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FERMENTATION-MEDIUM RHEOLOGY IN ANTIBIOTIC PRODUCTION

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Measurements have been made on the rheological characteristics of antibiotics under industrial conditions; a power-law relationship applies closely. Maxima occur in the consistency index and minima in the rheological index as the fermentation develops.

Many difficulties in designing and managing biological processes are due to the rheologically complicated behavior of fermentation media, as fibrous microorganisms are present even at low biomass concentrations, and they result in highly viscous and usually non-Newtonian suspensions. It has repeatedly been shown [1-17] that mycella suspensions show non-Newtonian behavior. In a fermentation, the rheological features vary because the mycella concentration alters, as do the morphological characteristics [1]. The data in the literature accessible to us on this are inadequate [1-4]. A major problem in such research concerns the reproducibility between cycles.

When rheological characteristics are measured with normal viscometers such as concentric cylinders, there are difficulties [5] arising from the sizes of the particles, which are comparable with the gap between the cylinders, which means that the structures are disrupted by the measurements; it is also possible for lower-density layers to form at the wall, which makes the results doubtful; the suspensions may also precipitate.

To eliminate these difficulties, it has been proposed to use a 6-blade [1, 5, 6] or 8-blade [7] propeller stirrer or spiral strip [13], which is coupled to the electromechanical part of a standard rotational viscometer. Also, one can assume to ±20% that the shear rate is a simple function of the speed and is independent of the rheological properties [18, 19]. The apparatus has however to be calibrated, which is done by the method given by Metzner and Otto [18] as developed in [1, 6] to suit the rheology. A deficiency is the comparatively narrow range in which the flow is laminar, which is particularly important for low-viscosity liquids.

Surveys [2, 8, 9, 20] deal with fermentation-medium rheology; Table 1 gives the main results from those sources and additional ones in recent years.

Even for a single species of microorganism such as Penicillium chrysogenum, there are various possible rheological models [1, 2, 4, 5, 10, 11], which is largely related to the shear rate range used, since the models are basically experimental ones, not theoretical flow laws. In a sufficiently narrow shear rate range, all those models gives satisfactory accuracy [1, 2, 4] (Fig. 1). As the descriptions are similar in accuracy, the model is chosen on the basis of simplicity.

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TABLE 1. Basic Rheological Data

Microorganism	Ref	Rheological equation	Effects of concn. on rheology	Notes
Penicillium chrysogenum	[1]	$\sqrt{\tau} = \sqrt{\tau_0} + K_c\sqrt{\dot{\gamma}}$ Casson equation (1)	$\tau_0 = \delta X^2$	Based on fibrous morphology, Casson's equation appropriate for the entire range in $\dot{\gamma}$
Penicillium chrysogenum	[10]	$\tau = \tau_0 + K\dot{\gamma}$ Bingham equation (2)	$\tau_0 = \alpha X$	Measurements also closely described by $\tau_0 = \alpha X^2$
Penicillium chrysogenum	[5]	$\tau = \tau_0 + K\dot{\gamma}$		
Penicillium chrysogenum	[2, 11]	$\tau = K\dot{\gamma}^n$ equation (3)		
Penicillium chrysogenum	[4]	$\tau = K\dot{\gamma}^n$ $\tau = \tau_0 + K\dot{\gamma}^n$ equation (4) $\sqrt{\tau} = \sqrt{\tau_0} + K_c\sqrt{\dot{\gamma}}$		All models applicable for narrow ranges, no quantitative relationships derived, numerous additional experiments
Aspergillus niger	[12]	$\tau = K\dot{\gamma}^n$ up a certain concentration, then (1) $\sqrt{\tau} = \sqrt{\tau_0} + K\sqrt{\dot{\gamma}}$		K increases but n falls as the dry mass increases, quantitative relationships not derived
Aspergillus niger	[2]	$\tau = \tau_0 + K\dot{\gamma}^n$, $\tau = K\dot{\gamma}^n$	$K = 0{,}03X^{2.48}$, $K \to \max$, $n \to \min$	Increase in dry mass with reduces n, but within the range covered, there are no large differences between the [1], [3], and [4] models
Aspergillus niger	[3]			Morphology examined for $\dot{\gamma}$ = constant. Pelletal and fibrous structures occur during trowth
Aureobasidium pullulans	[6]	$\tau = K\dot{\gamma}^n$	$K \to \max$ $n \to \min$	Calibration methods were examined for a 6-blade turbine
Aureobasidium pullulans	[2]	$\tau = K\dot{\gamma}^n$	Graphically, n passes through min and K passes through max	Graphs are given showing n passing through a minimum and K through a maximu, with the variations related to concentration and fiber morphology
Pullulans pullularia	[14]	$\tau = K\dot{\gamma}^n$		Curves given for n and K as functions of time and polysaccharide concentration
Kanamycin	[15]	$\tau = \tau_0 + K\dot{\gamma}$		
Endomyces sp.	[16]	$\tau = K\dot{\gamma}^n$		$0{,}3 \leqslant n \leqslant 0{,}9$ $2 \leqslant K \leqslant 35$ dyn/(cm·sec)

Some researchers have considered the relation between morphology in a biological system and rheological parameters [1, 10]; a theoretical correlation of satisfactory type has been formulated [1]. Mitard and Riba [3] also considered the morphology and growth rates for mycella structures at constant shear rate under conditions of Couette flow, as did Van Suijdam [17], who made a comprehensive study on how the shear stress affects the morphology in fibrous fermentation media.

Measurements have also been made on how the fermentation time affects the rheological parameters. Richards [21] found a sinusoidal variation in the yield point and plastic viscosity. It has been shown [2, 6, 12] that the consistency coefficient K passes through a maximum, while the rheological flow index passes through a minimum.

In [3], there is a reasonable explanation of these changes in K and n, where it was found that the mycella organisms pass through two forms: pelletal (spheroidal) and fibrous. As the fermentation proceeds, the mean shear stress for constant shear rate increases. The biomass growth curve has two peaks, which characterize the transition of the mycels from one structure to another, and with the pelletal form disrupted in the gap between them. This is a likely explanation for the sinusoidal variation in the rheological parameters.

In [1], it was suggested that the rheological parameters can be related to the morphol-

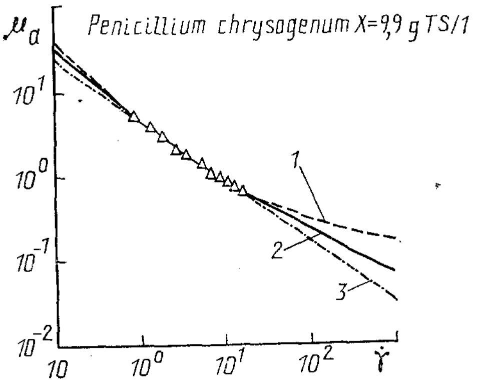

Fig. 1

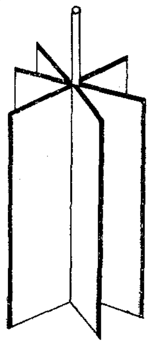

Fig. 2

Fig. 1. Comparison of rheological models for Penicillium chrysogenum suspensions (rheological data obtained with a turbine blade) [2]: 1) Casson's method; 2) Herschel's method; 3) power law; μ_a in Pa·sec and $\dot{\gamma}$ in sec^{-1}.

Fig. 2. The measuring component.

ogy via the morphological factor δ, which is a function of the yield point τ_0 and mycella concentration X:

$$\delta^* = \tau_0 X^{-2.5}.$$

The discussion concerned long fibrous structures (with high ratios of length to diameter), which were compared with polymer chains.

Various methods have been compared [2, 4, 12, 13] for measuring the viscosities of fermentation media; an ordinary rotational viscometer fitted with coaxial cylinders is unsuitable, and others have been used. Fairly common use has been made of a 6-blade stirrer viscometer developed by the Delft group [1, 5]. A capillary viscometer has also been described [4, 12] for high shear stresses, as well as a viscometer having a spiral strip [13]. A comparison has been made [12] of the results obtained with capillary and turbine viscometers, where the discrepancies were fairly substantial and were ascribed to the capillary viscometer measuring the wall properties and the rotational one, the properties of the entire mass. It has also been observed [2] that there are certain differences in apparent viscosity obtained with spiral and turbine viscometers, with the turbine giving higher results. Bjorkman [4] proposed a capillary viscometer for continuous monitoring, which gives consistent results for high shear rates. In it, the flow pattern is not homogeneous, in contrast to a concentric-cylinder apparatus, but is much simpler than in a turbine viscometer, and the results are satisfactory. Continuous monitoring is also possible with a vibrational viscometer [22], whose signal decreases nonlinearly as the apparent viscosity increases. This is a purely empirical instrument that requires calibration for each particular process.

We have made rheological studies on several antibiotic cultures in equipment of pilot-plant and industrial scales to examine the scope for using the viscosity for monitoring and regulating the fermentation.

The industrial experiments cover the complete fermentation cycles for two antibiotics: tylosine and gentamycin. To check the reproducibility, we examined the processes in two pairs of industrial equipments (volume 63 m^3) for two complete cycles in each.

An RV-2 rotational viscometer was used, with the inner cylinder replaced by a 6-blade turbine (Fig. 2); the system was calibrated by the [6] method. The rheological parameters were determined on average every 8 h during the complete cycle.

Figure 3 shows typical curves for tylosine; those for gentamycin are similar. The flow is nonnewtonian. The data were processed by least squares to show that the behavior was described with ±6% error by the Ostwald—de Weil equation (3) and the Casson model (1) throughout the shear rate range used (Table 1). The power-law relationship can thus be used to facilitate calculations.

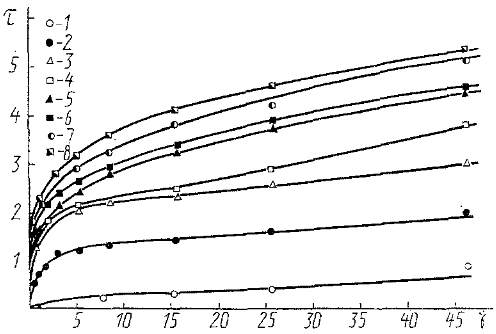

Fig. 3. Tylosine flow curves for various times from the start of fermentation in h: 1) 0; 2) 18; 3) 25; 4) 32; 5) 49, ≡90, ≡120; 6) 42; 7) 56; 8) 66; τ in Pa.

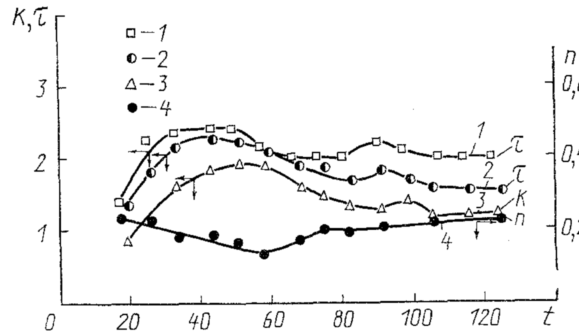

Fig. 4. Process parameters as functions of fermentation time for tylosine: 1 and 2) shear stress τ as a function of time t for two fermentation cycles; 3) consistency index K as a function of time t; 4) rheological flow index n as a function of time t; K in Pa·sec^{-n}, t in h.

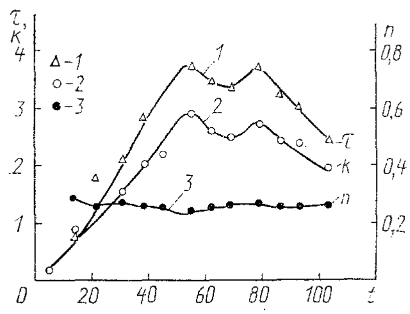

Fig. 5. Time dependence of process parameters for gentamycin: 1) τ = f(t); 2) K = f(t); 3) n = f(t).

Figure 3 shows that the shear stresses fall at the end, which suggests that there is a maximum as a function of shear rate. Figures 4 and 5 show how the rheological parameters and shear stress varied with time for tylosine and gentamycin. The results in Fig. 4 are of satisfactory reproducibility for industrial conditions. The stress–time curves have two peaks each, which are probably due to transition from pelletal structures to branched mycella ones. At that stage, however, no morphological studies were made, which form the subject of future research.

K and n also have turning points; n has a minimum when K has a maximum, which is natural in view of the physical significance, since the first characterizes the deviation from newtonian behavior and K characterizes the consistency.

Figures 4 and 5 also show good agreement over the instants when the turning points occur; the variation in shear stress with time is shown for constant shear rate, while the variations in n and K characterize the behavior throughout the cycle and for all shear rates.

These results can be used in biotechnology, but if they are employed for exact monitoring, it is necessary to perform numerous experiments. The next stage in the research is concerned with continuous monitoring via the rheological parameters.

NOTATION

δ morphological factor, dimensionless; $\dot{\gamma}$ shear rate in sec^{-1}; τ shear stress in Pa; τ_0 yield point in Pa; K consistency coefficient, $Pa \cdot sec^{-n}$; n rheological flow index, dimensionless; t time in h; X concentration.

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