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Steady-State Shear Characteristics of *Aspergillus niger* Broths

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ABSTRACT

It can be difficult to obtain reliable rheological data for filamentous fermentation broths using conventional instruments. One common approach is to measure the torque drawn by an impeller rotating in the suspension (*1–7*). Many previous workers have assumed that the applicable shear rate in such a device is related to the impeller speed by a fluid-independent constant determined by calibration with Newtonian and non-Newtonian fluids (*1–9*). The rheology of *Aspergillus niger* broths have been characterized using the impeller viscometer approach. The changes in the broth rheology were measured, and used to interpret the growth of biomass and the evolution of the microorganism morphology.

Index Entries: Impeller viscometer; *Aspergillus niger;* mycelial fermentations; rheology.

INTRODUCTION

Mycelial broths are used in the pharmaceutical industry in the production of penicillin, citric acid, amylase, modified steroids, and other products. Broths of filamentous mycelial organisms are known to exhibit highly viscous shear-thinning behavior as the biomass concentration increases. The filamentous nature of the microorganism leads to entanglement of the hyphae resulting in complex rheological behavior (*10*). The structure of the microorganism is quite important, since various researchers have described the marked difference in the apparent viscosity of

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broths of microorganisms with differing morphologies (*5-6,11-12*). It has been shown, for example, that filamentous suspensions of *Aspergillus niger* exhibited apparent viscosities three times greater than pelletal suspensions for similar biomass concentrations (*12*) and that broths of filamentous organisms with short, thin filaments exhibited lower yield stresses than did broths of mycelial organisms with longer hyphae (*6*).

Through their effect on the broth rheology, the concentration and morphology of the microorganism can profoundly affect the rates of mass transfer of oxygen and liquid nutrients within the fermentor. High viscosities tend to promote bubble coalescence, reducing the dispersion of gas throughout the reactor (*5*). Cellular agglomerates may be formed because of the high viscosity of the broth, resulting in possible diffusional limitation if the nutrients in the medium cannot rapidly diffuse into the pellet interior. Although the need for a better understanding of the interaction of the rheology of mycelial broths with oxygen-transfer rates, liquid mixing, and agitation power requirements has been well documented in the literature (*1,13*), advances in this area have been hindered because of the difficulty in obtaining consistent rheological data.

Traditional viscometers do not always provide reliable and accurate data for the rheology of filamentous suspensions. Common problems encountered with conventional instruments of the concentric cylinder, cone-and-plate, and rotating bob types include phase separation near the vicinity of the bob, particle settling, destruction of shear-sensitive organisms in the vicinity of the rotating device, and blockage of narrow gaps by large particles (*2-4*). In order to circumvent the difficulties encountered with traditional viscometers, it has been suggested that a turbine or helical ribbon impeller be used in place of the smooth bob employed in conventional rotational viscometers (*2-4*). Although this alleviates the problems of phase separation and particle settling to some extent, it produces a complicated flow field for which shear rates are not easily calculated (*1*). In order to obtain the constants relating the experimentally measured torques and rotational speeds to shear stresses and shear rates, Newtonian and non-Newtonian calibration fluids of known rheological properties must be used.

DATA ANALYSIS FOR THE IMPELLER VISCOMETER TECHNIQUE

In the laminar flow regime, where the impeller Reynolds number is < 10, the power number of an impeller is inversely proportional to the Reynolds number:

$$P_0 = (P / \rho N^3 D^5) = (c / \mathrm{Re}) \qquad (1)$$

where c is a dimensionless constant, P is the power consumption (W), ρ is the fluid density (kg/m^3), N is the impeller speed (s^{-1}), and D is the impeller diameter (m). For a non-Newtonian fluid, the Reynolds number is defined using an average viscosity:

$$\mathrm{Re} = (\rho N D^2 / \eta_a) \tag{2}$$

where η_a is the apparent viscosity of the fluid (N s/m^2). The power provided by a rotating shaft is related to the torque, Γ (N·m), and speed of rotation via:

$$P = 2\pi N \Gamma \tag{3}$$

Thus, for a given impeller, the torque is directly proportional to the impeller speed and the apparent viscosity:

$$\Gamma = (cD^3 / 2\pi)\, \eta_a N \tag{4}$$

Hence, if the torque is measured as a function of the impeller speed for a Newtonian fluid of known viscosity, the constant c can be obtained from a regression of the torque and impeller speed data. Once the constant, c, has been determined, the apparent viscosity for a non-Newtonian fluid can be determined from measurements of the impeller torque as a function of impeller speed from Eq. (4). In order to determine the shear rate at which this impeller speed is applicable, the assumption that the average shear rate is linearly related to the impeller speed by a fluid-independent constant is invoked (*8,9*):

$$\dot{\gamma}_{\mathrm{avg}} = kN \tag{5}$$

The validity of this assumption has been the subject of some debate. For example, some recent workers contended that the assumption was not valid for a turbine impeller viscometer for fluids that possessed a yield stress, although acceptable results were achieved if a helical ribbon was used instead of a turbine impeller (*6,7*).

METHODS

Microbial Cultures

Rheological data were obtained for broths of *A. niger* ATCC 9029. The composition of the media used for the various fermentations is summarized in Table 1. Deionized water was used in all media preparations. The inocula for the *A. niger* fermentations were grown from potato dextrose agar (PDA) slants in 375 mL of media in a 500-mL Erlenmeyer flask. The shake flasks were kept in a temperature-controlled shaker (New Brunswick Scientific) with the agitation maintained at approx 80 rpm

Table 1
Composition of the Fermentation Media

Component	Concentration, g/L
Glucose	100.0
Urea	0.10
KH_2PO_4	0.20
$MgSO_4 \cdot 7H_2O$	0.15
Corn steep liquor	20.0 mL/L
$(NH_4)_2PO_4$	0.40

since higher agitation rates led to pelletized growth. In order to prepare a 10% (v/v) inoculum, the contents of four flasks were combined after 48 h of incubation and transferred to a 20-L stirred-tank fermenter with a working volume of 15 L (L & H Fermentation). Most of the fermentations were carried out in batch for 5–6 d at a temperature of 30°C and pH of 6.5 maintained using 2*N* solutions of sodium hydroxide and sulfuric acid.

Equipment

The rheological investigations were carried out using three different Brookfield viscometers. Two of these were digital instruments with speed ranges from 0.1 to 250 rpm and torque ranges of $7.187 \times 10^{-4} N \cdot m$ and $5.7496 \times 10^{-3} N \cdot m$, respectively. The lower torque digital viscometer was a cone-and-plate instrument (DV-III RV), whereas the other (model DV-III HB) was of the standard Brookfield rotating bob design. A dial-reading LVT viscometer with a maximum spring torque of $6.737 \times 10^{-5} N \cdot m$ and eight speeds from 0.3 to 60 rpm was used to measure the rheology of less viscous suspensions and solutions. All three instruments accepted standard cylindrical and disk spindles, as well as customized turbine and vane impellers developed for rheology and yield stress studies. A Rushton disk turbine with a diameter of 0.0291 m was used for all impeller rheological tests performed in this study. Samples were kept at a constant temperature of 25°C by means of a circulating water bath with a stability of ±0.1°C. Silicone oils of different viscosities were employed as Newtonian calibration fluids for the determination of c, whereas xanthan gum and CMC solutions were the non-Newtonian fluids used for the determination of k. A summary of the properties of the calibration fluids is given in Table 2. The slight differences in the power-law parameters for the fluids for the different containers reflect the fact that the calibration fluids in each case were prepared separately.

Rheological Measurements

Rheological measurements were performed at 25°C with the cone-and-plate and the turbine impeller viscometers. Impeller viscometer

Table 2
Properties of Calibration Fluids

Solution	Shear rate range, s^{-1}	Power-law parameters: K, Ns^n/m^2	Power-law parameters: n
0.1035-m vessel			
1.0% Xanthan gum	0.2-50	8.85	0.127
1.5% Xanthan gum	0.2-50	11.0	0.170
2.0% Xanthan gum	0.2-14	15.6	0.160
0.072-m vessel			
1.0% Xanthan gum	0.2-50	8.20	0.133
1.5% Xanthan gum	0.2-50	12.1	0.159
2.0% Xanthan gum	0.2-10	16.6	0.149

measurements were performed in one of two vessels with diameters of 0.072 m (sample size = 0.30 L, liquid depth = 0.075 m) and 0.1035 m (sample size = 1.0 L, liquid depth = 0.120 m). The value for the shear rate constant, *k*, was determined for a given data point by calculating the shear rate that would correspond to the apparent viscosity calculated from the impeller viscometer torque-speed data using Eq. (4). Applicable shear rates were interpolated from the experimental viscosity vs shear rate data obtained with the cone-and-plate viscometer. The individual *k* values determined using this procedure were averaged to give a single value for a given fluid. Rheological measurements for the *A. niger* broths were performed solely with the turbine impeller viscometer.

RESULTS

From the silicone oil measurements, the value for the constant, *c*, was determined to be 68.8 for the 0.1035-m diameter container and 69.9 for the 0.072-m vessel. Excellent precision was obtained for all measurements with Re <10. A typical example of the torque-impeller speed curves obtained for Newtonian fluids is shown in Fig. 1. The value of the shear rate constant, *k*, was determined for solutions with nominal xanthan gum concentrations of 0.75, 1.0, 1.5, and 2.0 wt %. The cone-and-plate and impeller viscometer data obtained for 1% xanthan gum solutions in the two containers are displayed in Figs. 2 and 3. The average value for *k* determined for each solution is shown in Table 3 along with the power-law indices computed from the cone-and-plate and impeller viscometer data for each fluid. The data obtained for CMC solutions of 1.0 and 1.5 wt % are presented in Fig. 4. The 1% CMC solution was deemed unsuitable for determination of the shear rate constant for the reasons given in the discussion. Data obtained for the rheology of shake-flask cultures of *A. niger*

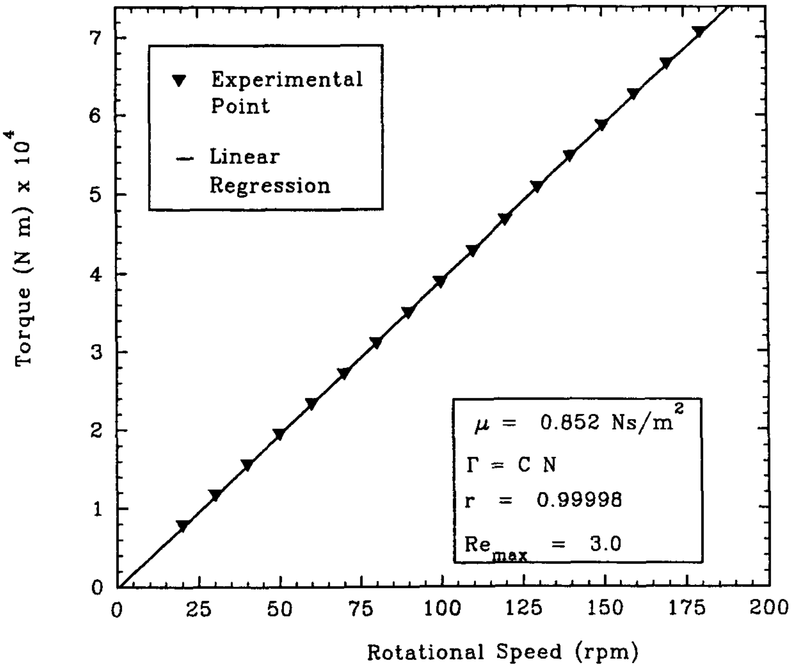

Fig. 1. Torque-speed relationship for a Newtonian silicone oil.

at two different initial substrate levels are shown in Figs. 5 and 6. Figure 7 presents the results of rheological analyses performed on samples taken from the stirred-tank vessel at two different points over the duration of the gluconic acid fermentation.

DISCUSSION

Calibration Procedure

There is no statistically significant difference in the *c* values determined for the 0.072- and the 0.104-m containers. This suggests that wall effects were unimportant over the range of Reynolds numbers encountered in the tests. There is likewise little difference in the shear rate constants determined for the two containers.

The basic premise of the impeller viscometer approach is that the shear rate constant is independent of the rheological properties of the fluid. This assumption is crucial, since it allows the impeller apparatus to be calibrated for homogeneous non-Newtonian fluids, which are amenable to analysis by conventional rheological instruments, and applied to filamentous suspensions, which are not. Recent data in the literature (*6*) indicated that this assumption might not be valid for fluids that possessed a

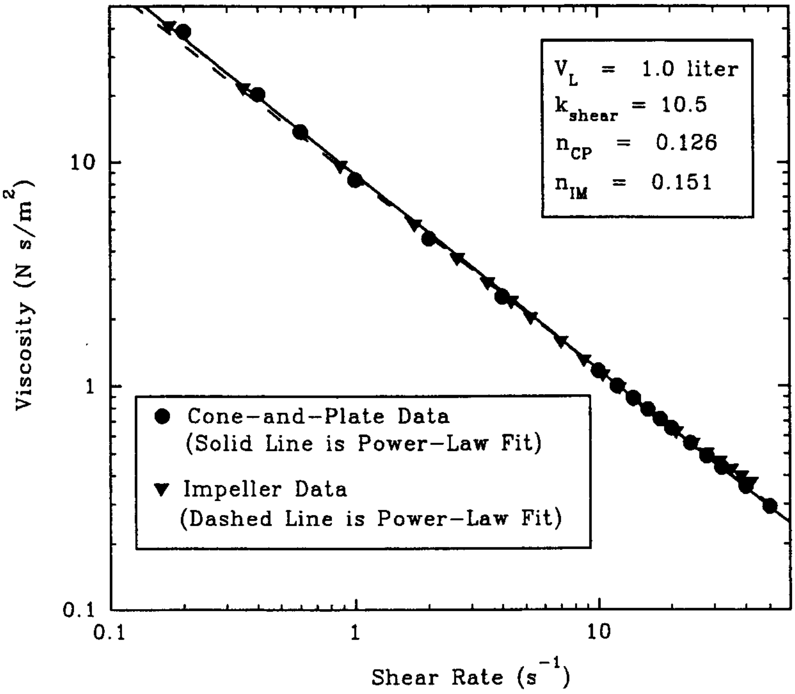

Fig. 2. Cone-and-plate and impeller data for 1.0% xanthan gum—1.0-L vessel.

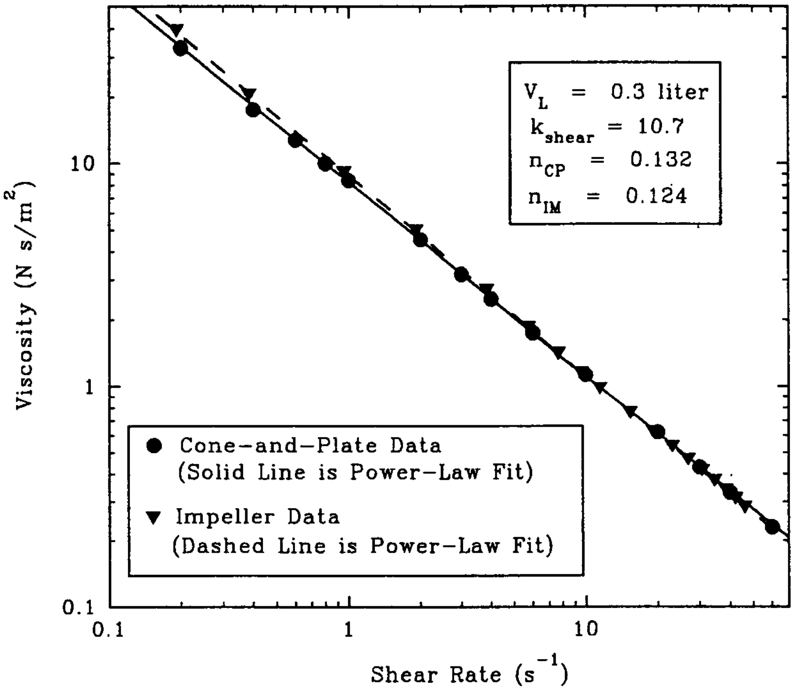

Fig. 3. Cone-and-plate and impeller data for 1.0% xanthan gum—0.3-L vessel.

Table 3
Values of the Shear Rate Constant for the Different Fluids and Vessels

	0.072-m Vessel			0.1035-m Vessel		
Solution	*k*	*n*, C & P	*n*, impeller	*k*	*n*, C & P	*n*, impeller
1.0% Xanthan gum	10.7	0.133	0.124	10.5	0.126	0.151
1.5% Xanthan gum	10.0	0.159	0.156	9.43	0.170	0.148
2.0% Xanthan gum	10.4	0.153	0.149	9.20	0.160	0.162
1.5% CMC	—	—	—	9.60	—	—

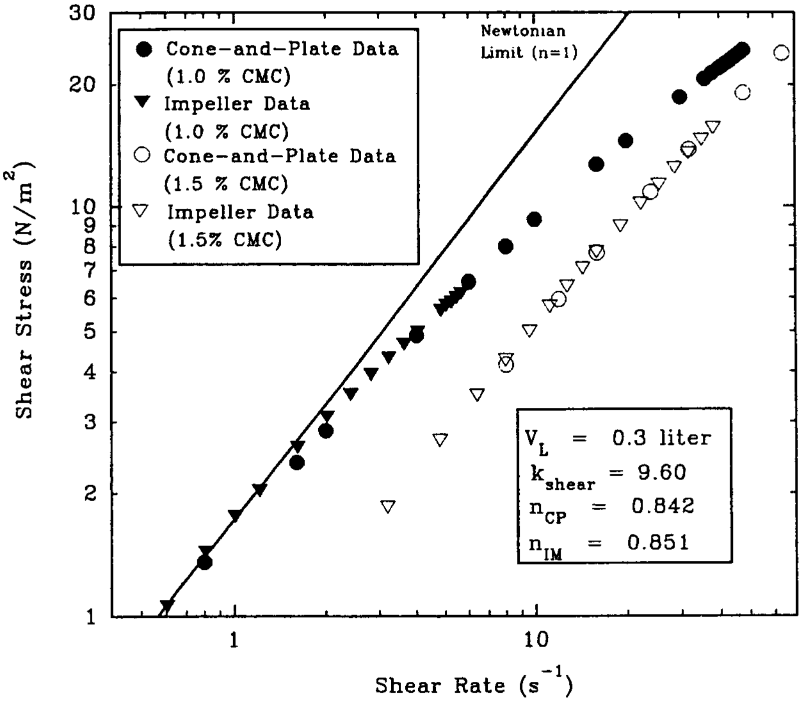

Fig. 4. Cone-and-plate and impeller data for CMC solutions.

yield stress. Xanthan gum solutions were therefore chosen as non-Newtonian calibration fluids, since their rheological behavior at low shear rates is similar to that of yield stress fluids (*14,15*). The calibration results for xanthan solutions ranging in concentration from 0.75 to 2.0 wt % appeared to be self-consistent, since a single value of *k* = 10.0 ± 0.8 would suffice to represent all the data. The power-law indices obtained from the data for the two techniques over the same range of shear rates are also virtually identical, as can be seen from Table 3. There is some discrepancy, however, when the *k* values determined for the xanthan solutions are compared with those found using CMC solutions. The difference

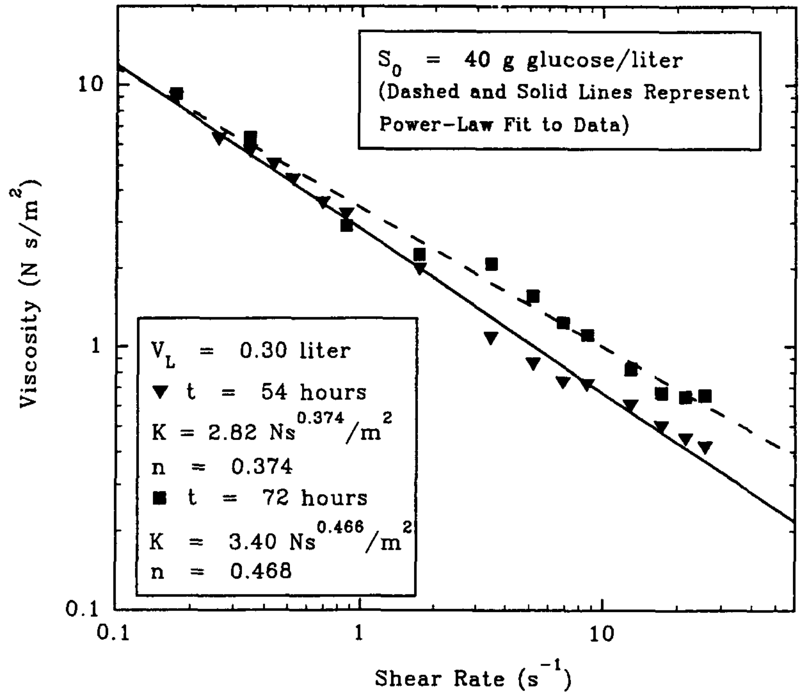

Fig. 5. Rheology data for *A. niger* broths—shake-flask culture.

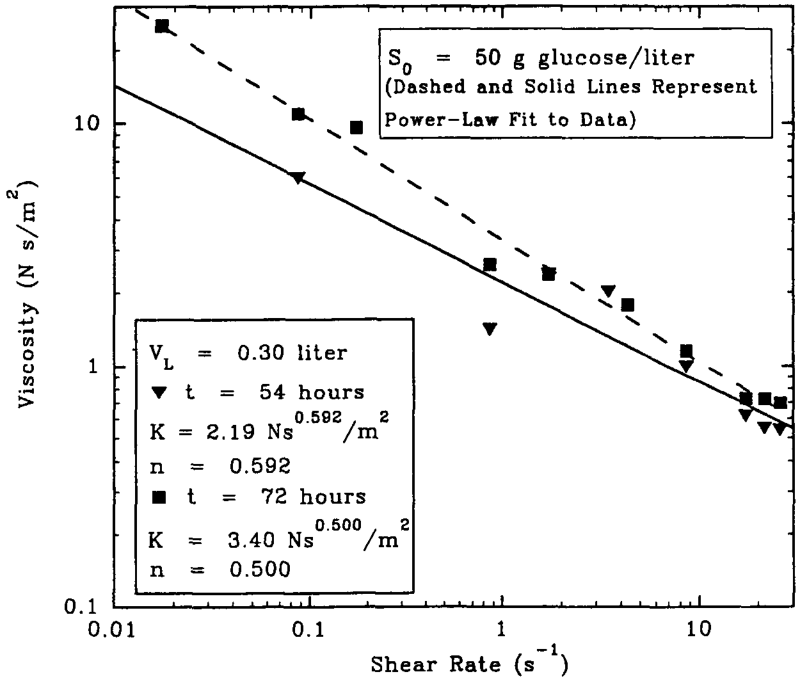

Fig. 6. Rheology data for *A. niger* broths—shake-flask culture.

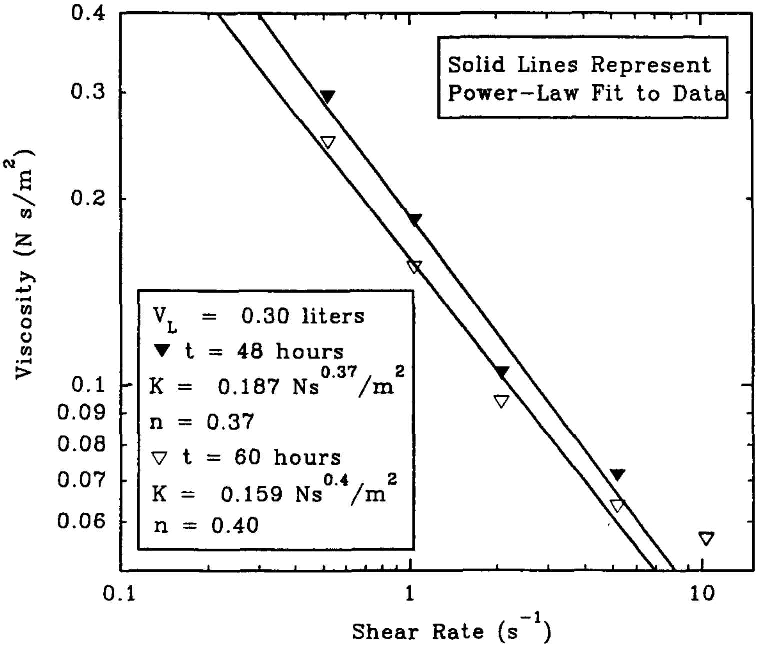

Fig. 7. Rheology data for *A. niger* broths—stirred-tank culture.

in *k* values may be at least partially explained by the particular rheological characteristics of CMS solutions. At low shear rates, the viscosity of almost all polymer melts and solutions approaches Newtonian behavior (*16*). This trend is clearly evident in both the cone-and-plate and impeller data for the CMC solutions given in Fig. 4 (the solid lines indicate the Newtonian limit). By contrast, the power-law model with a low value of *n* was sufficient to describe the rheology of xanthan solutions over the whole range of shear rates studied. In the vicinity of the low-shear Newtonian transition, the power-law flow index will be close to 1, and the viscosity will therefore be relatively insensitive to the shear rate, which is not desirable for determining *k*. The impeller data for the 1% CMC solution virtually all fell within this transition region and were therefore not used for the determination of *k*. The portion of the data for the 1.5% solution that fell within the transition region was excluded from the data set. With this exclusion, the value found for *k* (9.60) is close to the values determined for the xanthan solutions.

Aspergillus niger Broths

The results for the shake-flask rheological tests shown in Figs. 5 and 6 and summarized in Table 4 are relatively easy to interpret. Longer fermentation times correspond to greater apparent viscosities as would be expected from the increased biomass concentration. The difference in the viscosities for the cultures with different initial glucose concentrations is

Table 4
Power-Law Parameters for Selected *A. Niger* Cultures

Type of culture	Initial glucose concentration, g/L	K, $N\ s^n/m^2$	n
Shake flask (t = 54 h)	40	2.82	0.374
Shake flask (t = 72 h)	40	3.40	0.468
Shake flask (t = 54 h)	50	2.19	0.592
Shake flask (t = 72 h)	50	3.40	0.500
Stirred tank (t = 48 h)	100	0.187	0.370
Stirred tank (t = 60 h)	100	0.159	0.400

relatively slight, however. The morphology of the growth was such that a clump of biomass was formed.

The stirred-tank fermentation results are more difficult to interpret for a variety of reasons. Most of the stirred-tank fermentations were conducted with automatic control of pH. The amount of 2.0*N* sodium hydroxide required to neutralize the gluconic acid formed in the fermentation represents a significant fraction of the total fermenter working volume. Therefore, the biomass concentration was continuously being diluted by the addition of sodium hydroxide, and this effect was exacerbated by the periodic removal of 400-mL samples for rheological analysis, making it difficult to make a comparison between samples based on different fermentation times. The microorganisms in the stirred-tank fermentor also tended to grow in the form of pellets, resulting in a comparatively nonviscous broth rheology. It was usually not possible to measure the broth rheologies of the stirred-tank cultures owing to the low torque drawn by the impeller in the mycelial pellet suspensions. The stirred-tank data shown in Fig. 7 were obtained with an LVT Brookfield viscometer for a fermentation conducted without control of pH. There is relatively little difference in the rheological parameters determined for the two samples, which were taken 12 h apart. This might indicate that growth phase had ceased or slowed greatly (the biomass concentrations were 11.6 and 12.8 g/L, respectively). It is interesting to note the clear transition from the viscous flow regime that occurs in the figure. The last (highest speed) data point corresponds to an impeller Reynolds number of approx 15. The apparent viscosity at this point is substantially higher than the extrapolated correlation developed from the other points, indicating a transitional flow regime.

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REFERENCES

1. Charles, M. (1978), *Adv. Biochem. Eng.* **8,** 1-62.
2. Bongenaar, J., Kossen, N., Metz, B., and Meijboom, F. (1973), *Biotechnol. Bioeng.* **15,** 201-206.
3. Roels, J., VanDerBerg, J., and Voncken, R. M. (1974), *Biotech. Bioeng.* **16,** 181-208.
4. Kemblowski, Z. and Kristiansen, B. (1986), *Biotechnol. Bioeng.* **28,** 1474-1483.
5. Metz, B., Kossen, N., and van Suijdam, J. C. (1979), *Adv. Biochem. Eng.* **11,** 103-156.
6. Allen, D. G. and Robinson, C. W. (1990), *Chem. Eng. Sci.* **45(1),** 37-48.
7. Reuss, M., Debus, D., and Zoll, G. (1982), *Chem. Eng.* **June,** 233-236.
8. Metzner, A.B. and Otto, R. E. (1957), *AICHE J.* **3,** 3-10.
9. Calderbank, P. H. and Moo-Young, M. (1959), *Trans. Inst. Chem. Eng.* **37,** 26-33.
10. Pace, G. W. (1980), *Fungal Biotechnology,* Smith, J. E., Berry, D. R., and Kristiansen, B., eds., Academic, New York, pp. 95-110.
11. Deindoerder, F. H. and West, J. H. (1960), *Biotechnol. Bioeng.* **2,** 165.
12. Carrili, A., Chain, E. B., Gualandi, G., and Morisi, G. (1961), *Aeration Studies. III Science Reports. 1st Super. Sanita* 3, 177-189.
13. Blanch, H. W. and Bhavaraju, S. M. (1976), *Biotechnol. Bioeng.* **18,** 745-790.
14. Galindo, E., Torrestiana, B., and Garcia-Rejon, A. A. (1989), *Bioproc. Eng.* **4,** 113-118.
15. Hannote, M., Flores, F., Torres, L., and Galindo, E. (1991), *Chem. Eng. J.* **45,** B49-B56.
16. Rodriguez, F. (1982), *Principles of Polymer Systems.* Hemisphere, New York, p. 173.