Rolling-sphere viscometer for in situ monitoring of shake-flask fermentations

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A rolling-sphere viscometer (RSV) was constructed and evaluated for monitoring apparent viscosities of fermentation broths in shake flasks or test tubes. The apparatus was calibrated by measuring translational velocities of magnetic stainless steel spheres (0.16–0.95 cm diameter) rolling in Newtonian viscosity standards (50–12,500 mPa·s) on surfaces inclined 20° to 70°. The method was applied to fermentation broths of Aureobasidium pullulans. Apparent viscosities fit the power-law equation. Maximum viscosities and minimum power-law indices were reached within 4 to 6 days fermentation time, followed by a decrease in viscosity.

Keywords: Aureobasidium; pullulans; pullularia; polysaccharides; pseudoplastic; apparent viscosity; rolling-ball viscometer; Stokes’ law; power-law equation

Introduction
The change in viscosity of fermentation broths during production of microbial polysaccharides provides a useful indication of the progress of the fermentation. Samples must be removed periodically for measuring rheological properties of shear stress versus shear rate or of apparent viscosity versus a measure of shear rate. Apparent viscosities may be obtained by use of such instruments as the Brookfield viscometer. The rolling-sphere viscometer (RSV) described herein provides a rapid measure of apparent viscosity by use of relatively inexpensive equipment without invading the environment within the fermentation flask to remove samples.

Most polymeric solutions exhibit pseudoplastic rheological behavior, in which apparent viscosity decreases with increasing rotational speed or shear rate, and their viscosities are frequently fitted by a power function:

\[ \mu_a = K \sigma^n \]

where \( K \) is the consistency index (mPa·s\(^n\)), \( \sigma \) is the shear rate (s\(^{-1}\)), and \( n \) is the “power-law” index, calculated from the slope of a log-log graph of \( \mu_a \) versus \( \sigma \). The power function has been used to characterize the rheological properties of fermentation broths of A. pullulans, Xanthomonas campestris, and Leuconostoc mesenteroides. For monitoring changes in relative viscosities of fermentation broths, apparent viscosities at approximately the same shear rate as obtained with a Brookfield viscometer provide a useful comparison.

The concept of an RSV, first proposed by Flowers, provides the basis for an in situ method to monitor fermentations in shake flasks or other small conical or cylindrical fermentors with a straight side. A measure of relative viscosity can be obtained from the terminal rate of descent of a sphere on the inclined surface. The relation between translational velocity of the rolling sphere and viscosity cannot be calculated precisely, but the method can be calibrated with liquids of known viscosities. A modified form of the Stokes’ law equation for a sphere falling vertically under the influence of gravity has been applied for calculating apparent viscosity by use of an RSV:

\[ \mu = \frac{5.556 mD^2g \sin \theta}{\rho_s - \rho_l} u \]

where \( \mu \) is viscosity (mPa·s); \( m \) is an empirical correction factor to account for deviations from Stokes’ Law caused by friction and wall effects; \( D \) is the sphere diameter (cm); \( g \) is the gravitational constant, 980.1 cm s\(^{-2}\); \( \theta \) is the angle of inclination of the surface (degrees); \( \rho_s \) and \( \rho_l \) are densities of the sphere and liquid, respectively (g cm\(^{-3}\)); and \( u \) is the terminal transla-
tional velocity of the ball (cm s⁻¹). The velocity of the sphere in standard Newtonian fluids was used to determine the correction factor, \( m \). The viscometer consisted of a 0.064 cm-diameter steel sphere in a 100-μl micropipette (0.13 cm inside diameter) which could be inclined at several fixed angles from horizontal. That approach was extended and automated to include an electronic timing device to detect the elapsed time for the sphere to travel between two photodetectors. The micropipette was supported in a carriage that could be rotated to set the angle of inclination, although, in practice, the RSV was operated at standard angles of 30° or 40°, and the sphere transit time was measured to obtain relative comparisons of viscosities of biopolymers. Determination of apparent viscosity was less important than sensitivity to relative changes in viscosity.

For applications of the RSV to non-Newtonian fluids, measurements of apparent viscosity should be correlated with a measure of shear rate to more fully characterize the rheological behavior. However, there is no theoretical basis for calculating shear rate because of the nonsymmetrical pattern of fluid flow around a sphere rolling on an inclined surface. We have applied a purely empirical approach through comparison with the mathematical analysis for a sphere falling under the influence of gravity without wall effects, in which the shear rate is proportional to \( u/D \) (s⁻¹). This paper describes an RSV for measuring apparent viscosities of microbial polysaccharide solutions such as A. pullulans broths during the course of a fermentation. In our adaptation of the RSV, the sphere diameter is less than 15% of the diameter of the flask, and previous calibrations do not apply.

Materials and methods

The apparatus depicted in Figure 1 was constructed of clear polyacrylate plastic to support an Erlenmeyer flask or test tube with provision for adjusting the angle of inclination by means of laboratory clamps. A protractor (1° graduations) with a pendulum indicator was used to set the angle of inclination with a reproducibility of ±0.25°. Two pairs of fiberoptic cables spaced 5 cm apart were held with the ends in contact with the surface of the inclined container. A rolling sphere that traversed the upper sensor interrupted a beam of light that was transmitted to the surface of the flask through one fiberoptic cable. Light reflected from the sphere was transmitted through the second cable to the photoelectric sensor, which started a precision electronic timer sensitive to ±0.001 s. The elapsed time was displayed when the sphere interrupted a similar light beam from a fiberoptic cable mounted in its path 5 cm downstream. The circuit was designed to calculate the elapsed time for the center of a sphere to traverse each sensor, in order to reduce the error caused by changes in sensitivity of the sensors and to compensate for off-center paths where the sphere did not pass directly over the center of the fiberoptic cables.

Stainless steel spheres of nominal diameters [0.159 cm (½ in), 0.238 cm (½ in), 0.318 cm (1 in), 0.476 cm (1½ in), 0.635 cm (1 in), and 0.953 cm (3 in)] and constructed of a ferromagnetic stainless steel alloy (\( \rho_s = 7.64 \text{ g cm}^{-3} \)) were used for experimental tests. Measurements were conducted by manipulating the sphere manually with a magnet to the drop point above the first sensor. The time signals for five to ten replicates were transmitted to a personal computer for the mean and standard error of the mean (SEM) and coefficient of variation (CV) to be calculated and printed.

The viscometer was calibrated with standards of nominal viscosities of 50, 100, 500, 1,000, 5,000, and 12,500 mPa·s and 0.97 g cm⁻³ density (Brookfield Engineering Laboratory, Stoughton, MA) contained in 500-ml Erlenmeyer flasks. The average CV for \( n \) determinations of elapsed time with the viscosity standards were, for 50 to 12,500 mPa·s: 0.39% (\( n = 19 \)), 0.46% (\( n = 56 \)), 0.64% (\( n = 61 \)), 0.73% (\( n = 69 \)), 0.65% (\( n = 37 \)), and 0.85% (\( n = 17 \)). Exact viscosities at 25°C and an approximate viscosity—temperature relationship for each standard were provided by the manufacturer. Viscosities of the standards were verified in tests with calibrated glass capillary viscometers (Cannon Instrument Co., State College, PA). Experimental measurements were made at 21°C to 25°C and temperature-corrected viscosities were used to calculate \( m \), the empirical Stokes' Law correction factor, by rearranging equation (2). The calibration data were fitted with an equation of the type:

\[
m = a + bD^{ac} + d \sin \theta \tag{3}
\]

where \( a, b, c, \) and \( d \) are constants selected for equation (3) to fit the experimental data with minimum residual error.

Fermentation broths (150 ml) of A. pullulans, strain NRRL 6220, were prepared in 500-ml Erlenmeyer
flasks with 50 g l\(^{-1}\) carbohydrate, which was sucrose unless specified otherwise. Other components of the medium were 5 g l\(^{-1}\) \(K_2HPO_4\), 1.0 g l\(^{-1}\) NaCl, 0.6 g l\(^{-1}\) \((NH_4)_2SO_4\), 0.4 g l\(^{-1}\) \(MgSO_4 \cdot 7H_2O\), 0.4 g l\(^{-1}\) yeast extract (BBL), and 0.02 g l\(^{-1}\) thiamine. Before autoclaving, the pH was 5.4. Broths were incubated for 10 days at 28°C on a rotary shaker (200 min\(^{-1}\) with 3.8 cm eccentricity).

For daily measurements with polysaccharide fermentation broths, apparent viscosities were calculated by means of equation (5) from the terminal velocities for one or more spheres of different size and at several angles of inclination. A single measurement required less than 30 s, and the angle of inclination was changed in less than 1 min. Flasks were shaken by hand or on a laboratory shaker between replicate measurements to simulate the agitation and aeration conditions in the rotary shaker-incubator used for the fermentations. Apparent viscosities were plotted on logarithmic graph paper versus \(a/\ell D\) (s\(^{-1}\)). The data could be fitted with a linear regression model, and the power-law index, \(n\), was calculated from the slope. The consistency index, \(K\), could not be calculated because of the indeterminate relation between shear rate, \(\sigma\), and \(a/\ell D\).

Apparent viscosity of a 4-day-old pullulan broth was measured with a Brookfield Model LVT viscometer and with the RSV, to compare results with the two methods. The power-law index, \(n\), may be calculated from “Brookfield” viscosities by use of equation (1), in which spindle rotation, \(N\) (min\(^{-1}\)), is the measure of shear rate, \(\sigma\) (s\(^{-1}\)).

**Results and discussion**

**Calibration results**

Experimental determinations of velocity for six sizes of spheres with six viscosity standards and with the viscometer inclined at 30° are presented in **Figure 2**. The correlation of translational velocity with viscosity for each size is linear at velocities to approximately 10 cm s\(^{-1}\) on a logarithmic graph with a slope of approximately one. The correlation was not linear for velocities exceeding approximately 10 cm s\(^{-1}\), which occurred for the larger spheres in 50 and 100 mPa s viscosity standards, because the spheres had not yet reached terminal velocity and were continuing to accelerate in the space between sensors. **Figure 2** can be used to estimate apparent viscosities of unknown samples tested at 30° inclination, and similar calibration plots can be constructed for other angles of inclination.

**Figure 3** indicates that the Stokes’ Law correction factor, \(m\), increased with increasing sin \(\theta\), and the variation was approximately linear in the sin \(\theta\) range of 0.34 (20°) to 0.94 (70°). In addition, the slopes of the linear portions were approximately the same for the six sphere sizes, which indicated that an interaction term with both \(D\) and \(\theta\) would not be needed in a model equation to fit the experimental calibration data. In fitting equation (3) to the calibration data, for each assumed value of the exponent, \(c\), the optimum values of the constants \(a, b,\) and \(d\) were determined to provide the least residual error or deviation of the model from the experimental data. **Figure 4** indicates that the optimum model occurred with a reciprocal square root of \(D\) term in equation (3):

\[
m = 0.1900 - 0.0329D^{-0.5} + 0.0825 \sin \theta \quad (4)
\]

Substitution of equation (4) into equation (2) yields an equation for calculating apparent viscosity for spheres with diameters less than 15% of the diameter of the flask:

\[
\mu = 1035 \left[ (D^2 - 0.173D^{1.5}) \sin \theta + 0.434D^2 \sin \theta \right] (\rho_s - \rho_f)/\ell U \quad (5)
\]

Another calibration equation would be required for use of the rolling sphere method under conditions where the sphere diameter is a large fraction of the container diameter, such as for small test tubes. Once the calibration has been established for specific sphere

**Figure 2**  Logarithmic correlation of velocities of stainless steel spheres rolling in Newtonian viscosity standards contained in 500-ml Erlenmeyer flasks inclined 30°. Sphere diameters (cm): (■) 0.159, (▲) 0.318, (▲) 0.318, (▲) 0.159, (▲) 0.318, (▲) 0.238

**Figure 3**  Variation of the Stokes’ Law correction factor, \(m\) ± SEM, with sine of the angle of inclination, \(\sin \theta\), for stainless steel spheres of diameters (cm): (■) 0.159, (▲) 0.318, (▲) 0.636
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Figure 4 Effect of the value of the exponent, c, in the equation, \( m = a + bD^{-c} + d \sin \phi \), on residual error of the model for spheres rolling in Newtonian liquids.

Figure 5 Logarithmic correlation of apparent viscosities, \( \mu_a \) (mPa·s) for 4-day-old fermentation broth of A. pullulans, strain 6220: (■) measured with a Brookfield viscometer and plotted versus \( \log N \) (min\(^{-1}\)), and (△) measured with the rolling-sphere viscometer and plotted versus \( \log u/D \) (s\(^{-1}\)).

For comparing results with two methods, the term \((u/D)_{30}\) is defined as a reference measure of shear rate for the RSV, where the apparent viscosity equals the Brookfield viscosity at 30 min\(^{-1}\). For the pullulan broth of Figure 5, \((u/D)_{30}\) is approximately 3 s\(^{-1}\), and this value was used as the reference measure of shear rate for monitoring changes of apparent viscosity during pullulan fermentations. For monitoring fermentation broths, sphere sizes and angles of inclination should be chosen to provide values of \(u/D\) that bracket \((u/D)_{30}\) and permit \(\mu_a\) to be determined by interpolation.

Monitoring fermentation broths

Figure 6 presents apparent viscosities of a pullulan broth measured with the RSV for several days during the course of a fermentation. Apparent viscosities increased during the first six days and declined on succeeding days. The slope also increased early in the fermentation, which indicated a decrease in the value of the power-law index, \(n\), as the broth became more pseudoplastic, presumably as the concentration of polysaccharide and its molecular weight increased. Beyond 6 days, the slope decreased.

The effects of fermentation time on apparent viscosity at a value of \((u/D)_{30} = 3\) s\(^{-1}\) are indicated in Figure 7 for pullulan broths with different carbohydrate sources. Figure 8 presents the change in the power-law index, \(n\), for these broths as a function of fermentation time. The trends of a maximum viscosity and a minimum \(n\) with values reported in Table 1, are similar to those reported by others. Leduy\(^4\) reported a minimum \(n\) of 0.25–0.29 after 4–5 days for strain 2552 with sucrose as carbohydrate, and Kembowski and Kristiansen\(^1\) reported that \(n\) decreased to 0.35 after 3 days fermentation with an undisclosed strain of A. pullulans. The loss of viscosity was presumed to be caused by a depolymerase excreted late in the fermentation.\(^1\) The resulting decrease in molecular weight would cause the solution to become less pseudoplastic.

and test tube diameters, the RSV does not require standardization.

Comparison of Brookfield and rolling-sphere apparent viscosities

Figure 5 presents logarithmic graphs of apparent viscosities of a four-day-old A. pullulans fermentation broth versus measures of shear rate: (1) the spindle rotation \(N\) (min\(^{-1}\)) for the Brookfield instrument, and (2) the function \(u/D\) (s\(^{-1}\)) for the rolling-sphere viscometer. Both correlations are linear and of approximately the same slope, with power-law indices of 0.45 and 0.44 for the Brookfield and for the rolling-sphere, respectively. The correlation equations were: \(Y = 3.715 - 0.554X\), SD = 0.011, for the Brookfield and \(Y = 3.167 - 0.565X\), SD = 0.013, for the rolling-sphere.

The Brookfield viscosity at 30 min\(^{-1}\) was 790 mPa·s.
with time. In our study, the highest viscosity was reached with sucrose as carbohydrate source, followed by glucose, maltrin 250, and soluble starch. Maximum viscosities were reached within 4–6 days, while minimum n occurred a day earlier. The broth with glucose deviated most from Newtonian behavior, with a power-law index of 0.31, suggesting that the concentration of polysaccharide may have been lower than occurred with sucrose, but the molecular weight may have been higher.

Conclusions

The RSV provides a useful in situ method that uses inexpensive equipment to monitor apparent viscosities of polysaccharide fermentation broths in shake flasks or in other conical or cylindrical containers. The method can be calibrated with Newtonian viscosity standards by means of a modified form of the Stokes’ Law equation for a free-falling sphere and can be used to determine apparent viscosities of pseudoplastic solutions. Apparent viscosities of fermentation broths of A. pulpasans were shown to fit the power equation based on u/D (s⁻¹) as a measure of shear rate. Our results confirmed the findings of others that maximum viscosity and minimum power-law index, n, are reached within 4–6 days, followed by a loss of viscosity and a return to more nearly Newtonian rheological behavior as the broths age.

Nomenclature

- D: sphere diameter (cm)
- g: gravitational constant, 980.1 cm s⁻²
- K: power-law consistency index (mPa·sⁿ)
- m: correction factor for Stokes’ Law
- n: power-law index
- N: rotational speed of Brookfield spindle (min⁻¹)
- u: translational velocity of rolling sphere (cm s⁻¹)
- μ: viscosity of newtonian fluid (mPa·s) (centipoise)
- μₐ: apparent viscosity of fermentation broth (mPa·s) (centipoise)
- ρ₁: density of liquid (g cm⁻³)
- ρₛ: density of sphere (g cm⁻³)
- σ: shear rate (s⁻¹)
- θ: angle of inclination (°)

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References