in the whole range of shear rates tested. Where the Herschel-Bulkley equation applies, the value of flow behavior

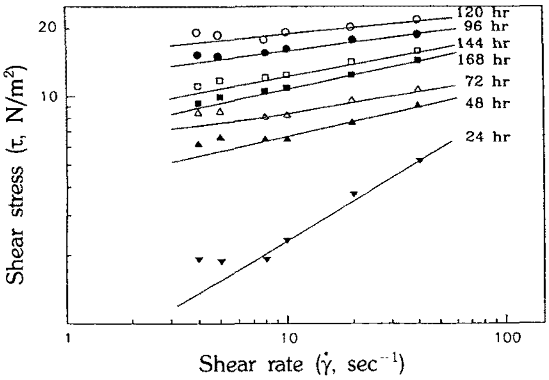

Fig. 1. Shear stress vs. shear rate plots made by the power law equation for the expression of the flow behavior of fermentation broth at different cultivation time.
Viscometer: The Haake Viscometer VT 181 (RS rotor)
Cultivation condition: 500 rpm, 1.0 vvm, 30℃ in 30 *l* jar fermentor.

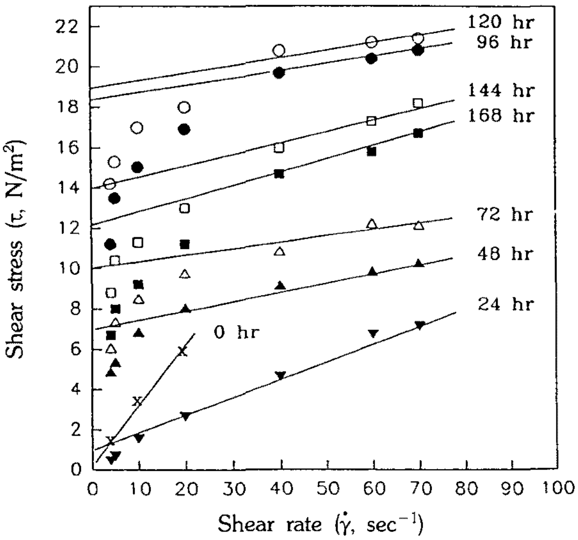

Fig. 2. Shear stress vs. shear rate plots made by the Bingham plastic equation for the expression of the flow behavior of fermentation broth at different cultivation time.
Viscometer: The Haake Viscometer VT 181 (RS rotor)
Cultivation condition: 500 rpm, 1.0 vvm, 30℃ in 30 *l* in jar fermentor.

index(n) and consistency index(K) can be read from the plot of log ($\tau-\tau_o$) against log $\dot{\gamma}$. The yield stress (τ_o) was calculated from Casson equation ($\tau^{1/2}=K'\dot{\gamma}^{1/2}+\tau_o^{1/2}$). The dependency of the consistency index, the flow behavior index and yield stress on fermentation time is given in Fig. 4. The flow behavior index falls rapidly and then rises slowly. Accordingly, the consistency index increases with the fermentation time. τ_o appears to increase in the first 120 hr of fermentation, then it tends to be unchanged during the fermentation time.

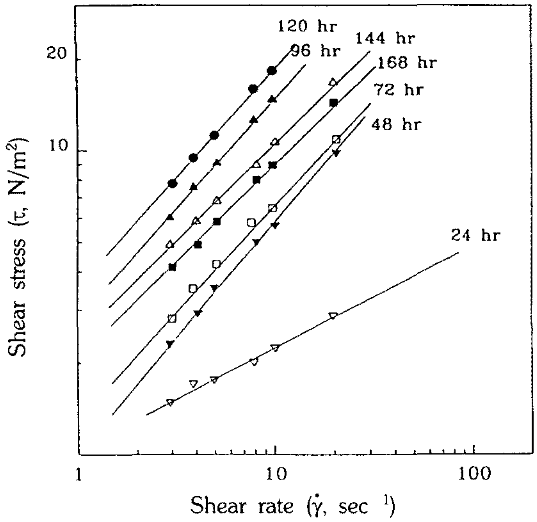

Fig. 3. Shear stress vs. shear rate plots made by the Herschel-Bulkley equation for the expression of the flow behavior of fermentation broth at different cultivation time.

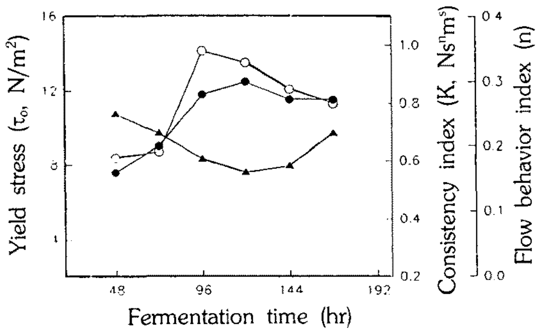

Fig. 4. Changes in flow behavior index, consistency index and yield stress of the fermentation broth by the cultivation time.
Cultivation condition: 500 rpm, 1.0 vvm, 30℃ in 30 *l* jar fermentor.
●−●: Yield stress, ○−○: Consistency index, ▲−▲: Flow behavior index

Effect of the Mycelial Concentration on Broth Rheology

Table 2 shows the influence of mycelial concentration on the yield stress, the consistency index, and flow behavior index of the broth sampled at 72 hr and 120 hr of cultivation. τ_o decreased rapidly as the dilution increased. The mycelial concentration against the consistency index made a straight line in log-log scales, as shown in Fig. 5. The apparent viscosity against the mycelial concentration formed also a straight line in the log-log scales. The mycelial concentration against the flow behavior

Table 2. Dependency of Herschel-Bulkley equation constants on mycelial content (Dilution experiment)

Mycelium concentration(g/l)		τ_o	K	n
Broth at 72 hr	2	22.9	0.2	1.50
	4	37.0	0.5	0.60
	6	48.0	1.0	0.43
	8	59.3	1.3	0.33
	10	74.0	1.8	0.17
	20	118.8	4.5	0.06
Broth at 120 hr	3	28.0	0.3	0.68
	5	43.0	0.9	0.26
	9	65.9	1.5	0.15
	15	100.0	4.0	0.05

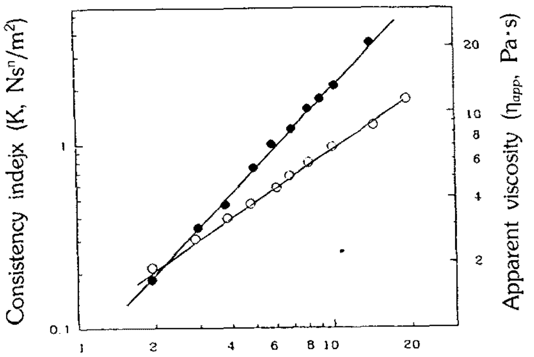

Fig. 5. Influence of mycelium concentration on the consistency index and apparent viscosity of mycelium suspensions of *Asp. niger* No. PFST-38.
Viscometer: The Haake Viscometer VT 181 (RS rotor)
Cultivation condition: 500 rpm, 1.0 vvm, 30℃ in 30 *l* jar fermentor.
The viscosity was measured at a shear rate 10 sec^{-1}.
●—●: Consistency index, ○—○: Apparent viscosity

index made a negative straight line in the log-log scales (Fig. 6).

Relationship Between the Rheological Parameters and Morphological Parameters

Efforts were made to correlate the morphological parameters of mycelia to the flow behavior index and the consistency index of the fermentation broth. The flow behavior index decreased with the mean hyphal thickness (L_d) and the hyphal growth unit increased. The plots of log flow behavior index versus log mean hyphal thickness and log flow behavior index versus log hyphal growth units could be approximated as a straight line. The apparent viscosity against the hyphal thickness showed a straight line in log-log scale, and the exponential value

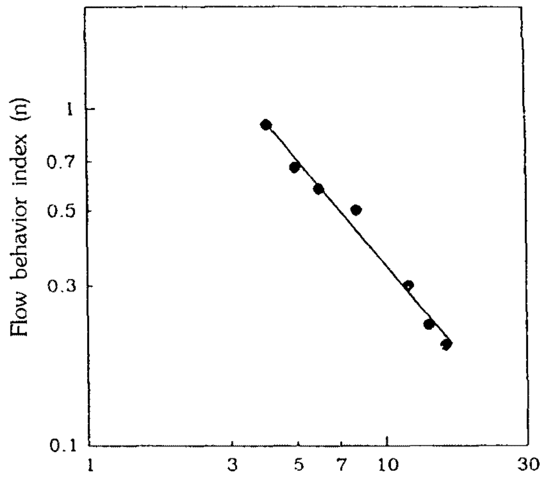

Fig. 6. Influence of mycelium concentration on the flow behavior index of mycelium suspensions of *Asp. niger* No. PFST-38.
Viscometer: The Haake Viscometer VT 181 (RS rotor)
Cultivation condition: 500 rpm, 1.0 vvm, 30℃

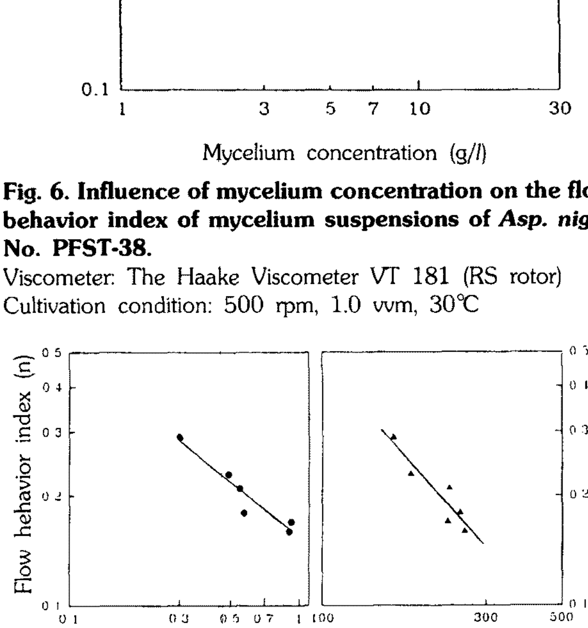

Fig. 7. The effects of mean hyphal thickness and hyphal growth unit to flow behavior index of fermentation broth.
Cultivation condition: 500 rpm, 1.0 vvm, 30℃

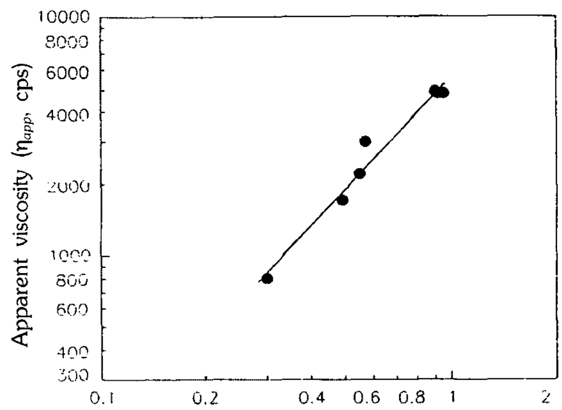

Fig. 8. Relationship between mean hyphal thickness and apparent viscosity.
Cultivation condition: 500 rpm, 1.0 vvm, 30℃

of the hyphal thickness was calculated to be 5.62 (Fig. 8), and the following empirical equation was obtained.

$$\eta_a = 1.51 \cdot L_d^{5.62}$$

The hyphal thickness measured by image analysis could be used as a good parameter for predicting the viscosity of the fermentation broths of *Asp. niger* for the production of glucoamylase.

DISCUSSION

From the analysis of the rheological characters, it could be concluded that the Herschel-Bulkley equation was able to provide an excellent description of the rheological behavior of glucoamylase broths of various morphologies. The power law and the Bingham plastic model failed to describe the behavior in the sufficiently large range of shear rates. It is , therefore, our belief that the Herschel-Bulkley equation can be used for an accurate description of the behavior of glucoamylase mycelial broths in physical operation in stirred vessels.

The viscosity measurements on *Aspergillus niger* broths have been reported by Solomons and Weston (14). Their data indicated a pseudoplastic behavior of the broth under consideration. In general, literature data on filamentous molds show that the consistency index tends to vary with biomass concentration to the power of 2~4 and with increasing biomass concentration the flow behavior index drops off rapidly from 1 to a value between 0.2 and 0.4 (2, 12, 14).

In previous study, a fully automatic image analysis method was applied to obtain detailed data on morphological parameters of the glucoamylase fermentation broth with *Asp. niger* No. PFST-38. We are suggesting that the hyphal thickness and other morphological parameters measured by image analysis can be used as a good parameter to indicate the viscosity of the glucoamylase broths.

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