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Plant Cell Suspension Culture Rheology

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The results of rheological measurements on 10 different plant cell suspension cultures are presented. *Nicotiana tabacum* (tobacco) suspension cultures grown in serial batch subculture display high viscosity and power law rheology. This "undesirable" rheology is shown to be a result of elongated cell morphology. The rheology of *Papaver somniferum* (poppy) cell suspensions is quite different; poppy suspensions behave as Newtonian fluids and have relatively low viscosity (less than 15 cP) at fresh cell densities up to 250 g/L. This flow behavior can be attributed to a lack of elongation in batch-grown poppy cells. A simple correlation for the viscosity as a function of cell density is developed for poppy suspensions up to 300 g fresh weight (FW)/L. It is shown that tobacco cells do not elongate when grown in semicontinuous culture (daily media replacement). These semicontinuously cultured cells have rheological behavior that is indistinguishable from that of poppy, further confirming the dependence of rheology on plant cell morphology. The rheology of a wide variety of other plant suspensions at 200 g FW/L is presented. Most cell suspensions, including soybean, cotton, bindweed, and potato, display low viscosities similar to poppy suspensions. Only carrot and atriplex exhibit slight pseudoplastic behavior which corresponded to a slight degree of cellular elongation for these cultures. This demonstrates that complex rheology associated with elongated cell morphology is much less common than low-viscosity Newtonian behavior. High viscosity in plant cell culture is therefore not an intrinsic characteristic of plant cells but, instead, is a result of the ability to grow cultures to extremely high cell densities due to low biological oxygen demand. © 1993 John Wiley & Sons, Inc.
Key words: viscosity correlation • morphology effects • aggregate effects • poppy cultures

INTRODUCTION

Plant suspension cultures are a potential source of important plant-derived chemical compounds. To scale-up such processes, the physical properties of plant suspensions must be determined. As in other submerged culture systems, suspension rheology is important in determining bioreactor power requirements and transport properties. Most rheological studies have focused on high-density culture in the range of 300 g fresh weight (FW)/L.[1,9,25,27,28] The interest in high-density suspensions is driven in part by the need to increase the volumetric productivities of plant cell suspension cultures in order to make these processes commercially viable.[19] Typical growth conditions for cultured plant cells involve inoculation at about 4 g FW/L and final cell densities of 200–250 g FW/L, particularly for non-Solanaceous plants. (Solanaceous plants such as tobacco, potato, and *H. muticus* which are often used as model systems may bias density expectations due to their ease of culturing.) While the density can be driven to levels of 300–400 g FW/L in shake flasks and modified small-scale fermentors (unpublished results), it is likely that enhancements achieved in high-density culture will be mitigated to a large extent to problems associated with scaleup. Taking the cue from commercialization of antibiotics, it can be anticipated that economic viability will hinge on the success of "brute force" screening programs, whereas reactor developments will focus primarily on making large-scale culture possible and maintaining economic competitiveness.

Plant cell suspensions have been reported to be rather viscous even at moderate tissue densities less than 300 g FW/L.[9,25] This has led to a proliferation of reviews citing the technological problems to be encountered in large-scale culture of plant cells. The purpose of this work is to provide a broad perspective on plant cell culture rheology focusing on moderately dense cultures (less than 300 g FW/L). In the present study, we show that most plant cell suspensions display Newtonian behavior at moderate cell densities, and non-Newtonian behavior is a result of cellular elongation observed in only a few cell lines. Newtonian viscosity is successfully correlated with cell density for poppy suspension cultures, and the potential application of on-line rheometry as a means of monitoring plant cell reactors is discussed.

MATERIALS AND APPARATUS

Viscometry

Two viscometers were used in the study. To facilitate a direct comparison to previous work,[9,25,29] a Brookfield syncro-electric viscometer was used for rheological comparison of tobacco to other plant cell suspension types.

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Shear rate was determined by the speed of the synchronous motor; shear stress is proportional to the torque on the rotor and is calibrated with dial deflection. Rotor 1, diameter 18.8 mm and height 65.2 mm, was used for all measurements. The Brookfield viscometer used with rotor 1 is treated mathematically as a concentric cylinder with infinite outer radius. As long as flow remains laminar, a plot of dial reading against rotor speed will directly demonstrate rheological behavior.[12] Sucrose solutions were used to determine a Newtonian calibration constant, K, of 0.137 dyn/cm^2:

$$\tau = \mathrm{K}\ (\text{dial reading}) \tag{1}$$

Data obtained with the Brookfield viscometer are presented here as dial readings to avoid complicating the interpretation with non-Newtonian shear rate expressions. Rheological characteristics of the suspensions were measured in a 500-mL glass graduate cylinder sawed off at 200 mL. This allowed the rotor 13 mm clearance from the bottom during measurements with 150 mL of suspension. Immediately after suspension by mixing, the apparent viscosity was measured (in triplicate) at 15, 30, 60, 90, and 120 s at each of the four rotation rates. The average of six readings, three at 30 s and three at 60 s, was used to calculate the average dial deflection reading.

The second viscometer used was a Stormer viscometer modified into a concentric cylinder arrangement. The outer baffled cup was replaced by a smooth cup, radius 17.2 mm, to facilitate calculation of shear rate. Different shear stresses were applied by hanging different weights of mass, W, on a pulley/gear system attached to a rotor (radius R_b = 15.9 mm and height h = 35 mm). The shear stress at the rotor is given by Eq. (2), with a mechanical gear constant Γ of 0.0815,[27]

$$\tau = \frac{\Gamma \cdot Wg}{2\pi R_b h} \tag{2}$$

To obtain an explicit equation for shear rate S, the flow behavior of the fluid must be known. Assuming the following constitutive equation for a Newtonian fluid,

$$\tau = \mu \cdot S \tag{3}$$

the shear rate in a concentric cylinder viscometer can be calculated from the rate of rotation N and the gap width k:

$$S = \frac{4\pi \cdot N}{(1 - k^2)} \tag{4}$$

(For a general analysis, see Krieger[11] or Krieger and Maron[12].) Shear rate is measured by timing steady state rotor rotation speed; all measurements were conducted two or more times. Deviations from ideal flow represented by Equations (3) and (4) were corrected by calibration with multiple sucrose solutions of different viscosities.

The maximum shear rates produced by the Brookfield viscometer are relatively low (1–10 s^{-1}) as compared to 100–500 s^{-1} for the modified Stormer viscometer. For comparison, the shear rate in benchtop fermentors which we have used to grow plant cell suspensions is about 150 s^{-1} (calculated based on a 7.5-cm impeller rotating at 200 rpm[17]). To test for any potential problems of shear damage to the cells in the Stormer viscometer, measurements of viscosity were performed first with increasing shear rates, followed by several measurements at reduced shear to test for hysteresis. No observable effects were noted within the accuracy of the measurements taken.

Suspension Cultures

The majority of data presented here was obtained with *Papaver somniferum* (poppy) suspension cultures grown in the dark on MS salts based media[14] and subcultured every 14 days as described previously.[5] Other batch-grown suspension cultures included tobacco (*Nicotiana tabacum* var. WI38), cotton (Gossypium), soybean (var. Kent), prosomillet, potato, carrot, and bindweed. This wide variety of plant suspension cultures was used in an attempt to get a broad perspective on rheological behavior. Original sources of plant suspension cultures and media used for maintaining cultures are given elsewhere.[3] Unless otherwise noted, rheology measurements were obtained from late-logarithmic to the stationary phase of batch culture (14–16 days) because the extent of cell expansion is greatest at that time. Also, to facilitate comparison of different cell cultures, suspensions were adjusted to 200 g FW/L by decantation. All measurements were conducted in growth medium to avoid possible osmotic or electrolytic effects.

Semicontinuous tobacco cultures were maintained by daily removal and replacement of a small fraction of the culture as described previously.[20,21] This procedure was intended to approximate continuous-flow culture conditions. The tobacco suspensions were maintained in semicontinuous culture for 85 days before use.

Time course of batch culture viscosity was determined by inoculating multiple 250-mL flasks. At designated intervals, one flask was harvested for fresh and dry cell density determination and viscosity of the filtrate was measured. A replicate flask was used for total broth viscosity measurements.

Plant cell aggregate frequency distributions were determined by repetitive screening of cells through standard Tyler sieves. Cells were gently rinsed through sieves with isotonic NaCl (6 g/L) 10–20 times to minimize overestimation of aggregate size.

The viscosity of a suspension of particles is dependent upon the proportion of the suspension volume occupied by the suspended particles. The volume fraction occupied by the cells was calculated by measuring filtrate volume from a known volume of culture. Careful measurements of all volumes and weights permitted correction for media entrained in filter and filter paper (Whatman No. 4). To develop a viscosity correlation, poppy cells from all phases of growth were resuspended at various densities in their own medium to account for variability caused by

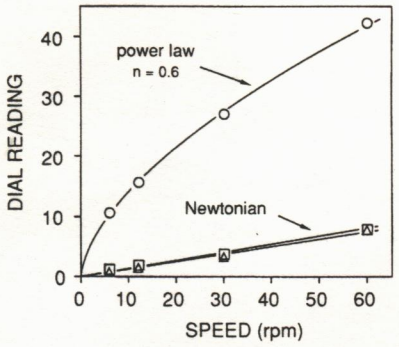

Figure 1. Comparison of rheology of (○) batch-cultured tobacco, (△) semicontinuously cultured tobacco, and (□) batch-cultured poppy plant suspension cultures at a cell concentration of 200 g FW/L. Measurements of shear stress (proportional to dial reading) at different shear rates (proportional to rotor speed) were obtained with a Brookfield viscometer. Batch-cultured tobacco displays power law flow behavior, whereas poppy and semicontinuously cultured tobacco display essentially indistinguishable Newtonian flow behavior.

inherent differences in cell morphology throughout a batch growth cycle.

RESULTS AND DISCUSSION

Tobacco versus Poppy

Rheological diagrams for batch-grown poppy, batch-grown tobacco, and semicontinuous grown tobacco are shown in Figure 1. Both batch poppy and semicontinuous tobacco display Newtonian behavior. Tobacco suspensions grown by sequential batch subculturing display power law behavior and much higher apparent viscosity than poppy. A power law index of 0.6 is in excellent agreement with previous results for batch tobacco suspension cultures.[9,25]

In Figure 2 a microscopic comparison of poppy and the two tobacco cultures demonstrates the elongated morphology of batch-cultured tobacco. Both batch-grown poppy and semi-continuously cultured tobacco cells are nearly spherical in shape. A power-law rheological behavior for elongated cell morphology is consistent with the results of rheological studies on mycelial fungi.[10,18]

Viscosity Survey

A wide variety of plant cell cultures including carrot, bindweed, millet, cotton, soybean, atriplex, and potato are compared in Figure 3. All cultures displayed relatively low viscosities comparable to batch-grown poppy. Microscopic examination revealed that only atriplex and carrot displayed significant cellular elongation. Only these two cultures displayed moderately pseudoplastic behavior (Fig. 3b), further substantiating the relationship between cell morphology and non-Newtonian behavior. The observation that semicontinuous tobacco cultures do not elongate, whereas batch grown cultures are significantly elongated throughout the culture period, suggests that elongation is related to

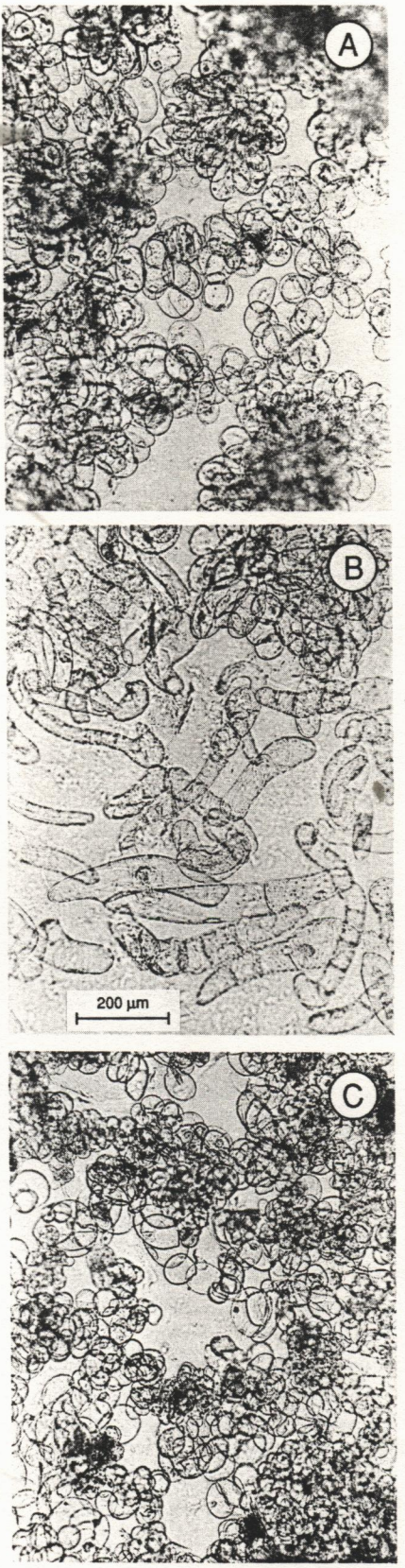

Figure 2. Photomicrographs of suspension cultures used in rheology measurements of Figure 1: (A) batch-cultured poppy, (B) batch-cultured tobacco, and (C) semi-continuous cultured tobacco. All micrographs were taken at the same magnification. Elongated morphology of batch-cultured tobacco results in power law flow behavior.

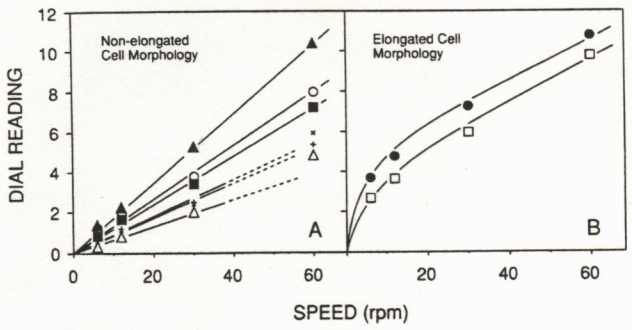

Figure 3. Survey of rheological behavior of nine different plant cell suspensions at a culture density of 200 g FW/L. (A) Newtonian flow behavior is observed for (▲) bindwood, (○) poppy, (■) cotton, (+) soybean, (×) millet, and (△) potato. (B) Slightly elongated cells of (●) carrot and (□) Atriplex display weak pseudoplastic behavior.

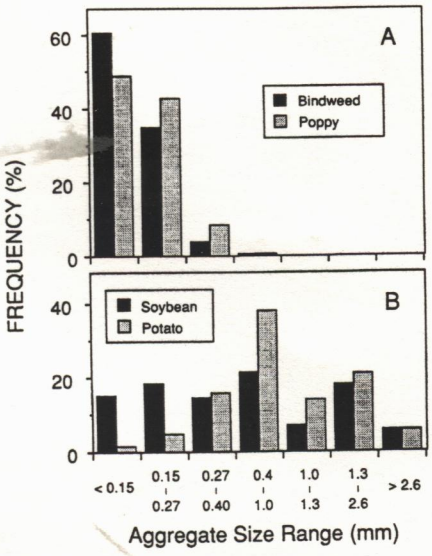

Figure 4. Aggregate frequency distributions are shown for (A) bindweed and poppy cultures which have narrow distribution and (B) soybean and potato cultures which display a broad distribution. By comparison to Figure 3, a broad aggregate distribution results in a lower apparent Newtonian viscosity.

nutrient availability. This is further substantiated by our observation that carrot cultures maintained on a 7-day subculture interval change from spherical to elongated morphology when switched to a 14-day subculture interval. It should also be noted that atriplex, bindweed, cotton, soybean, and cowpea are maintained on MS medium,[14] which is the same growth medium as tobacco; therefore, the growth medium per se is not the factor controlling cell elongation.

The nonelongated cell suspensions displayed Newtonian flow behavior (Fig. 3a). The onset of turbulence for the lowest viscosity suspensions is indicated by the dashed lines. (At this viscosity sucrose solutions also displayed turbulent transition.) Differences in viscosity displayed by these cultures at a constant fresh cell density can be explained by differences in aggregate size distribution. There are numerous experimental observations of decreased viscosity with increasing particle size.[23] It has also been shown that viscosity is reduced for broad aggregate size distributions as compared to suspensions of monodisperse particles.[7] The results of this work with plant suspensions are consistent with these findings. Comparing plant cell aggregate frequency distributions (Fig. 4), it is apparent that the Newtonian suspensions of lowest apparent viscosity (soybean and potato) are also the cultures that have much higher degree of aggregation and a broader aggregate size distribution as compared to poppy and bindweed suspensions which displayed higher apparent viscosity.

A high degree of cellular elongation and concomitant high-viscosity characteristic of tobacco are not common in plant cell suspension cultures. Tobacco cell cultures have been used widely as model systems for plant suspension cultures due to optimization of culture conditions by Murashige[14] and relative ease of plant regeneration. This situation has led to the erroneous conclusion that plant cell cultures in general are highly viscous and display complex rheological behavior. Another factor that has led to this perception is the ability to grow plant cell suspensions to high densities due to their slow growth. It should be kept in mind that plant tissue culture can be maintained as a solid phase callus (of extremely high viscosity indeed). Highly concentrated suspensions of any material will result in high viscosity and non-Newtonian behavior.

Batch Rheology Time Course

Further studies of poppy suspension rheology were conducted with the modified Stormer viscometer for several reasons. First, even at the relatively high density of 200 g FW/L shown in Figure 1, the viscosity of the resulting suspension is less than 10 cP, which is below the range of recommended accuracy for the Brookfield viscometer. This limitation of the Brookfield viscometer would not permit accurate measurement of rheology throughout the batch culture period. Another limitation of the Brookfield viscometer is that it provided only four data points for construction of the shear rate/shear stress diagrams, whereas a large number of shear rates can be obtained with small weight increments using the Stormer viscometer. In addition, the simple construction of the Stormer viscometer facilitated rapid insertion of the rotor and completion of shear rate measurements within a minute. This avoids problems of sedimentation experienced by others using concentric cylinder viscometers[22] by permitting resuspension between each replicated measurement at every shear rate.

Newtonian flow behavior of poppy suspensions was verified in the Stormer viscometer in Figure 5. Because rate of rotations (N) and pulley weight (W) are proportional to shear rate (S) and shear stress (τ), respectively [equations (2) and (4)], a plot of $\ln(N)$ versus $\ln(W)$ for a Newtonian fluid will have unit slope. In Figure 5, the experimental data fall parallel to the diagonal lines of unit slope, indicating that the cell suspensions are displaying

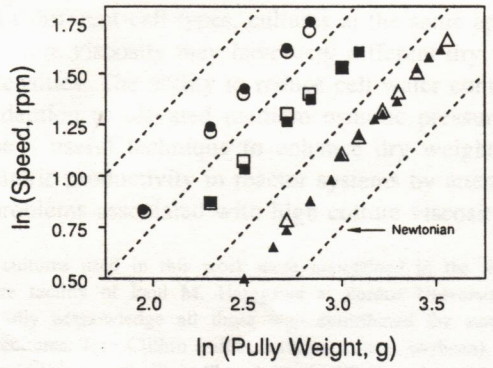

Figure 5. Demonstration of Newtonian flow behavior of poppy plant suspensions in a modified Stormer concentric cylinder viscometer: suspension cultures at cell concentrations of (●) 39 g FW/l, (■) 192 g FW/L, and (▲) 260 g FW/L; sucrose solutions at (○) 0 wt %, (□) 34 wt %, and (Δ) 46 wt %. Both plant cell suspensions and sucrose solutions fall parallel to theoretical unit slope of a Newtonian fluid.

Newtonian behavior. Sucrose solutions of comparable viscosity are included to verify rheological behavior. The suspension culture data fall parallel to the data of the sucrose solutions, providing direct experimental verification of Newtonian flow behavior, independent of viscometer nonidealities.

Having verified Newtonian behavior, the absolute shear diagrams can be constructed using Equations (2) and (3) and apparent Newtonian viscosity calculated from the slope. The viscosity of filtered media was determined to be Newtonian in a similar manner. The time course of cell density, media viscosity and culture viscosity are shown in Figure 6. The viscosity of the media remains below 2 cP throughout the batch culture period. The viscosity of media was low at the end of culture periods for other cell lines tested as well (data not shown). These results demonstrate that, in general, the media do not become highly viscous due to the secretion of polysaccharides. Instead, the increase in culture viscosity directly parallels the gain in fresh cell density.

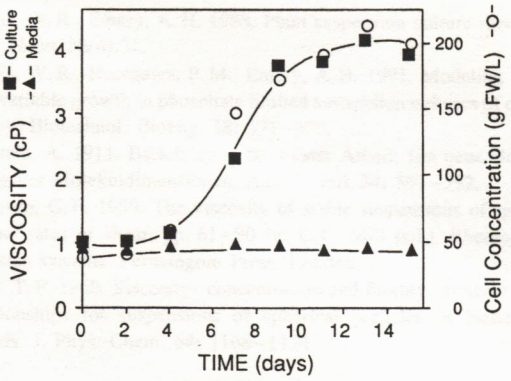

Figure 6. Time course of (○) culture growth and (■) culture viscosity during batch cultivation of poppy plant cell suspension cultures. Since viscosity of medium (▲) does not change appreciably during culture, the increase in viscosity parallels cell mass accumulation.

Viscosity Correlation

The preceding results suggest that the culture viscosity can be correlated with fresh cell density. Theoretically, the fluidity (apparent viscosity of a suspension, μ, relative to the viscosity of the suspending medium, μ_0) is directly related to the volume fraction of the suspended particle, ϕ. A correlation close to unity between fresh cell density and volume fraction occupied by the cells of poppy suspension cultures is demonstrated in Figure 7. Most theoretical fluidity correlations can be expressed as a power series in volume fraction.[8,26] We chose to use the following power series:

$$\frac{\mu}{\mu_0} = 1 + 2.5\phi + 10.05\phi^2 + A\phi^3 + B\phi^5 \qquad (5)$$

The first three terms are based on theoretical developments[6,13]; the last are empirical additions. The constants A and B were determined by least squares analysis to be 247 and 2150, respectively. The results in Figure 8 demonstrate that cell viscosity can be correlated very well with fresh cell density. Since this correlation was obtained with cells from all growth phases resuspended at different densities, the correlation remains valid for all periods of batch culture growth. A previous report noted a lack of correlation between viscosity and dry cell density at the end of batch culture[29]; however, this should be expected since plant cells go through a period of expansion after depletion of nutrients. As a result, the volume fraction occupied by the cells increases without a proportional increase in the dry cell mass. Since there is a reasonably good correspondence between increases in fresh and dry cell density throughout the *growth* phase, culture viscosity is a reasonably good indicator of culture growth.

The ability to correlate cell density with viscosity may provide a means of monitoring and controlling growth using in-line viscometers.[15,24] This would be particularly useful for plant cell suspensions due to their high degree of aggregation and frequent media cloudiness, which precludes the

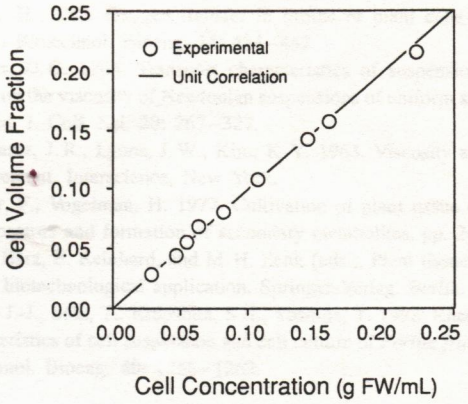

Figure 7. Unit correlation between fresh cell concentration and volume fraction occupied by cells. Since viscosity is theoretically related to suspended particle volume fraction, a correlation of viscosity to fresh cell concentration is theoretically sound.

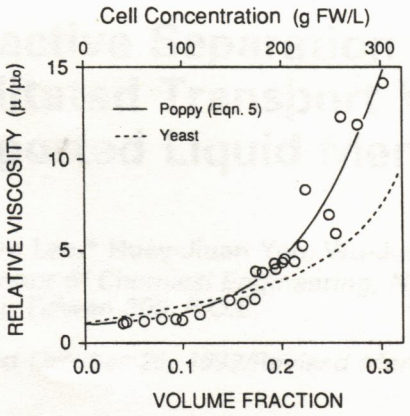

Figure 8. Empirical correlation of relative culture viscosity of poppy plant cell suspension is compared to a correlation developed for yeast suspensions at an osmotic pressure of 18.7 bars.[16] Apparent viscosity of poppy plant suspensions is comparable in magnitude to suspension of yeast cells at the same fresh cell concentration.

use of optical methods for on-line density assessment. To be utilized as a bioreactor control parameter, not only must viscosity correlate with cell density, but the derivative of that correlation must fall between certain limits to provide stability. At fresh cell densities less than 150 g FW/L there is a very weak dependence of viscosity on cell density; on the other hand, at cell densities above 300 g FW/L the slope is extremely large. Neither of these two conditions is conducive to adequate control. Based on this qualitative analysis of the viscosity correlation, viscosity appears to be a good parameter for control of poppy (and many other) plant suspension bioreactors in the moderate to high cell density range (150–300 g FW/L).

At sufficiently high densities, suspensions become pseudoplastic. While shear diagrams remained Newtonian in this work up to 300 g/L, it should be kept in mind that most shear thinning materials have a Newtonian asymptote at high shear rates, and measurements of rheology at the shear rates of the Stormer viscometer (100–500 s^{-1}) may mask rheological behavior observed in lower shear regions of the reactor.

To compare the rheology of plant cells to a more traditional cell suspension, a previously reported fluidity correlation for yeast[16] is also presented in Figure 8. Figures 3 and 8 demonstrate that many plant suspension cultures display culture viscosities that are comparable to microbial suspensions at the same fresh cell density. The perception of a highly viscous nature for plant cells arises from the routine culturing practices for plant cells at cell densities which can exceed 200 g FW/L. Microbial systems are not typically cultured at these biotic phase densities because it is not practical to provide sufficient oxygen to the culture. Plant cells, by comparison, have relatively low biological oxygen demand, which permits high density culture.

Nine of the ten cultures examined displayed approximately the same apparent viscosity at a fresh cell density of 200 g FW/L (Fig. 3). Since viscosity has a large influence on transport properties of reactors, the maximum fresh cell

Table I. Fresh to dry weight ratios of various plant cell suspensions at 14–16 days.

Tobacco (batch)	22.3	Tobacco (semicontinuous)	25.3
Poppy	17.9	Bindweed	8.7
Carrot	14.0	Atriplex	7.4
Soybean	7.7	Millet	8.6
Cotton	7.7	Potato	17.6

density achievable in reactors will likely be nearly the same for different suspensions of plant cells. However, the maximum dry cell densities will vary greatly because of the tremendous variation in water content of different plant cell suspensions. As shown in Table I, the fresh-to-dry weight ratios for the cells examined in this study varied by a factor of 4. Therefore, at a reactor fresh cell density of 300 g FW/L, the dry cell densities would vary between 12 and 41 g dry weight (DW)/L. These results suggest that a reduced water content of plant cells may be an effective means of enhancing the productivity in large-scale culture. By adapting tobacco cell suspensions to 25 g/L NaCl, Binzel et al.[2] were able to reduce the water content cells by 75% without reducing the rate of growth. Subsequently, we were able to show that the carbon use efficiency did not change significantly because the maintenance coefficient remained constant upon adaptation to these high osmotic conditions.[21] Since growth and cell yields can be maintained under high osmotic conditions, several-fold enhancements in dry weight productivities should be achievable with the added benefit of lower water content for downstream processing. Combining osmotic adaptation with nutrient/growth conditions which avoid elongated cell morphologies, dry cell productivities of 40 g DW/L should be achievable in large-scale commercial plant cell suspension culture.

CONCLUSIONS

1. Poppy suspension cultures display Newtonian flow behavior and relatively low viscosity to cell densities as high as 250 g FW/L. The viscosity of the culture can be correlated with fresh cell density. The viscosity displayed is comparable to apparent viscosities of yeast cell suspensions at the same volume fractions.
2. Tobacco grown by batch subculture is much more viscous than poppy and displays power law rheology with a flow behavior index of 0.6. Both the higher viscosity and non-Newtonian behavior can be attributed to cellular elongation. When grown by semicontinuous culture techniques, both the morphological and rheological characteristics of tobacco approach that of poppy.
3. Low viscosities comparable to poppy were measured in seven other plant cell suspension types, suggesting that the highly viscous, non-Newtonian behavior of tobacco is an exception rather than a rule.
4. Since viscosity correlates with fresh cell density, and the water content of plant cells varies tremendously

for different cell types, cultures at the same apparent culture viscosity may have very different dry weight densities. The ability to reduce cell water content by adaption to elevated medium osmotic pressure may be a useful technique to enhance dry weight volumetric productivity in reactor systems by attenuating problems associated with high culture viscosity.

The cultures used in this work were maintained in the tissue culture facility of Paul M. Hasegawa at Purdue University. I gratefully acknowledge all those who contributed the suspension cultures: Jean Clithro (millet, atriplex, cotton, soybean), Bill Kosinski (bindweed), Elain Shea (carrot), Bill Dyer (potato), and Sherry Rae Schnapp (batch and semicontinuous tobacco). This article was presented at the 1988 annual meeting of the American Society of Industrial Microbiology, Chicago, IL, and the abstract was previously published in reference 4. The work was supported in part by a Shell Foundation Fellowship and a Dupont Ph.D. Fellowship. I acknowledge the support of the National Science Foundation (NSF Grant No. BCS-9110288), which freed up the time to finally publish the work.

NOMENCLATURE

- g gravitational constant ($cm \cdot s^{-2}$)
- h height of viscometer rotor (cm)
- k viscometer gap width (cm)
- K Brookfield viscometer calibration constant (Newtonian) ($dyn \cdot cm^{-2}$)
- N rotation rate ($rev \cdot s^{-1}$)
- R_b radius of viscometer rotor (bob) (cm)
- S shear rate (s^{-1})
- W pully weight (g)
- Γ Stormer viscometer mechanical gear constant (dimensionless)
- μ apparent viscosity ($g \cdot cm^{-1} \cdot s^{-1}$)
- ϕ volume fraction occupied by cells (dimensionless)
- τ shear stress ($dyn \cdot cm^{-2}$)

References

1. Ballica, R., Ryu, D. D. Y., Powell, R.L., Owen, D. 1992. Rheological properties of plant cell suspensions. Biotechnol. Prog. **8:** 413–420.
2. Binzel, M. L., Hasegawa, P. M., Handa, A. K., Bressan, R. A. 1985. Adaptation of tobacco cells to NaCl. Plant Physiol. **79:** 118–125.
3. Curtis, W. R. 1988. Kinetics of phosphate limited growth of poppy plant suspension cultures. Ph.D. Thesis, Purdue University, Lafayette, IN.
4. Curtis, W. R., Emery, A. H. 1988. Plant suspension culture rheology. SIM News **38**(4):31.
5. Curtis, W. R., Hasegawa, P. M., Emery, A. H. 1991. Modeling linear and variable growth in phosphate limited suspension cultures of opium poppy. Biotechnol. Bioeng. **38:** 371–379.
6. Einstein, A. 1911. Berichtigung zu meiner Arbeit: Ein neue Bestimmung der Molekuldimensionen. Ann. Physik **34:** 591–592.
7. Evanson, G. F. 1959. The viscosity of stable suspensions of spheres at low rates of shear. pp. 61–80 In: C. C. Mill (ed.), Rheology of disperse systems. Permangom Press, London.
8. Ford, T. F. 1960. Viscosity–concentration and fluidity–concentration relationships for suspensions of spherical particles in Newtonian liquids. J. Phys. Chem. **64:** 1168–1174.
9. Kato, A., Kawazoe, S., Soh, Y. 1978. Viscosity of the broth of tobacco cells in suspension culture. J. Ferment. Technol. **56**(3): 224–228.
10. Kim, J. H., Lebeault, J. M., Reuss, M. 1983. Comparative study on rheological properties of mycelial broth in filamentous and pelleted form. Eur. J. Appl. Microbiol. Biotechnol. **18:** 11–16.
11. Krieger, I. M. 1969. Computation of shear rate in the Couette viscometer. pp. 511–516 In: S. Onogi (ed.), Proceedings of the fifth international congress on rheology, vol. 1. University Park Press, Baltimore, MD.
12. Krieger, I. M., Maron, S. H. 1954. Direct determination of the flow of non-Newtonian fluids. III. Standard treatment of viscometric data. J. Appl. Physics **25**(1): 72–75.
13. Manley, R. J., Mason, S. G. 1955. Particle motions in sheared suspensions. III. Further observations on collisions of spheres. Can. J. Chem. **33:** 736–773.
14. Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. **15:** 473–497.
15. Picque, D., Corriew, G. 1988. New instrument for on-line viscosity measurement of fermentation media. Biotechnol. Bioeng. **31**(1): 19–23
16. Reuss, M., Josic, D., Popovic, M., Bronn, W. K. 1979. Viscosity of yeast suspensions. Appl. Microbiol. Biotechnol. **8:** 167–175.
17. Robertson, B., Ulbrecht, J. J. 1987. Measurement of shear rate on an agitator in a fermentation broth. pp. 31–35 In: C. S. Ho and J. Y. Oldshue (eds.), Biotechnology processes: Scale-up and mixing. American Institute of Chemical Engineers, New York.
18. Roels, J. A., Van den Berg, J., Voncken, R. M. 1974. The rheology of mycelial broths. Biotechnol. Bioeng. **16:** 181–208.
19. Sahai, O., Knuth, M. 1985. Commercialization plant tissue culture processes: Economics, problems, and prospects. Biotechnol. Prog. **1**(1): 1–9.
20. Schnapp, S. R., Curtis, W. R., Bressan, R. A., Hasegawa, P. M. 1991. Estimation of growth yield and maintenance coefficient of plant cell suspensions. Biotechnol. Bioeng. **38:** 1131–1136.
21. Schnapp, S. R., Curtis, W. R., Bressan, R. A., Hasegawa, P. M. 1991. Growth yields and maintenance coefficients of unadapted and NaCl-adapted tobacco cells grown in semi-continuous culture. Plant Physiol. **96:** 1289–1293.
22. Scragg, A. H., Allen, E. J., Bond, P. A., Smart, N. J., 1986. Rheological properties of plant cell suspension cultures. pp. 178–194 In: P. Morris, A. H. Scragg, A. Stafford, and M. W. Fowler (eds.), Secondary metabolism in plant cell cultures. Cambridge University Press. Cambridge, England.
23. Sherman, P. 1970. Industrial rheology, pp. 141–151. Academic Press, New York.
24. Shimmons, B. W., Svrcek, J. P., Zajie, J. E. 1976. Cell concentration control by viscosity. Biotechnol. Bioeng. **18:** 1793–1805.
25. Tanaka, H. 1982. Oxygen transfer in broths of plant cells at high density. Biotechnol. Bioeng. **24:** 424–442.
26. Thomas, D. G. 1965. Transport characteristics of suspension: VIII. A note on the viscosity of Newtonian suspensions of uniform spherical particles. J. Coll. Sci. **20:** 267–327.
27. Van Waser, J. R., Lyons, J. W., Kim, K. Y. 1963. Viscosity and flow measurement. Interscience, New York.
28. Wagner, F., Vogelman, H. 1977. Cultivation of plant tissue cultures in bioreactors and formation of secondary metabolites. pp. 245–252 In: W. Barz, E. Reinhard, and M. H. Zenk (eds.), Plant tissue culture and its biotechnological application. Springer-Verlag, Berlin.
29. Zhong, J.-J., Seki, T., Kinoshita, S.-I., Yoshida, T. 1992. Rheological characteristics of cell suspension and cell culture of *Perilla frutescens.* Biotechnol. Bioeng. **40:** 1256–1262.

Extractive Separation of Penicillin G by Facilitated Transport Via Carrier Supported Liquid Membranes

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The facilitated transport of penicillin G (Pen G), through a supported liquid membrane with Amberlite LA-2 dissolved in 1-decanol, supported on a microporous polypropylene membrane, were studied. The distribution coefficient was obtained from a batch extraction experiment. The effects of flow rate, carrier concentration, initial concentration of Pen G, and the pH of feed and stripping phases on the transport rate of Pen G through the supported liquid membrane were also investigated. The results are in agreement with theoretical predictions, and it is demonstrated that the transport of Pen G through the supported liquid membrane is controlled simultaneously by mass transfer across both aqueous and liquid membranes. © 1993 John Wiley & Sons, Inc.
Key words: penicillin G • liquid membrane • facilitated transport • separation process • Amberlite LA-2

INTRODUCTION

Production of Pen G is far greater than that of any other antibiotics in terms of total capital invested in plant facilities, return on capital investment, and total volume of antibiotics produced. Traditionally, it is obtained from fermentation broth by extractive separation with an organic solvent at low pH, followed by re-extraction at about pH 6.0 using a buffer solution. Considerable amounts of Pen G are lost during separation and purification steps because of its instability at low pH. Accordingly, the separation/purification step plays a critical role in the recovery and efficient production of Pen G in the antibiotics industry. Much research has been conducted toward a more improved separation/purification method for efficient production of Pen G, including the extractive method with amine at pH values ranging from 5.0 to 8.0.[10,15–19]

In this study, a supported liquid membrane (SLM) system is utilized to study the extractive separation of Pen G from fermentation broth. The carrier-facilitated transport mechanism of the SLM system would help to enhance the efficiency of separation and recovery of Pen G.

In general, the liquid membrane systems may be classified in two basic types of operations, namely, the emulsion liquid membrane (ELM) and the supported liquid membrane (SLM). The former is known to offer large specific mass transfer areas but with less guaranteed operational stability. The SLM system is more suitable for fundamental studies on the mass transfer mechanism, i.e., mass transfer flux and selectivity measurements. It also offers better feasibility for scale-up and adaptability for continuous operation. Further enhancement of the liquid membrane can be accomplished by using a complexing agent (carrier) in the liquid membrane.[13,14] The carrier molecule can selectively and reversibly react with the solute, and reversible reaction provides a means of enhancing the solute flux and improving the selectivity at the same time.

Because of the superior characteristics of simultaneous extraction and stripping, liquid membrane systems have found wide applications in hydrometallurgy,[1,12,21–23] hydrocarbon separation,[2,8,9] wastewater treatment,[3,29] biomedical engineering,[28] and some bioseparation processes.[4,5,7,11,24–26] The application of liquid membrane system to the extraction of Pen G, using the tetrabutyl ammonium cations as the ionic pair of Pen G anion in a SLM, was first performed by Marchese et al.[11] The ELM process using various amines as carriers was applied by Hano et al.[4] and the effects of various operational conditions in a batch system were studied by Lee and Lee.[7] Tsikas et al.[25] studied the kinetics of the combined extraction of Pen G and the enzymatic hydrolysis to 6-aminopenicillanic acid (6-APA) using an ELM system. A hollow-fiber SLM system was also utilized to study the same problem, by the same authors, in a recently published study.[26]

In this report, experimental results on the extractive equilibrium and the facilitated transport of Pen G through the carrier (Amberlite LA-2)-supported liquid membrane system are presented. Theoretical analysis of the mechanism of facilitated transport as well as of the extractive equilibrium are also discussed in light of the experimental data.

THEORY AND MODELING

Extractive Equilibrium

According to a study by Reschke and Schueger,[15] the reactive extraction of Pen G with an amine corresponds to a neutralization reaction and is expressed in Eq. (1).

$$A_{(org)} + H^+_{(aq)} + P^-_{(aq)} \longrightarrow AHP_{(org)} \qquad (1)$$

Because the reaction is very rapid (almost instantaneous), it is assumed that the rate of extraction is controlled by mass

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