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Rheology of culture broths and exopolysaccharide of *Cyanospira capsulata* at different stages of growth†

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This study deals with the rheological behaviour of the culture broths of the exocellular polysaccharide producer cyanobacterium *Cyanospira capsulata* at different culture times. The non-Newtonian character of the culture broths is mainly due to the presence of the exopolysaccharide solubilized into the medium but the contribution of the microbial cells cannot be considered to be negligible, particularly at the early stages of the microbial growth. The culture broths exhibit shear-thinning behaviour and the Carreau viscosity equation appears to fit the experimental data fairly well. The culture broths show elastic behaviour as well. Non-linear viscoelastic behaviour has also been investigated. The shear viscosity curve as well as oscillatory flow tests of the old culture (28 days after the inoculum) show some 'anomalies' which can be ascribed to a decrease in the exocellular polysaccharide molecular weight.

INTRODUCTION

The production of an exocellular polysaccharide by the filamentous nitrogen fixing cyanobacterium *Cyanospira capsulata*, isolated from the alkaline soda Magadi Lake in Kenya (Florenzano *et al.*, 1985), has been reported by Sili *et al.* (1984) and studied in detail by De Philippis *et al.* (1989).

C. capsulata grows in the form of helical trichomes (Sili *et al.*, 1984) surrounded by an external capsular polysaccharidic layer of considerable thickness. During its growth, the microorganism continuously excretes a polysaccharide (named *Cyanospira capsulata* exocellular polysaccharide, CC-EPS) into the medium, resulting in an increase in the viscosity of corresponding culture broths as a function of the culture time.

A rheological study (Navarini *et al.*, 1990) of aqueous solutions of CC-EPS has revealed that, even at low polymer concentration, this polysaccharide shows some features typical of a good suspending agent, and that its rheological behaviour shows some similarities with that of xanthan gum, a high molecular weight exopolysaccharide secreted by the bacterium *Xanthomonas campestris*.

Many investigations have been carried out in order to understand the rheology of aqueous solutions of

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microbial polysaccharides produced by a variety of microorganisms including species of bacteria, moulds and yeasts, but there are only a few reports in literature concerning the description of polysaccharide culture broths rheology including viscoelastic properties (Noel *et al.*, 1984; Choplin & LeDuy, 1986; Santore & Prud'homme, 1990).

The study of the rheological behaviour of polysaccharide culture broths is very important since it may give information on the cellular kinetics. In the case of xanthan gum, it has been shown that the evolution of the rheological properties in the course of the fermentation process greatly influences the productivity of the microorganisms (Peters *et al.*, 1989).

Moreover, an analysis of the rheological properties of the culture broths is of practical interest for the engineering design of fermenters (Noel *et al.*, 1984) and it may suggest a direct application of the broths in processes such as enhanced oil recovery (Yen *et al.*, 1985; Santore & Prud'homme, 1990).

The aim of our study was to follow the culture process of *C. capsulata* by means of the evolution of the rheological properties of its culture broths in order to find the best conditions, in terms of culture time, for the recovery process used in the production of the CC-EPS.

The viscous nature of the polysaccharide fermentation broths is due to the presence of the exocellular polysaccharide and particularly in the case of bacteria or yeast cultures the microorganism cells contribute little to the viscosity (Pace, 1978; Noel *et al.*, 1984). On the other hand, the viscosity of mycelial fermentation broths is mainly due to the presence of the microorganisms and in particular to their morphology (Metz *et al.*, 1979). Since, to our knowledge, a paper on the rheological properties of filamentous cyanobacterial polysaccharide fermentation broths has not been published yet, it is not possible to obtain information from literature on the influence of the morphology of the biomass on rheological behaviour. In order to clarify this point, in the present work, a comparison of the rheological properties of the whole culture broths and of the corresponding supernatants has been carried out.

MATERIALS AND METHODS

Culture broths

C. capsulata ATCC 43193 was cultivated under photoautotrophic and nitrogen fixing conditions at 30°C in small 'raceway' ponds (0·13 m^2 of illuminated surface, 5 cm height of culture) using a medium (pH *c.* 10·5) described elsewhere (Tredici *et al.*, 1987). Continuous illumination was provided by 1000 W incandescent lamps giving a light intensity of about 18·000 lx. The water loss due to evaporation was daily restored to maintain the volume at the initial value. The culture suspension was stirred with a rotating arm (10 rev/min). *C. capsulata* grows diffusely, does not adhere to the surface of the culture devices and it does not require medium or fermenter sterilization. For practical reasons four cultures were set up with a delay time of 7 days between each other using the same inoculum in the exponential phase of growth. In such a way, cultures 7, 14, 21, 28 days old were collected at the same time after 4 weeks.

Supernatants of the four cultures were obtained by centrifugation of the broths at 30 000 rpm, 10°C, 60 min. In order to carry out the separation of the biomass from the culture broth, centrifugation was used since ultrafiltration and filtration techniques were not practicable.

For the sake of brevity, culture broths sampled at 7, 14, 21, and 28 days after inoculum and corresponding supernatants hereafter will be indicated as culture broths 7d, 14d, 21d, 28d and supernatants 7d, 14d, 21d respectively.

The actual amount of CC-EPS in the broths and in the supernatants has been evaluated on the basis of the free sugar content.

The sugar content in the supernatants and in the whole broths was determined by the phenol–sulphuric acid method of Dubois *et al.* (1956) using glucose as a standard. An empirical factor of 2 has been found by Sili *et al.* (1984) to convert the amount of soluble sugars, expressed as glucose equivalents, into the content of polymeric glycan.

Bacterial growth was followed by determining the protein content of the cultures according to the Lowry method (Lowry *et al.*, 1951).

Microscopic observations were carried out in order to investigate the morphology of the microorganisms and to estimate the number density of microorganisms in the culture broths.

Apparatus and procedures

All rheological measurements were carried out at 25°C, with a rotational torsionally oscillating rheometer Haake RV100 (measuring device CV100), which was equipped with a coaxial sensor system ZB15 (Couette type). The inner cylinder used with this coaxial sensor system had the following dimensions: 13·91 mm diameter, 32·30 mm length; the gap was of 0·545 mm. During the tests, while the outer cup was driven, the inner cylinder was mechanically positioned and centred by an air bearing. The top and bottom surfaces of the inner cylinder are especially designed to minimize 'end effects' (Dickinson & Stainsby, 1982).

Shear-dependent measurements were performed by using triangular and multi-stepwise procedures. According to the triangular procedure, the shear rate is varied from zero to a maximum value and, immediately afterwards, to zero in a preset time under constant shear acceleration and deceleration. In the second procedure, a sequence of constant shear rate steps is applied, each shear rate being maintained for a sufficient time for a steady value of the shear stress to be attained.

It has to be taken into account that the relationship between the shear rate $\dot{\gamma}$ and the shear stress τ (i.e. the shear viscosity) can be directly obtained from the raw experimental data only in the case of Newtonian fluids. Indeed, for non-Newtonian fluids it is necessary to calculate the 'effective shear rate' sheared between rotating coaxial cylinders. The angular velocity Ω is related to the effective shear rate through the following integral equation:

$$\Omega = \int_{\tau_0}^{\tau_i} \dot{\gamma}(\tau)/2\tau \, d\tau \qquad (1)$$

where τ_i and τ_o are the tangential stresses at the inner and outer cylinder walls, respectively, and $\dot{\gamma}(\tau)$ is the unknown quantity. For the calculation of the effective shear rate the method suggested by Yang and Krieger (1978) was used. The method is based on the derivation of both members of the eqn (1) and on the expression of $\dot{\gamma}(\tau)$ as a sum of a convergent infinite series. The series truncation suggested by Yang and Krieger was considered here. Effective shear rates have been reported throughout this paper.

Flow curves (shear viscosity versus effective shear rate plots) were fitted by using a computerized non-linear regression analysis.

The analysis of time-dependent properties (stress growth after initiation of a constant shear rate as a function of time) has been performed according to the single step procedure suggested by Trapeznikov and Fedotova (1954). The same shear rate is applied after different rest times and until a steady value of stress is achieved. In such a way the rheological history of the samples is thoroughly defined by the shear rate value and the rest time before the sudden application of shear rate. The results obtained by using transient experiment can generally be affected by errors larger than would be considered really satisfactory due to instrument start-up effects. Great caution has been taken in making the above measurements, however the obtained experimental results have not been corrected.

Dynamic measurements were carried out in order to investigate the viscoelastic properties. The outer cylinder is forced to oscillate sinusoidally at a frequency ω and the corresponding oscillation of the inner cylinder, which is proportional to the resultant torque, is recorded. The stress responses were corrected for the inertial effects due to the instrument and the fluid. The relationship between stress and strain is represented by the complex modulus G* (or alternatively by the complex viscosity η^*) and its elastic and viscous components G′ (or η'') and G″ (or η'), respectively. The loss tangent, $\tan\delta = G''/G'$ is a measure of the ratio of energy lost to energy stored in a cyclic deformation. The phase angle δ between stress and strain, which is a function of the frequency of oscillation, can be also used for the description of viscoelastic properties.

The dynamic tests were carried out at a strain amplitude (0·175 rad) which is well above the upper limit of the linear viscoelasticity regime in the case of the fermentation broths. It implies that the viscous and the elastic moduli as well as the phase angle are functions of both the strain and the frequency of oscillation. Previous investigations performed on coal suspensions (Lapasin & Pricl, submitted) and polysaccharide systems (Delben *et al.*, 1990, Lapasin *et al.*, 1990) showed that a discrimination can be made between the rheological behaviours peculiar to the different classes (concentrated suspensions, solutions and gels) under the experimental conditions considered. Consequently, the G′ and G″ profiles can serve to compare the viscoelastic properties of the systems examined under dynamic conditions.

RESULTS AND DISCUSSION

Characterization of culture broths

C. capsulata grows in the form of helical trichomes which are small chains of spherical vegetative cells (diameter 6–7 μm (Florenzano *et al.*, 1985)). The vegetative cells can be differentiated into two types of spherical cells: heterocysts (responsible for nitrogen fixation) and akinetes (responsible for the survival in harsh environmental conditions). The trichomes are surrounded by an external polysaccharidic layer of considerable thickness, termed a capsule. This has an overall ellipsoidal shape.

Growth of *C. capsulata* occurs as an even suspension in liquid culture. During its growth, *C. capsulata* continuously excretes an EPS causing a large increase in the viscosity of the culture medium.

Microscopic observations show that in the first days of growth, the trichomes have an ellipsoidal size of about 60 × 120 μm, after *c.* 7 days, the size decreases to about 30 × 60 μm due to random trichome breakage (reproduction mechanism). In old cultures (25–30 days), in addition to the helical trichomes, dispersed spherical akinete cells are abundant.

Table 1 shows that the biomass (total protein + total carbohydrate) concentration regularly increases in the first 21 days of cultivation; only a small increase is observed after 21 days. On the other hand, the EPS concentration into the medium, regularly increases as a function of the time of cultivation. These findings are in good agreement with the published results indicating that *C. capsulata* excretes polysaccharide in the medium independently of the growth phase (De Philippis *et al.*, 1989).

Table 1. The concentration of biomass and of exocellular polysaccharide in the culture broths at different culture times

Culture time (Days)	Biomass (g/litre)	Polysaccharide (g/litre)
7	1·56	0·85
14	2·73	1·99
21	4·02	2·54
28	4·14	3·27

Microscopic observations allow the number of trichomes per 1 ml of culture broth (microorganisms number density) to be counted. In the samples 7d, 14d, 21d and 28d the microorganisms number densities were 1×10^6, $1{\cdot}5 \times 10^6$, $2{\cdot}8 \times 10^6$ and $2{\cdot}4 \times 10^6$, respectively; in the case of the 28d sample the number density estimation was difficult due to the high viscosity of the medium.

Shear-dependent properties

The preliminary characterization of the rheological behaviour of culture broths has been carried out using the triangular procedure. The hysteresis cycles (shear stress versus shear rate curves), obtained in all cases by applying the triangular procedure, emphasize two features: the shear-thinning behaviour in the explored range of shear-rate and the amplitude of the obtained hysteresis cycle which depends on both the 'preset time' and the shear history of the samples (non-linear viscoelastic behaviour). The latter findings lead the triangular procedure to be considered insufficient for the quantitative description of the non-Newtonian behaviour of *C. capsulata* culture broths. As a matter of fact, the proper experimental procedure for characterizing a non-Newtonian fluid should give results independent of the rheological history experienced by the sample under investigation.

The stress steady-state values obtained by applying the multi-stepwise procedure are more appropriate for a quantitative rheological characterization as the imposed shear rates are maintained for sufficient time to delete any previous rheological history.

All experimental raw data have been corrected taking into account the effective shear rate calculations by using the Yang and Krieger method (see the Materials and methods section).

In Fig. 1 the shear-thinning behaviour of the *C. capsulata* culture broths is shown. In the range of shear rate investigated the viscosity is a decreasing function of the shear rate. During the course of the fermentation the deviations from the Newtonian behaviour become more marked.

In order to obtain a suitable extrapolation of the zero-shear rate viscosity (η_0) from experimental data it

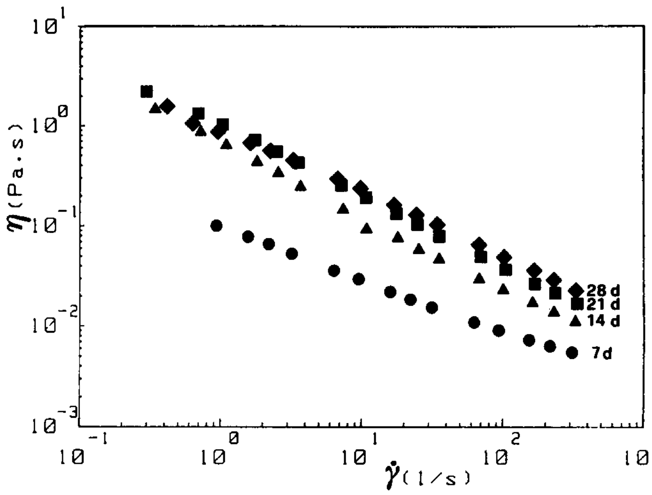

Fig. 1. Steady shear viscosity (η) as a function of shear rate ($\dot{\gamma}$) for the culture broths at different culture times.

is necessary to resort to non-Newtonian mathematical models. The best fitting of the whole set of experimental data has been obtained by using the four adjustable parameters equation proposed by Carreau (1968):

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{(1 + (\lambda\dot{\gamma})^2)^{(1-n)/2}} \tag{2}$$

where η_0 is the zero-shear rate viscosity (upper Newtonian plateau), η_∞ is the infinite-shear rate viscosity (lower Newtonian plateau), λ is a constant parameter with the dimension of time and n is a dimensionless exponent. The value of η_∞ is a few orders of magnitude smaller than η_0 and η, it has therefore been set equal to the pure solvent viscosity, 0·001 Pa·s. Although physically more acceptable, this choice does not differ practically from that of Chauveteau (1982), who has suggested a reduced equation in which the infinite-shear rate viscosity was set to zero.

The Cross (1965) equation which has previously been used to fit the experimental data for CC-EPS aqueous solutions (Navarini *et al.*, 1990) as well as pullulan fermentation broths (Choplin & LeDuy, 1986), was only able to fit the experimental data of the supernatants.

The power law equation has frequently been used to fit the experimental flow curves of polysaccharide fermentation broths (pullulan and xanthan fermentation broths) (Pace, 1978; LeDuy *et al.*, 1974). However, the power law equation predicts an infinite viscosity at zero shear rate instead of Newtonian behaviour, which is the typical behaviour of a pseudoplastic fluid.

The zero-shear rate viscosity values as well as the other parameters of the Carreau equation for the culture broths are reported in Table 2. An analysis of these values shows that the variation of all the parameters as a function of culture age is not monotonic but it reaches a maximum in η_0 and λ and a minimum in n at a time of 14 days. Moreover the 28d culture broth in addition to an unexpectedly low zero-shear rate

Table 2. The parameters in the Carreau equation obtained from viscosity measurements on the culture broths and the corresponding supernatants

	η_0 (Pa·s)	η_∞ (Pa·s)	λ (s)	n
Broths				
7d	0·162	0·001	1·98	0·416
14d	3·470	0·001	8·63	0·232
21d	3·260	0·001	4·98	0·280
28d	1·600	0·001	2·38	0·368
Supernatants				
7d	0·070	0·001	1·94	0·454
14d	0·845	0·001	4·34	0·324
21d	1·820	0·001	3·67	0·322
28d	0·686	0·001	1·56	0·424

viscosity value shows a flow curve profile which is not similar to that of the other samples.

Figure 2 shows the shear viscosity as a function of the culture age at different shear rates. The increase in shear viscosity with the culture time depends markedly upon the shear rate. At low values of the shear rate, the shear viscosity reaches a maximum for the 14d sample.

During the course of the culture, the concentration of the EPS increases (see Table 1). If the contribution of the suspended microorganisms to the non-Newtonian behaviour of the culture broths were negligible, the rheological behaviour of the corresponding supernatants should be equal to that of Fig. 1.

As expected, the flow curves of the supernatants shown in Fig. 3 are similar to those of culture broths; nevertheless the shear viscosity values obtained for the supernatants are lower than those obtained for the culture broths. In Table 2 the zero-shear rate viscosities are reported as well as the other parameters of the Carreau equation for the supernatants. It has to be

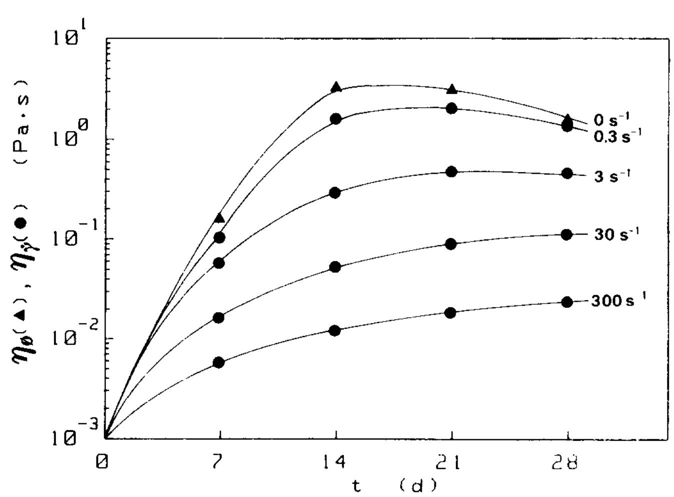

Fig. 2. Zero-shear rate viscosity (η_0) and steady shear viscosity (η) at different shear rates ($\dot{\gamma}$) as a function of the culture time.

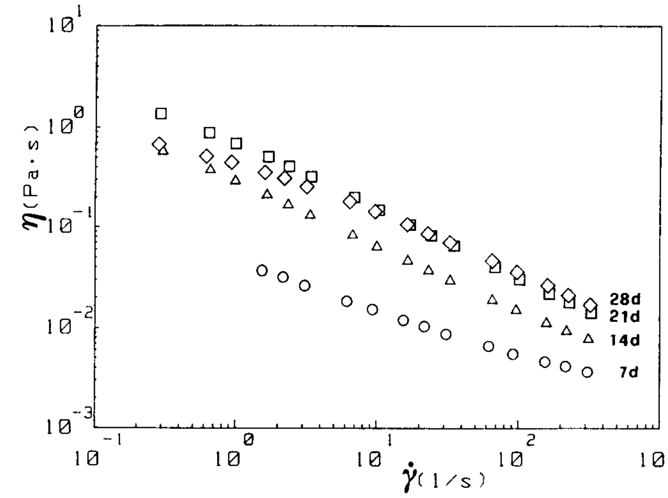

Fig. 3. Steady shear viscosity (η) as a function of the shear rate ($\dot{\gamma}$) for the supernatants at different culture times.

pointed out that the rheological behaviour of the supernatants is very close to that of the CC-EPS aqueous salt solutions (Navarini *et al.*, 1990) at similar polymer concentrations, except for the 28d sample.

Accordingly, the rheological behaviour of the culture broths is largely dominated by the presence of the EPS even if the presence of the microorganisms cannot be considered as being negligible.

However, the maximum value of the zero-shear rate viscosity of the 14d culture broth (Fig. 2) cannot be ascribed to a variation in the EPS concentration. In fact, the data reported in Table 1 show a regular increase of the polysaccharide concentration in the medium.

In order to clarify this point, it is necessary to attempt to quantify the contribution of the disperse phase to the total rheological behaviour of the culture broths on the basis of the microscopic observations on the number, size and shape of the *C. capsulata* trichomes and in terms of relative shear viscosity.

The relative shear viscosity of a suspension at a given shear rate can be expressed as

$$\eta_r = \eta/\eta_s = f(\phi) \tag{3}$$

where η is the viscosity of the suspension, η_s is the viscosity of the continuous phase (supernatant), and $f(\phi)$ a function of the volume fraction of the disperse phase (microorganisms). According to Quemada (1978) eqn (3) becomes (at the upper Newtonian plateau and in the limit of monodisperse systems)

$$\eta_{r,0} = \eta/\eta_s = (1 - 0{\cdot}5k\phi_{\text{eff}})^{-2} \tag{4}$$

where $\eta_{r,0}$ is the zero-shear rate relative viscosity, k is a constant related to the 'structural state' of the system (shape) and ϕ_{eff} an 'effective' volume fraction (a volume fraction which takes into account changes of particle effective volume especially through aggregation effects) (Quemada, 1978).

Assuming the trichomes as prolate ellipsoids with an axial ratio of 2, then the value of k results 2·908 (Cantor & Shimmel, 1980), and eqn (4) can be used to evaluate from the experimental rheological data the effective volume fraction ϕ_{eff} for all samples.

On the other hand, on the basis of microscopic observation, it is possible to evaluate the 'normal' volume fraction, ϕ, corresponding to the product between the volume of a trichome, in the approximation of the prolate ellipsoids, and the microorganism number density. The nominal volume fraction can be considered equal to the effective volume fraction only in the absence of perturbation effects (e.g. aggregation).

In Table 3 effective and nominal volume fractions are reported for the different culture broth samples.

For the 7d sample the effective volume fraction is very close to the nominal one indicating a negligible aggregation. For the other samples, the effective volume fraction always results larger than the nominal volume fraction; it suggests that an 'interaction' among the suspended bodies has to occur in order to increase the effective volume fraction as calculated from the rheological results.

From the data of Tables 1 and 3 it can be also seen that the ratio of effective to nominal volume fraction increases with the mass of polysaccharide in the supernatant, indicating a non-negligible contribution from the polysaccharide/disperse phase interaction.

In particular the 14d broth shows the greatest difference between effective and nominal volume fraction; therefore, this system, might contain the largest amount of aggregation in the disperse phase. The high zero-shear rate viscosity for the 14d broth can be mainly ascribed to this feature.

On the other hand, the largest change in the morphology of the suspended cells occurs between 7 days and 14 days after the inoculum, as judged from Table 3. This difference of morphology can be evidenced by scrutinizing the relative viscosity of the broths with respect to the supernatants. In Fig. 4 the relative viscosity as a function of the shear rate is reported. The shear-dependent behaviour of the relative viscosity of the 7d broth is very different from that of the other

Table 3. Effective (ϕ_{eff}) and nominal (ϕ) volume fraction for the culture broths at different culture times

Broth	ϕ_{eff}	ϕ
7d	0·230	0·226
14d	0·350	0·042
21d	0·170	0·079
28d	0·240	0·068

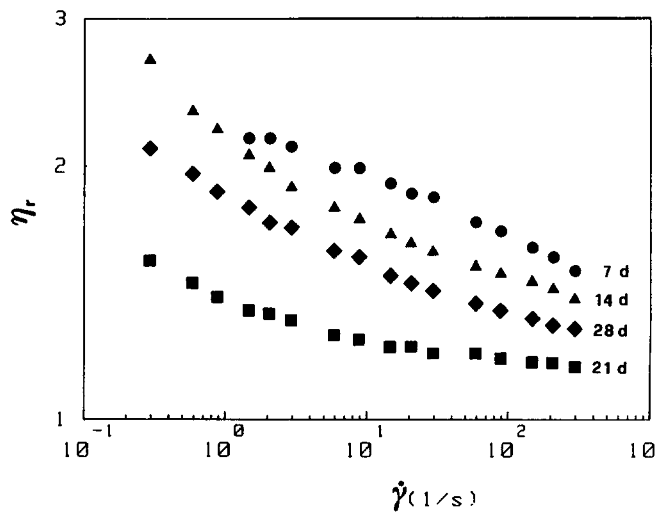

Fig. 4. Relative viscosity (η_r) as a function of the shear rate ($\dot{\gamma}$) at different culture times.

culture broths. In the 7d broth the shear rate dependence of the relative viscosity can be attributed to the shear induced orientation of the trichomes; in the other cases the shear rate dependence of the relative viscosity can be related to a sort of aggregation–disaggregation process occurring under shear flow in addition to orientation and deformation effects of disperse phase.

Coming back to the rheological behaviour of the 28d supernatant, its flow curve crosses the curve corresponding to the 21d sample and, at low shear rates, also the curve of the 14d sample. Since the polysaccharide concentration is the higher for the 28d supernatant, this 'anomalous' rheological behaviour, which is appreciable also in the case of the 28d culture broth, suggests a decrease in the average molecular weight of the EPS in the older cultures. Such an evidence was obtained by direct molecular weight determinations of the polysaccharides extracted from old cultures.

Evidence for a change in the molecular weight of CC-EPS extracted from cultures of different growth times (26d and 37d) has been already given (Cesàro *et al.*, 1990). The macromolecular characterization gave a dramatic decrease of the intrinsic viscosity (2·26 and 0·13 litre/g) and molecular weight (MW = $1·4 \times 10^6$ and $0·13 \times 10^6$) for the two polysaccharides at 26d and 37d, respectively.

In the case of the fermentation broths of *Aureobasidium pullulans*, a yeast-like fungus producing an EPS, the decrease in zero-shear rate viscosity at the end of the batch fermentation has been attributed to the reduction of the polysaccharide molecular weight by the action of the enzyme pullulanase which is synthesized by the fungus (Noel *et al.*, 1984; Choplin & LeDuy, 1986). A similar process could occur also in the course of the culture of *C. capsulata*, but it is at present only an hypothesis which has to be tested by further studies.

Time-dependent properties

Transient shear experiments have been performed in order to investigate qualitatively the time-dependent properties shown by using the triangular procedure as described above. If a constant shear rate is imposed on a fluid characterized by a linear viscoelastic behaviour, the stress rises monotonically approaching the steady-state value as steady flow is reached. In the case of non-linear viscoelastic behaviour the stress does not increase monotonically but passes through a maximum.

A comparison with the results obtained from an analysis performed at the same shear rates on Newtonian fluids having comparable viscosities to those of the systems presently investigated, clearly showed that the stress overshoots do correspond to the material properties and that their values are underestimated because of the response of the instrument used. Accordingly, the stress overshoots can be properly used for a comparative study of the time-dependent properties of the systems examined.

Figure 5 shows, in addition to a sketch of the experimental procedure, the evolution of the stress overshoot in the shear stress growth upon inception of steady shear flow for the 7d, 14d and 21d culture broths and corresponding supernatants (shear rate = $3\ s^{-1}$; rest time = 60 s; $\tau_{red} = \tau/\tau_e$ where τ_e is the steady shear stress equilibrium value). This complex behaviour is close to that exhibited by CC-EPS aqueous solutions previously investigated (Navarini *et al.*, 1990). The stress overshoot is strongly affected by both shear rate and rest time preceding the application of the constant shear rate and in all samples investigated the stress overshoot is an increasing function of the rest time until a rest time of about 300 s. For rest time greater than 300 s the stress overshoot does not vary. The maximum stress overshoot is obtained for the 14d broth. The relevant contribution of the disperse phase to the non-linear viscoelastic behaviour of the broths is remarkable at 7 and 14 days as it can be evinced from the comparison between the broth and corresponding supernatant transients. Afterwards, it decreases, thereby becoming negligible after 21 days.

All these considerations are pertinent here before proceeding with the results obtained in oscillatory flow conditions.

Oscillatory flow tests

Besides non-Newtonian viscous behaviour, *C. capsulata* culture broths exhibit elastic properties. Since the elasticity of the culture broths influences the mixing

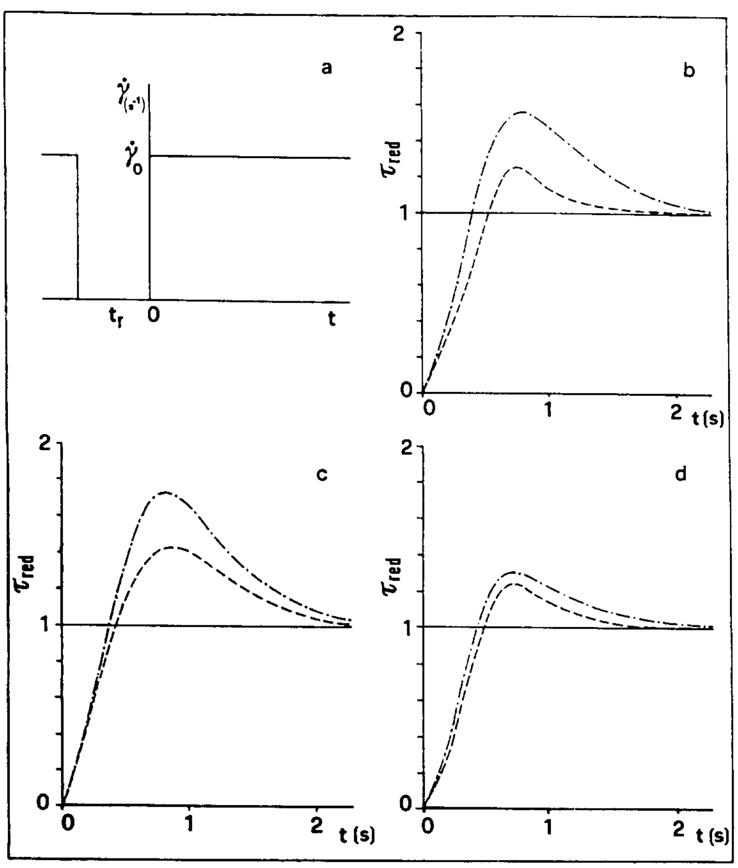

Fig. 5. Stress growth resulting from steady shear flow for (·——) the culture broths and (----) the corresponding supernatants at different culture time: (a) The test procedure ($\dot{\gamma} = 3\ s^{-1}$, rest time = 60 s); (b) 7d; (c) 14d; (d) 21d. ($\tau_{red} = \tau/\tau_e$ where τ_e is the steady shear stress equilibrium value).

efficiency (Choplin & LeDuy, 1986), the investigation of fluid elasticity is of practical interest for the engineering design of fermenters.

Figure 6 shows the frequency dependence of the elastic modulus G′ for all the culture broths examined. The elastic modulus is an increasing function of the frequency and the profile of the curves is similar for the 7d, 14d and 21d culture broths, being G′ at any frequency, an increasing function of the culture time (i.e. of both the EPS and the biomass concentration). On the other hand, the behaviour of the 28d sample is noticeably different: not only does the profile of the curve show a higher slope but there is also a large decrease in the values of the elastic modulus at low frequency. The data for the supernatants (not reported) show a similar behaviour.

In order to investigate the contribution of the disperse phase to the elastic properties of the culture broths it may be useful to resort to the relative elastic modulus $G'_r = G'/G'_s$ where G′ and G'_s are the elastic moduli of the broths and the supernatants, respectively. Figure 7 shows the frequency dependence of the relative elastic modulus for the four samples. The figure clearly shows that the contribution of the disperse phase to the elastic properties of the culture broths is very marked at the lower frequencies of oscillation and decreases in the course of the culture, being always relevant in the early stages of the process (7 days). In the 21d and 28d samples the presence of the biomass contributes little to the elastic properties of the systems.

The frequency dependence of the viscous modulus G″ is less marked in comparison with that of the elastic modulus for the culture broths, as shown in Fig. 8.

The viscous modulus increases in the course of the culture; the profile of the 28d sample curve is different to that of the other samples, in agreement with the other reported findings. Similar results have been obtained for the supernatants.

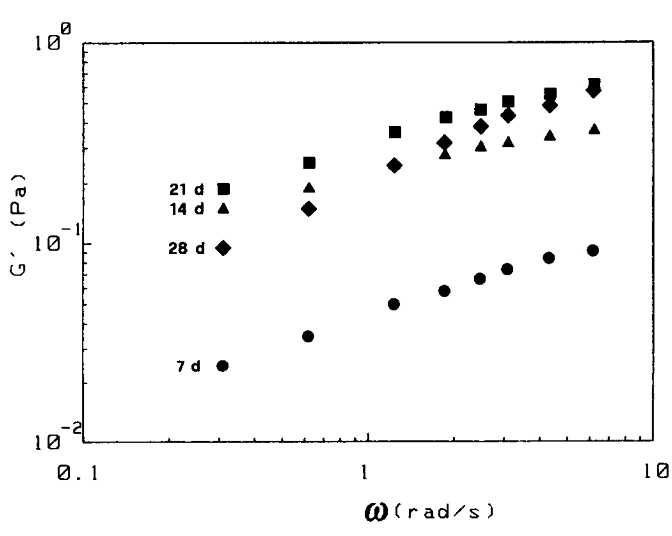

Fig. 6. The storage modulus (G′) as a function of frequency (ω) for the culture broths at different culture times.

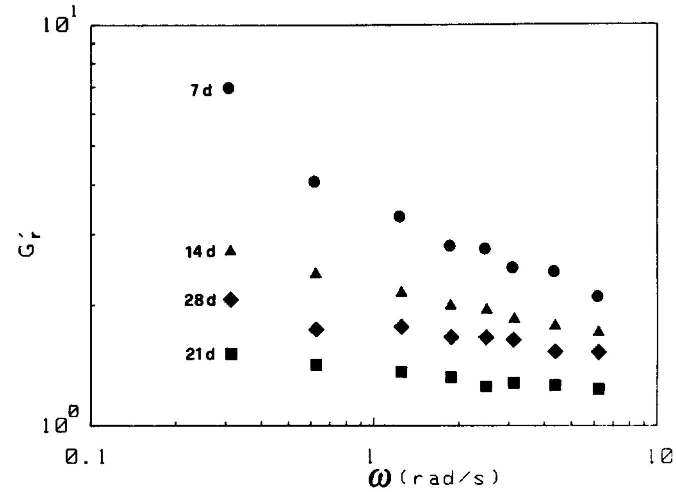

Fig. 7. The relative storage modulus (G'_r) as a function of frequency (ω) for the culture broths at different culture times.

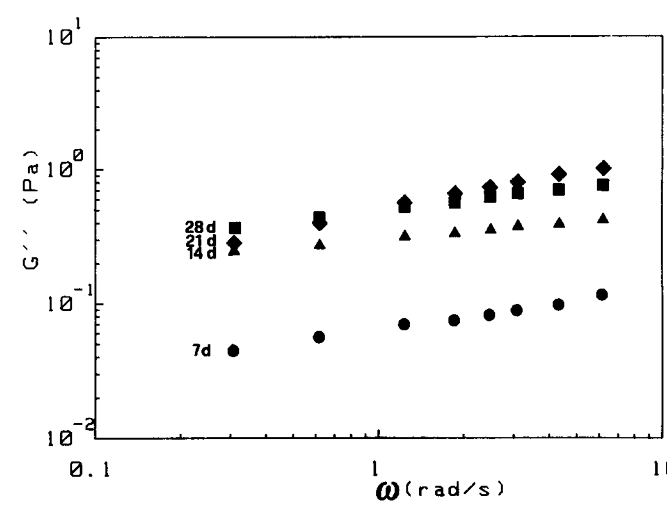

Fig. 8. The loss modulus (G″) as a function of frequency (ω) for the culture broths at different culture times.

The viscoelastic behaviour of both culture broths and corresponding supernatants may be properly summarized in terms of phase lag, δ, as a function of the culture time, as shown in Fig. 9 at two different frequency values. By definition, at a constant frequency, the phase lag in the absence of elastic behaviour assumes the value 1·57 and this value decreases as the elastic component increases reaching the value 0·785 when G′ = G″. As shown in Fig. 9 all the samples investigated exhibit a liquid-like behaviour (G′ < G″) largely dominated by the continuous phase; moreover the contribution of the elastic component to the viscoelasticity is larger for the system with a culture time of 14 days. This behaviour is consistent with the time evolution of the non-linear viscoelastic properties observed in the shear stress growth experiments and may be mainly related to the disperse phase, which at

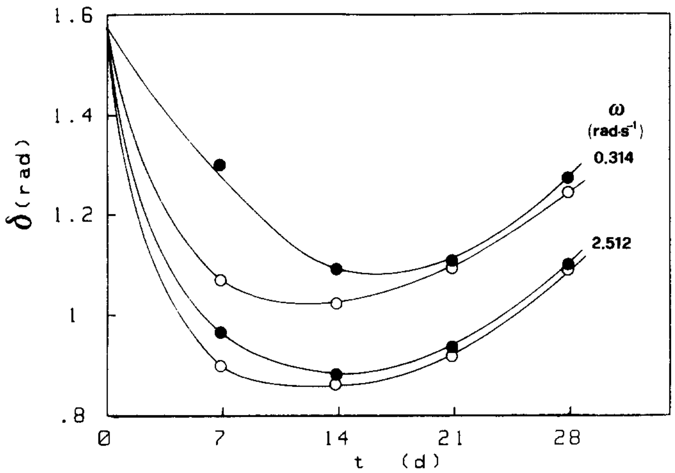

Fig. 9. Phase lag variation with culture time for the culture broths (○) and the corresponding supernatants (●) at two different frequencies.

this stage of the culture process shows the higher level of aggregation. The decrease of the elastic component after 14 days, in addition to morphological changes of the biomass, may be related to the molecular weight changes of the polysaccharide solubilized in the continuous phase.

CONCLUSIONS

The viscous nature of the *C. capsulata* culture broths varies with time as a consequence of the morphological and mass modifications occurring in both the disperse and continuous phase during the course of the microbial growth.

The rheological behaviour under continuous shear flow is shear-thinning and is described by means of the Carreau equation. The non-Newtonian behaviour is largely dominated by the EPS released into the culture medium but the rheological results, in particular the η_{rel} versus $\dot{\gamma}$ and G'_r versus ω curves, clearly show that the presence of the microorganisms cannot be considered negligible particularly during the initial stages of the culture.

The zero-shear rate viscosity as well as the elastic component reaches a maximum value 14 days after inoculation when the biomass shows maximum aggregation as estimated when comparing the broths and the corresponding supernatants.

The rheological behaviour of the broth after 28 days of growth is slightly anomalous: in addition to an unexpected low zero-shear rate viscosity the profile of the experimental curves obtained in both steady shear and oscillatory flow is not comparable with that obtained in the other cases. All these anomalies may be ascribed to the morphology of the disperse phase (dispersed spherical akinete cells are abundant) in addition to a polysaccharide depolymerization probably due to the action of an enzyme. This hypothesis, which has to be tested by further studies, suggests that the molecular weight of the EPS is affected by the culture time.

This work suggests that the optimum time for recovery of the EPS is between 14 and 21 days after innoculation.

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