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Section: Bioprocessing and Bioengineering

Applicability of *Penicillium chrysogenum* rheological correlations to broths of other fungal strains

G.L. Riley • C.R. Thomas

Abstract

Existing correlations of Power law consistency index with *Penicillium chrysogenum* biomass concentration and morphology were revised using a microscope magnification of 50 times to characterize the latter, rather than the 80 times used previously. This allowed tests of the correlations on broths of *Aspergillus oryzae* and *Aspergillus niger*, which have such large mycelial sizes that a lower magnification is required for accurate morphological analysis. The new correlations were successful at predicting the rheology of *A. oryzae* broths but not *A. niger* broths, which may be because of a change in the exponent on the biomass concentration in the correlations for the latter. Because the mean maximum dimension of clumps is magnification independent, the preferred correlation was:

$$K = C_m^2 \times [4 \times 10^{-5} D - 9 \times 10^{-4}]$$

where K is the consistency index (Pa s^n), C_m is the biomass concentration as dry cell weight (gL^{-1}) and D is the mean maximum dimension (μm).

Keywords Consistency index, Fungi, Morphology, Rheology

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Nomenclature

n	flow behaviour index
A	mean mycelial projected area (μm^2)
C_m	biomass concentration (g (dry cell weight) L^{-1})
D	mean mycelial maximum dimension (μm)
K	consistency index (Pa s^n)
α	exponent on biomass in consistency index correlations
γ	shear rate
τ	shear stress

Introduction

The morphology of filamentous micro-organisms in submerged fermentations plays a crucial role in determining their productivity (Papagianni 2004). The dispersed morphological form (Paul and Thomas 1998) is common in industrial filamentous fermentations, especially in the production of antibiotics such as penicillin. In such fermentations hyphal entanglement (Metz *et al.* 1979) can result in significant changes in broth rheology i.e. the relationship between stresses and strains in the suspension. Sometimes large increases in broth (apparent) viscosity are observed and broths often become "pseudoplastic" or "shear thinning" i.e. the apparent viscosity decreases as the shear rate increases. The results of high viscosity and pseudoplastic behaviour can be a reduction in fermenter mixing performance and process control (Amanullah *et al.* 2004), with a consequential decrease in productivity and/or the production of undesirable metabolites (Papagianni 2004).

The interactions between morphology, broth rheology and fermenter performance are complex (Metz *et al.* 1979) but are amenable to study because morphology can be characterised by image analysis (Paul and Thomas 1998). This method has allowed useful correlations to be sought between morphology and rheology. Pseudoplasticity is usually assumed in such studies so that the shear stress τ is related to the shear rate γ by a Power law relationship:

$$\tau = K\gamma^n \tag{1}$$

where the rheological parameters K and n are called the consistency index and the flow behaviour index respectively.

In an extensive study on an industrial strain of *Penicillium chrysogenum*, Riley *et al.* (2000) proposed an average value of the flow behaviour index of 0.35 was proposed for broths of >10 g (dry cell weight) L^{-1} . Successful predictions of the Power law consistency index were possible using correlations with the biomass concentration and the (dispersed form) morphological parameters mean mycelial projected area and mean maximum dimension (Paul and Thomas 1998). The correlations were:

$$K = C_m^2 \times (1 \times 10^{-6}A + 3 \times 10^{-3}) \quad (2)$$

$$K = C_m^2 \times (5 \times 10^{-5}D - 10^{-3}) \quad (3)$$

where K is the consistency index ($Pa \ s^n$), C_m is the biomass concentration as dry cell weight (gL^{-1}), D is the mean maximum dimension (μm) of the mycelia and A is their mean projected area (μm^2). The morphology was characterised by image analysis according to the method described in Paul and Thomas (1998).

For the design and control of submerged fermentations of fungi growing in dispersed form, it would be very useful to have correlations applicable to all or many strains, for example *Aspergillus niger* and *Aspergillus oryzae* as well as *P. chrysogenum*. Clumps of the *Aspergillus* strains studied here were generally much larger than those of *P. chrysogenum* and consequently a lower magnification was required for image analysis, if truncation of the mycelial clumps by the image frame was to be avoided. Magnification has a strong influence on the measured mean projected area in particular, so correlations (2) and (3) were revised for a lower magnification and then tested on *A. niger* and *A. oryzae* broths.

Materials and methods

Fermentations and morphological characterization

Penicillium chrysogenum

P. chrysogenum fermentations and morphological analysis were carried out as detailed in Riley *et al.* (2000) except a microscope magnification of 50 times was used.

Aspergillus oryzae

An industrial strain of *A. oryzae* (Novo Nordisk, Lyngby, Denmark) was grown on a semi-defined medium in 5.8 l (AO1), 10.8 l (AO2) and 60 l (AO3) working volume batch fermentations. Further details can be found in Riley (1999). Characterization of mycelial morphology was by the method of Riley *et al.* (2000) using a 50 times magnification. The concentration of mycelia on the slide was around 0.25 g l⁻¹ after appropriate dilution with a 20% (w/v) sucrose solution.

Aspergillus niger

Samples were taken from two 600 l *A. niger* (A1233/citrate, IMI number 075353) batch fermentations (AN1 and AN2) reported by John (1997). The vessel contained an independently-driven, dual impeller system with draft tube. Only limited monosepsis could be achieved in the vessel but a pH of 2 reduced the contamination risk and ensured dispersed growth. Full details of these fermentations are given in John (1997). Two 4 l working volume aseptic batch fermentations were carried out at pH 2, 25°C, impeller diameter 100 mm, agitation rate 800 rpm (AN3) or 200-750 rpm (AN4), aeration rate 3 l min⁻¹. The medium was that of Roehr *et al.* (1981). Morphology was characterized as for *A. oryzae*, with a magnification of x 50 and a biomass concentration on the slide of around 0.25 g l⁻¹ after dilution with 20% (w/v) sucrose.

Morphological analysis was carried out on a Quantimet 570 (Leica Cambridge Ltd., Cambridge, UK) with a Polyvar optical microscope (Reichert Jung Opstische Werke AG, Austria)

Rheology

The rheological characteristics of the fermentation broths were determined using a Rheomat 30 (Contraves, Zurich, Switzerland) fitted with a six-blade, 53 mm diam, Rushton turbine rotating in a 600 ml glass beaker containing 400 ml sample (Riley *et al.* 2000). The shear rate range was 3.3 s⁻¹ to the maximum possible value whilst the flow remained laminar. This depended on the sample, but never exceeded 75.8 s⁻¹. The sample temperature was controlled at 30 °C ± 1 °C for *A. oryzae*, and 23 ± 1°C for *P. chrysogenum* and *A. niger*, i.e. close to the fermentation temperature.

Correlations

Penicillium chrysogenum

For revision of the *P. chrysogenum* correlations, the dependence of the consistency index K on the biomass concentration C_m was not altered (i.e. $K \propto C_m^2$) because this parameter should be magnification independent. Using 50 times morphological data, linear regression was then used to find correlations between K/C_m^2 and the mean projected area or the mean maximum dimension. The new correlations were tested on *Aspergillus* broths.

Dependence of rheology on biomass concentration was also investigated to see if the exponent (α) of 2 on C_m in the correlations applied to *Aspergillus* broths. Even small changes, even minor, in the exponent might result in failure of the correlations. Reconstitution experiments similar to those undertaken by Riley *et al.* (2000) on *P. chrysogenum* broths were carried out on broth samples taken at the end of the rapid growth phase of fermentation AO1 and at various times from fermentations AO3, AN1 and AN2. These were reconstituted to biomass concentrations between 6-30 gL⁻¹, the rheology measured, and the exponent values found.

Results and discussion

Consistency index of *Penicillium chrysogenum* (50 times magnification)

Fig. 1 shows the morphological parameters mean mycelial projected area and mean maximum dimension measured at 50 times magnification for the *P. chrysogenum* fermentations. The higher magnification data of Riley *et al.* (2000) are given for comparison. The differences between the mean projected areas are clear, but differences between the mean maximum dimensions were small. The lower areas at higher magnification are because more gaps between the hyphae are detected and existing gaps become larger.

Fig. 2 shows K/C_m^2 versus the mean projected area and the mean dimension at the lower magnification. Table 1 shows the slopes and intercepts of the regression lines. The correlations for a magnification of 50 times were:

$$K = C_m^2 \times [5 \times 10^{-7} A + 3 \times 10^{-3}] \quad (4)$$

$$K = C_m^2 \times [4 \times 10^{-5} D - 9 \times 10^{-4}] \quad (5)$$

where the symbols have the same meanings as earlier. It can be seen that many of the data points in Fig. 2 are clustered between values of K/C_m of 0.4 and 0.6. As discussed in Riley *et al.* (2000), these points were mainly

measurements taken after the end of the rapid growth phase, when most of the samples were taken due to the extended nature of fed-batch fermentations. Measurements made early in the fermentations when mean projected area and maximum dimension were high have a strong influence on the form of the regression lines. Riley *et al.* (2000) discussed this and considered the possibility of having separate correlations for low and high mycelial sizes. However, they decided that the form of correlations (4) and (5) would be of most practical use given the imprecision of morphological data. This approach was also accepted here.

As the mean maximum dimension D is nearly independent of magnification (Fig. 1), it was expected that there would be no significant difference in the relationship between K/C_m^2 and the mean maximum dimension whatever the magnification. Correlations (3) and (5) give similar predictions within the range of their validity. That this was so can be seen from the values of the slopes and intercepts in Table 1, with no differences found at the 95% confidence level. A larger and significant difference was observed in the relationships between K/C_m^2 and the mean projected areas (correlations (2) and (3)), as this parameter is magnification dependent. The intercepts of the regression lines were identical whereas the slopes differed. The most likely reason for this is that the mycelia towards the end of the fermentation were smaller and less complex and were therefore less influenced by magnification changes, leading to the same intercept. When the mycelia were larger and more complex, the higher magnification permitted discrimination of gaps between hyphae that were not apparent at lower magnifications, leading to apparently lower projected areas.

Tests of the consistency index correlations on *A. oryzae* and *A. niger*

Correlations (4) and (5) were tested on the *A. oryzae* and the *A. niger* fermentations. The predicted values of the consistency index were plotted against the measured values (Fig. 3 and 4). The error bars on the predicted points represent the combined error on the biomass concentration, its exponent in the correlation, the mean projected area or mean maximum dimension and the constants of the correlation. Error analysis is discussed more fully in Riley *et al.* (2000). A reasonable agreement was observed between predicted and measured values of the consistency index for *A. oryzae* broths, but neither correlation described the rheological behaviour of *A. niger* broths well.

α Values for *A. niger* and *A. oryzae*

The mean α values and their standard deviations, standard errors on the mean, and the number of reconstitution experiments from which these were determined are given in Table 2. The mean α values were tested for significant differences at the 95 % level. There was no significant difference between the mean α value for *P. chrysogenum* and that for *A. oryzae*, but a significant difference was found for *A. niger*. As α is an exponent, even small changes would have a significant effect on predictions. It is therefore unsurprising that correlations (4) and (5) are poor for *A. niger* broths. However, it is not possible to conclude that the failure of the correlations for *A. niger* broths was solely due to a variation in α , as a different morphological relationship may also exist. It may be that α varies so much between batch and fed batch fermentations of this organism (Olsvik and Kristiansen 1994) that to develop useful rheological correlations would require extensive and specific studies of *A. niger* fermentations. These were not undertaken in this work.

As mentioned earlier, it would be very useful to have rheological correlations applicable to all or many fungal strains, to be used in the design and control of their submerged fermentations. Because of unpredictable changes in α , this is clearly not yet achievable even with respect to just one of the rheological parameters i.e. the consistency index.

Flow behaviour index

As for *P. chrysogenum* (Riley *et al.* 2000), no simple relationship was found between the flow behaviour index and the biomass concentration for *A. oryzae* or *A. niger*. For *P. chrysogenum* broths with a biomass concentration above 10 g L^{-1} , Riley *et al.* (2000) proposed that an average value of 0.35 ± 0.1 be used for the flow behaviour index. The values of the flow behaviour index were much higher for the more viscous *A. oryzae* broths (around 0.5) and were generally lower for *A. niger* around 0.2 (Fig. 5). As the flow behaviour index is an exponent in the Power law, the inability to find any global value for this parameter is disturbing and probably means that separate flow behaviour and consistency index correlations will be need for every species, and possibly for different strains.

It should be noted that Petersen *et al.* (2008) have used a light scattering technique to determine biomass particle size distributions in *Aspergillus oryzae* broths from 550 L fermentations and to develop useful rheological correlations. This fast and practical technique does not classify particles into morphological forms but uses principal component analysis to extract size information from the data. A mean value of the flow behaviour index of about 0.4 was observed, which did not depend on the principal components. This is lower than the value reported here for unknown reasons. Long run, this light scattering technique may be superior to image analysis for rheological studies on fungal broths but it needs further testing on other strains and fermentation conditions.

Conclusions

Correlations (4) and (5) obtained by using lower magnification *P. chrysogenum* morphological data were successful in predicting the consistency index of *A. oryzae* fermentation broths at the same magnification but gave poor predictions for *A. niger* broths. It is promising that these correlations worked well for the *A. oryzae* broths, as these mycelia were much larger and well outside the morphological range used to develop the correlations. The failure for *A. niger* broths and the requirement for different flow behaviour index values for the different species means that a globally applicable method to predict fungal broth rheology remains elusive, despite the power of morphological characterization by image analysis. Further progress will probably require a far better understanding of how mycelial interactions in broths lead to high viscosities and non-Newtonian behaviour.

Because mean maximum dimension was not magnification dependent, no significant difference was found between the predictions of correlations (3) and (5). Because they are magnification independent, these correlations are preferred.

Acknowledgements

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Table 1: Slopes, intercepts and standard deviations for the regression of K/C_m against mean projected area A and mean maximum dimension D (Fig. 2).

K/C_m^2 versus:	slope	intercept
mean projected area (50 times)	$5 \times 10^{-7} \pm 6 \times 10^{-8}$	$3 \times 10^{-3} \pm 4 \times 10^{-4}$
mean projected area (80 times)	$10^{-6} \pm 10^{-7}$	$3 \times 10^{-3} \pm 4 \times 10^{-4}$
mean maximum dimension (50 times)	$4 \times 10^{-5} \pm 6 \times 10^{-6}$	$-9 \times 10^{-4} \pm 10^{-3}$
mean maximum dimension (80 times)	$5 \times 10^{-5} \pm 8 \times 10^{-5}$	$-1 \times 10^{-3} \pm 10^{-3}$

Table 2: Summary of mean exponent α on biomass for *Penicillium chrysogenum*, *Aspergillus niger* and *Aspergillus oryzae*, σ : standard deviation, σ_m : standard error on the mean and N: number of reconstitution experiments.

	<i>Penicillium chrysogenum</i>	<i>Aspergillus oryzae</i>	<i>Aspergillus niger</i>
mean α	2.0	1.7	2.7
σ	0.3	0.2	0.3
σ_m	0.1	0.1	0.1
N	20	4	7

Figure legends

Fig. 1: Mean projected areas and maximum dimensions for *Penicillium chrysogenum* fermentations F1 (Δ ▲), F2 (\square ■) and F3 (\circ ●). Open symbols are for 80 times magnification (Riley *et al.* 2000); closed symbols are for 50 times magnification

Fig. 2: K/C_m^2 versus mean projected area and mean maximum dimension at 50 times magnification for *Penicillium chrysogenum* fermentations F1 (▲), F2 (■) and F3 (●)

Fig. 3: Actual and predicted values of the consistency index (Pa s^n) using correlations (4) and (5) for the *Aspergillus oryzae* fermentations AO1 (●), AO2 (■) and AO3 (▲)

Fig. 4: Actual and predicted values of the consistency index (Pa s^n) using correlations (4) and (5) for the *Aspergillus niger* fermentations AN1 (\square), AN2 (Δ), AN3 (\circ) and AN4 (\diamond)

Fig. 5: Time profile of the values of the flow behaviour index (n) for fermentations of *Aspergillus oryzae* (open symbols) and *Aspergillus niger* (closed symbols). The mean value of 0.35 advocated by Riley *et al.* (2000) for *Penicillium chrysogenum* broths over 10 g (dry cell weight) L^{-1} is shown by the solid line and ± 0.1 (standard deviation) by the dotted lines

Fig. 1

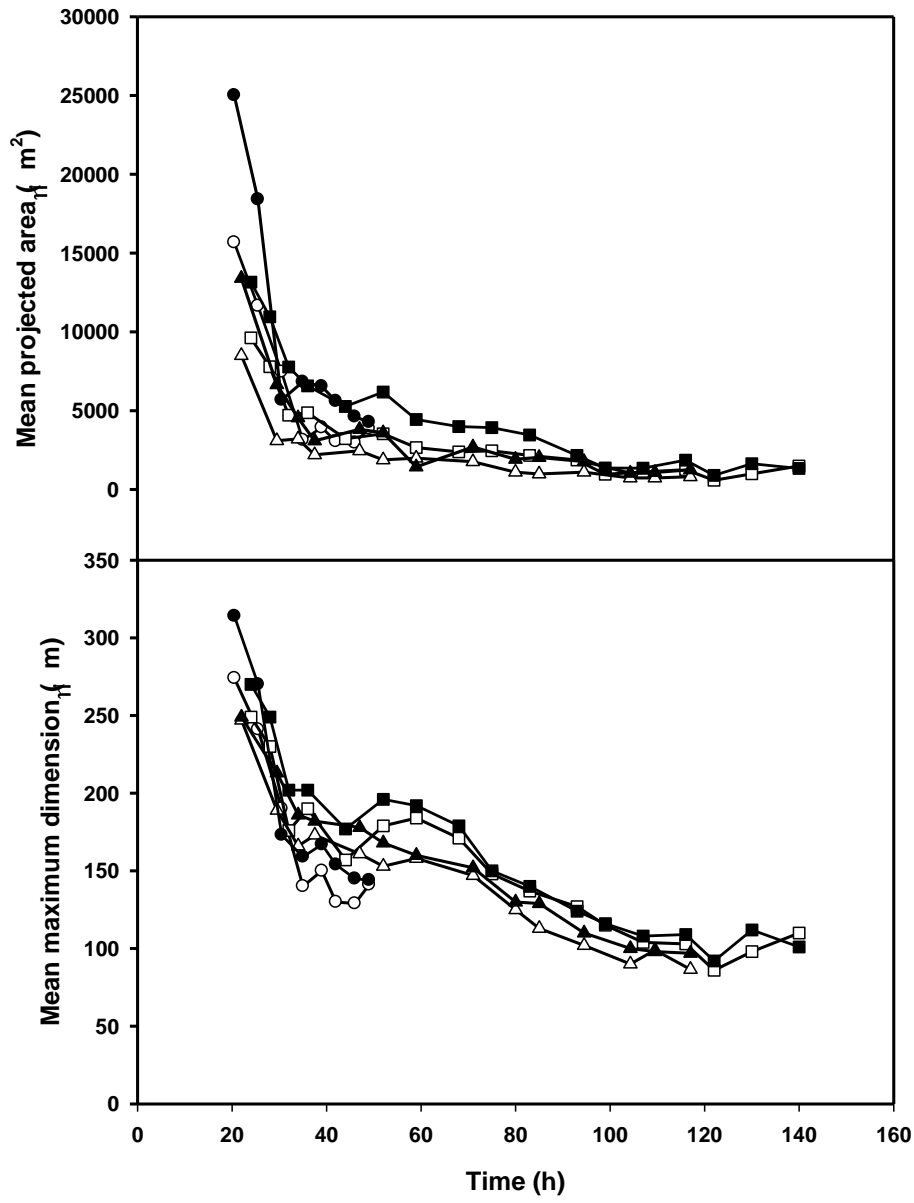


Fig. 2

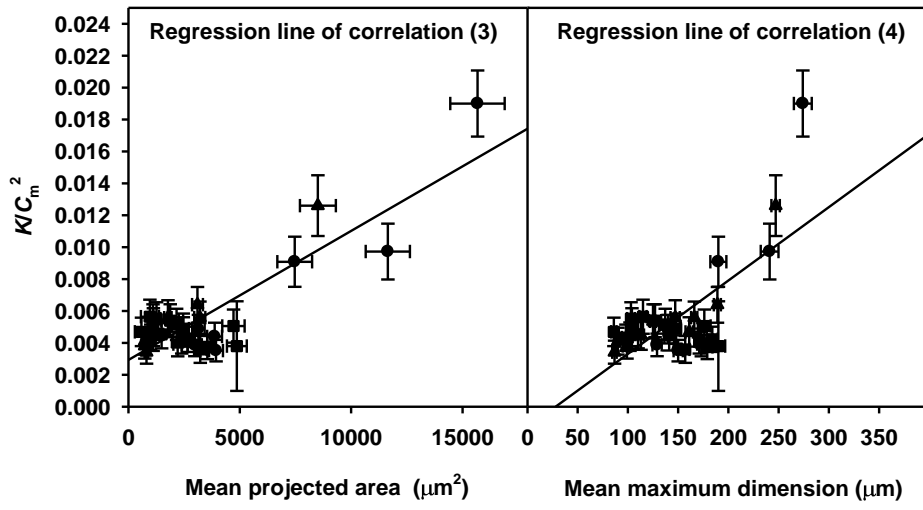


Fig. 3

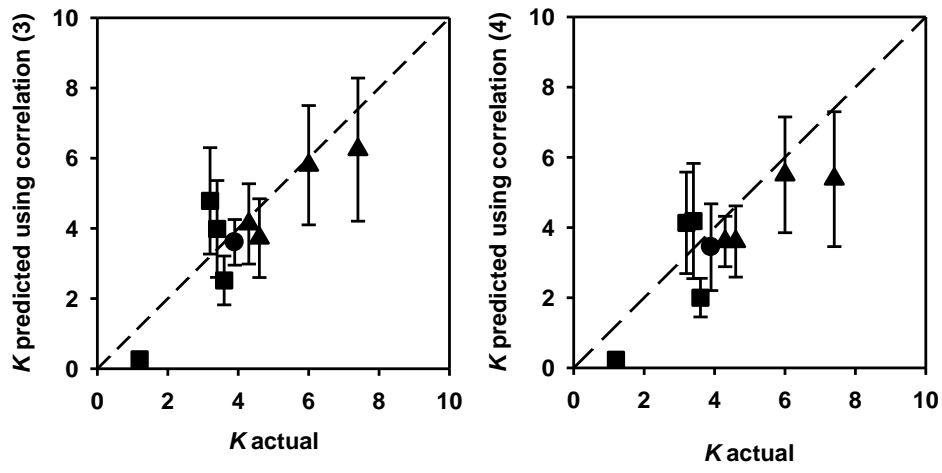


Fig. 4

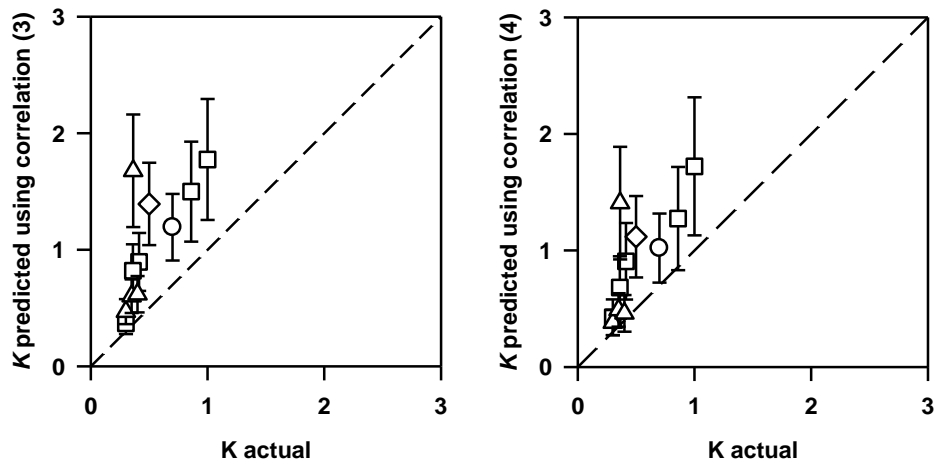


Fig. 5

