www.nature.com/jim

# Hydrodynamic characteristics and mixing behaviour of *Sclerotium glucanicum* culture fluids in an airlift reactor with an internal loop used for scleroglucan production

X Kang<sup>1</sup>, H Wang<sup>1</sup>, Y Wang<sup>1</sup>, LM Harvey<sup>2</sup> and B McNeil<sup>2</sup>

<sup>1</sup>State Key Laboratory of Biochemical Engineering, Institute of Chemical Metallurgy, Chinese Academy of Sciences, P.O. Box 353, Beijing 100080, China; <sup>2</sup>Strathclyde Fermentation Centre, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow G1 1XW, UK

The filamentous fungus, *Sclerotium glucanicum* NRRL 3006, was cultivated in a 0.008 m<sup>3</sup> airlift bioreactor with internal recirculation loop (ARL-IL) for production of the biopolymer, scleroglucan. The rheological behaviour of the culture fluid was characterised by measurement of the fluid consistency coefficient (*K*) and the flow behaviour index (*n*). Based on these measurements, the culture fluid changed from a low viscosity Newtonian system early in the process, to a viscous non-Newtonian (pseudoplastic) system. In addition, reactor hydrodynamics and mixing behaviour were characterised by measurement of whole mean gas hold-up ( $\varepsilon_g$ ), liquid re-circulation velocity ( $U_{ld}$ ) and mixing time ( $t_m$ ). Under identical process conditions, the effects of the viscosity of the culture fluid and air flow rate on  $\varepsilon_g$ ,  $U_{ld}$  and  $t_m$  were examined and empirical correlations for  $\varepsilon_g$ ,  $U_{ld}$  and  $t_m$  with both superficial velocity  $U_g$  and consistency coefficient *K* were obtained and expressed separately. The correlations obtained are likely to describe the behaviour of real fungal culture fluids more accurately than previous correlations based on Newtonian or simulated non-Newtonian systems. *Journal of Industrial Microbiology & Biotechnology* (2001) **27**, 208–214.

Keywords: scleroglucan; airlift reactor with an internal loop (ALR-IL); gas hold-up; liquid recirculation velocity; mixing time

# Introduction

The workhorse of the bioprocessing industry is still the conventional aerated stirred tank reactor (STR). The STR, although often considered a well-characterised reactor type, is acknowledged to have a number of drawbacks or limitations [9,12,16]. In general, these drawbacks worsen as scale of operation increases, and are especially apparent when the process fluid exhibits high viscosity and/or pronounced non-Newtonian (usually pseudoplastic) characteristics. Among the drawbacks are: complicated and costly construction arising from the need to give an effective sterile seal on a moving agitator shaft, high running costs due to continuous mechanical agitation, and ineffective mass and momentum transfer in highly non-Newtonian process fluids, such that, in extreme cases, regions of the process fluid may be static and anoxic [15].

Given these acknowledged limitations, there has been a search for simpler, less expensive reactors, especially in the processing of highly viscous non-Newtonian fluids [2,4,11,14]. Such fluid types are most commonly encountered in the cultivation of filamentous bacteria or fungi, or when cultivating exopolysaccharide-producing organisms. Since the former is arguably the single most important group of organisms in biotechnology, and from the latter comes such valuable products as xanthan and gellan gum, the need for alternative reactors is apparent.

One reactor type, which has shown much promise, is the airlift reactor (ALR), where agitation and mixing are achieved by aeration alone. Such low-cost reactors have been previously shown to be at least the equal of STR in the production of a range of exopolysaccharides [3,16].

However, by contrast with the STR (or at least the common perception of the STR), the ALR is generally considered a poorly characterised reactor system. In ALR's gas hold-up ( $\varepsilon_g$ ), liquid circulation velocity (Vld) and mixing are key parameters affecting product formation. In this context, the dearth of studies involving real, as opposed to simulated, viscous non-Newtonian fluids is especially striking. In fact, most studies on fluid dynamics and mixing in ALRs focus on relatively simple Newtonian fluid types and the relevance of the correlations between  $\varepsilon_{g}$ ,  $V_{ld}$  and  $t_{m}$  (mixing time) and process fluid viscosity, and gas velocity is questionable. Conversely, Wang and McNeil [16] used complex, heterogeneous, highly non-Newtonian fermentation fluids to measure  $\varepsilon_{g}$ ,  $V_{ld}$  and  $t_{m}$  in a pilot scale ALR with external loop.  $\varepsilon_{g}$ ,  $V_{ld}$  and  $t_{m}$  were strongly influenced by fluid characteristics such as viscosity and superficial gas velocity. The models developed were more accurate in predicting behaviour of real filamentous cultures than those extrapolated from correlations based on Newtonian or simulated non-Newtonian fluids.

Other advantages to using ALRs to produce scleroglucan (an immunostimulant exopolysaccharide, also of potential use in oil recovery) is that low  $O_2$  tensions are reported to stimulate biopolymer synthesis; thus, the commonly cited ALR disadvantage (modest  $O_2$  transfer rates, *cf.* STR) is not a drawback.

A further consideration is the effect of shear stress on biopolymer release. Rau *et al* [13] showed that under low shear conditions, only low molecular weight biopolymer was released; the high molecular weight material (especially desirable in many

Correspondence: Dr B McNeil, Strathclyde Fermentation Centre, Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow G1 1XW, UK Received 5 June 2000; accepted 18 March 2001

applications) remained adherent to the hyphae. Conversely, very high shear rates could lead to cell damage and reduced product. In this context, ALR with an internal re-circulation loop (ALR-IL) may offer advantages over previous ALR external loop reactors (ALR-EL) used for viscous processes. In particular, for scleroglucan release, although ALRs are often considered "low shear" systems, the ALR-IL, with a more violent bubble zone between baffle and draught tube top (central) than in the ALR-EL, may offer better mixing and higher shearing.

However, to date, there are no reports linking the hydrodynamics and mixing behaviour of such ALR-EL with the process performance. In this paper, we report on the effects of superficial gas velocity  $(U_g)$  and the fluid consistency coefficient (K) on  $\varepsilon_g$ ,  $V_{\rm ld}$  and  $t_{\rm m}$  during cultivation of the filamentous fungus, *Sclerotium glucanicum*.

# Materials and methods

## Airlift reactor with an internal loop (ARL-IL)

The ARL-IL used in this work (Figure 1) was made up of two borosilicate glass sections sealed with Teflon gaskets and held together by mild steel flanges. The height of the reactor was 0.9 m and its aspect ratio approximately 9:1. The nominal diameter of the draft tube (central tube) was 0.036 m. The internal diameter of the expanding section located at the upward part of the reactor was 0.176 m. The total volume of the reactor was 0.008 m<sup>3</sup> and the working volume was 0.0056 m<sup>3</sup>. Temperature was monitored by a Pt resistance thermometer fixed on top of the reactor and controlled by means of an electrical heating element fixed on the bottom of the reactor. Running water through a cooling finger was used to cool the reactor. A pH probe was located in the reactor to control the pH of the broth. The pH of the broth was controlled by automatic addition of either 1 M NaOH or 10% H<sub>2</sub>SO<sub>4</sub>. At the base of the vessel, sterile air was introduced into the central tube via a simple tubular sparger. Air flowing up the central tube eventually collided with the baffle above. The height of the baffle was determined by the amount of broth in the reactor.

#### Materials

The system used in these studies is the viscous, non-Newtonian fermentation fluid resulting from the cultivation of *S. glucanium* NRRL 3006, which mainly contained fungal mycelium and the extracellular polysaccharide, scleroglucan. The composition of the culture medium was  $(kg/m^3)$ : sucrose 30.0;  $(NH_4)_2SO_4$  1.0;  $KH_2PO_4$  1.0;  $MgSO_4.7H_2O$  0.5; yeast extract 1.0;  $FeSO_4.7H_2O$  0.01.

## Methods

The sterile medium in the fermenter was inoculated using 200 ml of a 48-h culture of *S. glucanicum*, which was cultivated on a rotary shaker at 28°C, 175 rpm. Process conditions were: 28°C, air flow rate 1.4 vvm, pH 4.5 $\pm$ 0.2. Biomass concentration was measured by dry weight estimation using GF/C filters (Whatman, Maidstone, UK). Extracellular polysaccharide was measured by precipitating scleroglucan from cell-free broth samples by addition of 2 vol of absolute alcohol. The precipitated biopolymer was then filtered, washed, dried and weighed. A viscometer Rheotest 2.1 was used to measure rheological properties of the broth at 28°C.



Figure 1 Line diagram of the airlift reactor with internal loop. (1) Liquid exit. (2) Cooling finger. (3) Gas nozzle. (4) Central tube. (5) Expanding section. (6) DO probe. (7) pH probe. (8) Pressure gauge. (9) Sampling port. (10) Heating element. (11) Reactor body. (12) Thermometer. (13) Baffle. (14) Liquid inlet. (15) Alkali and acid input. (16) Condenser. (17) Gas exit. (18) DO meter. (19) pH controller and recorder. (20) Temperature controller.

Total mean gas hold-up,  $\varepsilon_g$ , was calculated from the difference between the volumes of aerated and non-aerated phases, and can be expressed as:

$$\varepsilon_{\rm g} = (V_{\rm t} - V_{\rm l})/V_{\rm t} \times 100\% \tag{1}$$

One method for determination of the liquid circulation velocity is known as the "measuring ball method" [14], which was based on length and time measurements. Thereby, the liquid circulation velocity,  $U_{\rm ld}$ , can be calculated from the equation:

$$U_{\rm ld} = L/t_{\rm a} \tag{2}$$

with L in [m] and  $t_a$  in [s]. In the present work, the measurement of  $U_{ld}$  was carried out using fungal pellets as tracers and calculation using the above equation.

Previous studies have indicated that once the available carbon source is consumed, culture pH is stable due to cessation of organic 1

209

acid synthesis [17]. Thus, it is possible to estimate the mixing time by addition of a pulse of 5 ml of 10 M NaOH, and a response curve of pH against time can be obtained. In order to determine mixing time,  $t_m$ , when the broth viscosity is low and at high air flow rate, when mixing time is short, the degree of inhomogeneity, h, was set at 1%. When the fermentation fluid was highly viscous, and airflow rates were low (i.e., mixing times were lengthy), h was assumed to be 5%. Lehnert [6] defined h as the relative deviation of actual tracer concentration C from the mean value  $C_{\infty}$ , which expressed the tracer concentration upon reaching perfect mixing:

$$h = (C - C_{\alpha})/C_{\alpha} \tag{3}$$

In the present work, from the above equation, h can be defined as follows:

$$h = (pH - pH_{\alpha})/pH_{\alpha} \tag{4}$$

# **Results and discussion**

#### Rheological behaviour of culture fluid

After 10 days of cultivation of *S. glucanicum* at an airflow rate of 1.4 vvm in the ALR-IL, the biomass was 7.4 kg/m<sup>3</sup> and the concentration of scleroglucan was 7.9 kg/m<sup>3</sup>. During the process, the fungus grew in a dispersed hyphal form with few large pellets. The rheological nature of the liquid was thus highly complex, with fungal biomass, water, nutrients and metabolic products such as exopolysaccharide in the highly viscous fluid. To broaden the range of fluid viscosities examined, the final process fluid was diluted with varying amounts of sterile water (Table 1).

By the process end, most of the carbon and nitrogen sources had been used up. Thus, the concentration of components in the broth and pH value of the system did not change significantly during the final stages. Moreover, the concentration of by-products, e.g., oxalic acid, had little effect on the system rheology, which was mainly determined by fungal biomass and polysaccharide.

The viscosity of varying dilutions was measured at  $28^{\circ}$ C. The relationship between the rheological data (fluid consistency coefficient, *K*; flow behaviour index, *n*) and the fluid percentage is shown in Figure 2. It can be seen from data in Table 1 and Figure 2 that the fluid consistency coefficient, *K*, increases with increasing concentration of the fungal biomass and polysaccharide in the fluid. When the broth percentage exceeds 60%, *K* increases very sharply, while the flow behaviour index, *n*, decreases with the increasing concentration of the fungal biomass and polysaccharide in the broth. The undiluted broth has the highest consistency coefficient of 3.70 Pa s<sup>n</sup> and lowest flow behaviour index of 0.315, presenting markedly pseudoplastic behaviour. Figure 2 also shows the transformation from a Newtonian fluid with less biomass and polysaccharide towards the pseudoplastic fluid with more cell mass and scleroglucan.

 Table 1 Concentration of biomass and scleroglucan at various dilutions

Product	Culture fluid (%)				
	100	80	60	40	20
Biomass (kg/m <sup>3</sup> ) Scleroglucan (kg/m <sup>3</sup> )	7.4 7.9	5.9 6.3	4.4 4.7	3.0 3.1	1.5 1.6



Figure 2 The influence of broth dilution upon fluid consistency coefficient (K) and flow behaviour index (n).

## Overall mean gas hold-up in ALR-IL

The effect of changes in the value of the consistency coefficient (K) and of changes in the superficial gas velocity  $(U_g)$  upon overall mean gas hold-up is shown, respectively, in Figures 3 and 4. Based on the curves of  $\varepsilon_g$  versus  $U_g$ ,  $\varepsilon_g$  can be expressed simply by the following equation:

$$\varepsilon_{\rm g} = a + bU_{\rm g} \tag{5}$$

Both a and b in Eq. (5) are themselves the functions of consistency coefficient (K). By correlating a and b with K, we have:

$$a = 2.4882 \times 10^{-3} + 1.5437 \times 10^{-3} \ln K \tag{6}$$

$$b = 0.1379 + 7.8284 \times 10^{-3} \ln K \tag{7}$$

By combining Eqs. (5) and (6) with Eq. (7), an empirical



Figure 3 The influence of K on mean gas hold-up.

210



Figure 4 The influence of superficial gas velocity on mean gas hold-up.

relationship between  $\varepsilon_g$  and both  $U_g$  and K is obtained and can be expressed as:

$$\varepsilon_{\rm g} = 2.4882 \times 10^{-3} + 1.5437 \times 10^{-3} \ln K + (0.1379 + 7.8284 \times 10^{-3} \ln K) U_{\rm g}$$
(8)

This equation indicates that  $\varepsilon_g$  changes in direct proportions to  $U_g$ . The relative standard deviation between "calculated" and measured values is 5.7%.

As shown in Figure 3, mean gas hold-up increased with increasing consistency coefficient (K) of the fluid. At the beginning of the process (low biomass and negligible polysaccharide), K was very low and the value of n approached unity. The fermentation fluid exhibited near-Newtonian characteristics. When the superficial gas velocity was low, the fluid basically exhibited a pseudohomogeneous flow regime. The gas ejecting into the central tube from the spray nozzle formed a continuous phase, and gushed from the top of the tube to the baffle above. The gas plug was dispersed by the baffle and obliquely collided with the internal wall of the reactor — thereafter was deflected downward and then flew upward, thus forming a bubble concentrating zone between the baffle and the top of the draft tube. In the low-viscosity fluid, because of the violent turbulence of fluid in the bubble zone, bubbles tended to collide with each other with sufficient energy to cause aggregation. Meanwhile, because of the low viscosity, the bubbles were easily carried downwards in the annular space between draft tube and the inside wall of the reactor. Furthermore, due to the low frictional resistance between liquid and bubbles, the residence time of the bubbles was short. However, as viscosity increased, the surface tension of bubbles increased, which decreased the bubbles' tendency to coalesce, prolonging the residence time of the bubbles in the liquid. At the same time, the increase in frictional resistance between bubbles and the viscous liquid will lead to more bubbles being entrained by the down flow of the liquid in annular space; thus, mean gas hold-up increased. Figure 3 also reveals that the gas hold-up increases sharply with increasing viscosity when the value of the consistency coefficient is relatively low. But when the consistency coefficient reached a value of 0.323, the gas hold-up increased smoothly with increasing viscosity. The main reason for this phenomenon is that at high viscosity, after the gas exits past the baffle over the central tube, a fraction of the gas is broken up to give large bubbles, which ascend faster than smaller ones, so residence time is shorter, counteracting the increase in mean gas hold-up.

It is striking to note the profound differences in the relationship between  $\varepsilon_g$  and viscosity in the ALR-IL (this study) and in similar process fluids in an ALR-EL, where generally  $\varepsilon_g$  fell with increasing apparent viscosity [1].

In the ALR-EL, the pattern of gas distribution led to the generation of large numbers of dispersed small bubbles, which rose in a co-current fashion in the riser; conversely, in the present study, gas and liquid phase movements in the annular space are countercurrent and the baffles in the ALR-IL tend to minimise or reduce the pronounced gas disengagement noted on top of the riser in the ALR-EL.

Figure 4 reveals that in the range of gas flow rates studied, the gas hold-up was significantly affected by the superficial gas velocity, which was in agreement with results obtained from simulated fermentation systems [7,11,18]. The gas exiting from the central tube collided with the baffle more energetically with increasing gas velocity; thus, more small bubbles came into being. Meanwhile, since the liquid circulation velocity increases with increasing gas velocity, the amount of circulating small bubbles carried by the liquid increases, which leads to a longer residence time of bubbles in the liquid and an increase in overall mean gas hold-up.

It was noted that as superficial gas velocity rose in an ALR-EL, the increased turbulence increased back mixing and vortex mixing, which led to a longer residence time of gas in the liquid. Thus, even when K is very high, gas hold-up increases linearly with the increase of superficial gas velocity in the present study.

## Liquid circulation velocity in the ALR-IL

The liquid circulation velocity in the annular space of the ALR-IL is shown in Figure 5 as a function of the consistency coefficient of



Figure 5 The influence of the consistency coefficient on the liquid recirculation velocity in the annular space.



**Figure 6** The effect of superficial gas velocity on liquid recirculation velocity in the annular space.

0.20

Ug (m/s)

0.10

0.00

0.00

the process fluid and in Figure 6 as a function of the superficial gas velocity. From Figure 6, an expression for  $U_{ld}$  and  $U_g$  can be described:

$$U_{\rm ld} = A \exp(BU_{\rm g}) \tag{9}$$

0.30

0.40

Both A and B change with consistency coefficient K and can be expressed by the following equations:

$$A = 8.1923 \times 10^{-3} K^{-0.4211} \tag{10}$$

$$B = 0.6824 \ln K + 6.4842 \tag{11}$$

By substituting the values of A and B into Eq. (9), empirical correlations for liquid circulation velocity in the annular space can be obtained as a function to both K and  $U_g$ :

$$U_{\rm ld} = 8.1923 \times 10^{-3} K^{-0.4211} \\ \times \exp[(0.6824 \ln K + 6.4842) U_{\rm g}]$$
(12)

The mean relative standard deviation between "calculated" and measured values is 4.64% (Figures 5 and 6).

Figure 5 shows that the liquid circulation velocity decreases with the increase in consistency coefficient K. When K < 0.323, the liquid circulation velocity decreased particularly sharply with increased K. In this case, there is a pseudohomogeneous flow regime due to the low concentration of biomass and polysaccharide. The liquid circulation velocity depends largely on the difference in hydrostatic pressure in the central tube and the annular space. Increased viscosity causes an increase in whole circulation path flow resistance, particularly the frictional resistance between liquid and the reactor wall. Thus, the pressure drop due to friction increases and liquid velocity decreases. However, in the heterogeneous flow regime, liquid velocities are very low and decline smoothly to almost zero with increasing fluid viscosity. There are several possible explanations for this. Firstly, in this highly viscous liquid system, large bubbles tend to be produced in the bubble zone and the flow pattern is almost plug flow due to poor gas dispersion. Disengagement of these large bubbles from the liquid phase, which occurs on top of the central tube, leads to energy dissipation. Secondly, some energy loss is associated with viscous dissipation [18]. Thirdly, with higher broth viscosity, large numbers of small bubbles will be kept in the broth and entrapped in the annular space. Higher viscosity diminishes the bubble rise velocity and keeps the bubble residence time longer, which leads to a high gas hold-up in the annular space and a decrease in the difference in mean density between the central tube and the annular space. Because of the factors affecting the driving force, a reduced amount of energy is left to accelerate the broth flow. As a result, when K > 0.323, further increase in broth viscosity does not strongly influence the liquid recirculation velocity.

Figure 6 shows the effect of gas flow rate on the liquid circulation velocity in the annular space. As in Figure 6, the curves also show the same two markedly different regimes divided by a critical *K* value. When K < 0.323, in the pseudohomogeneous flow regime, liquid velocities increase markedly with the increase in superficial gas flow rate. Wang and McNeil [16] also observed, in a *S. glucanicum* process, that  $U_{1d}$  was much higher at lower biomass concentrations. In the present work, at the highest  $U_g$  with a value of 0.33 m/s and lowest *K* being 0.018 Pa s<sup>n</sup>, liquid velocity reached a maximum value of 0.15 m/s. Conversely, in the heterogeneous flow regime when K > 0.323, liquid circulation velocities are very low and not significantly affected by superficial gas velocity.

When K < 0.323, a higher air flow rate will produce a high gas hold-up in the central tube, which results in an increase in the density difference, thus increasing the driving force for liquid circulation; subsequently, liquid velocity in the annular space increases.

However, when K > 0.323 due to the frictional losses in the circuit and poor gas dispersion, as well as the rapidly escaping large gas bubbles, less energy is available to promote circulation between the annular space and the central tube. This may explain why  $U_{\rm g}$  has little impact upon the liquid velocity in annular space when  $K \ge 0.323$ . When K was at its highest value of 3.697, liquid velocity was close to zero and seemed completely unaffected by gas velocity. In such cases, the fluid in the annular space appeared to be largely stagnant. In addition, at such high viscosities, there was no visible flow in the annular space when gas velocity was low. In addition to the pronounced effect of frictional resistance in the annular space due to the high viscosity of the broth, another possible explanation for the annular flow stoppage may relate to the yield stress property of mycelial process fluids. Results obtained by Wang and McNeil [16] in 0.12 m<sup>3</sup> ALR-EL showed increasingly pseudoplastic behaviour and yield stress with increasing biomass concentration. Comparing  $U_{\rm ld}$  in this work with  $U_{\rm ld}$  obtained by Wang and McNeil [16], the former is lower than the latter though the  $U_{g}$  in the ALR-IL is higher than the  $U_{\rm g}$  in the ALR-EL. The main reasons for this are as follows. Firstly, the energy introduced by the air in the ALR-IL is partly dissipated by the collision among gas, liquid and the baffle in the gas bubble zone. Secondly, the consistency coefficient of the broth used in the ALR-IL is higher than that in the ALR-EL studies. Thirdly, the sectional cross area of the annular space in ALR-IL  $(0.027 \text{ m}^2)$  is also larger than that of the downcomer in the ALR-EL ( $0.018 \text{ m}^2$ ).

#### Mixing time in the ALR-IL

The mixing times are plotted in Figures 7 and 8 for the various airflow rates and different viscosities investigated. The figures show the variation in  $t_m$  as a function of superficial gas velocity,



Figure 7 The effect of superficial gas velocity on mixing time.

and of the viscosity of the broth. Mixing time varies according to the relationship:

$$t_{\rm m} = m U_{\rm g}^n \tag{13}$$

The values of m and n change with the consistency coefficient K. They can be expressed as:

$$m = 12.3668 K^{0.05931} \tag{14}$$

$$n = -0.9664 - 0.0791 \ln K \tag{15}$$

By combining Eq. (13) with Eqs. (14) and (15), an empirical correlation for  $t_m$  in terms of  $U_g$  and K is obtained, which can be described thus as:

$$t_{\rm m} = 12.3668 K^{0.05931} U_{\rm g}^{-0.9664 - 0.0791 \ln K} \tag{16}$$

The values of  $t_{\rm m}$  obtained from Eq. (16) are also shown in Figures 7 and 8. The mean standard deviation compared to measured values is 8.19%.

Figure 7 shows that mixing time decreases with the increased superficial gas velocity. Two different regimes of mixing can be discerned. Once again, the critical value of K, which divides these two regimes, is 0.323. Below this value, mixing times are short and decrease smoothly with increasing  $U_g$ , but when K is above 0.323,  $t_m$  values are lengthy and decrease significantly with increasing  $U_g$ .

At a given viscosity, the mixing process is strongly influenced by the superficial gas velocity. On one hand, the increase in superficial gas velocity increases the density difference between annular space and central tube, which increases the driving force for liquid circulation flow, and thus increases liquid circulation velocity [5,8]. Conversely, the increase in superficial gas velocity increases radial dispersion and back mixing, and the chaos in the bubble zone, enhancing mixing and shortening the mixing time.

The influence of increased superficial gas velocity is significant in the case of a relatively low consistency coefficient (Figure 6); thus, the mixing time is short. At high K, however, increased gas velocity has only a weak influence on circulation and thus on overall mixing time. When gas velocity increases, factors such as increasing turbulence, back mixing and other factors, especially mixing in the bubble zone, have a significant contribution to mixing in the reactor.

Figure 8 describes the effect of consistency coefficient K on mixing time. At any given gas velocity,  $t_{\rm m}$  increases with increased viscosity. There may be two main contributors to the increased  $t_{\rm m}$ under these circumstances. Firstly, the large amount of small bubbles carried in the annular space, together with the frictional resistance, leads to a decrease in the driving force for liquid circulation, and thus to decreased liquid circulation velocity. Meanwhile, the back mixing and turbulence in the gas bubble zone decrease correspondingly. The change in  $t_{\rm m}$  with gas velocity is pronounced at high viscosity (Figure 7). Thus, the mixing process in the ALR-IL is strongly affected by superficial gas velocity, liquid circulation velocity and the consistency coefficient. With the lowest viscosity and the highest superficial gas velocity, the shortest mixing time was obtained (18 s), while the longest mixing time was 516 s, corresponding to the highest K and the lowest superficial gas velocity (3.697 Pa  $s^n$  and 0.033 m/s, respectively).

Comparing these findings with the results obtained by others [7,8,13], it is clear that the liquid velocities in this high viscosity system are lower than those of Newtonian fluids or non-Newtonian fluids, which are weakly pseudoplastic. Comparing our experimental data with those obtained in simulated high-viscosity systems (aqueous CMC [carboxymethyl cellulose] solutions with no cells present) [10,11], it is apparent that the liquid circulation velocities of real fungal systems are significantly lower and mixing times correspondingly higher than in the simulated systems.

While this difference may be partly due to reactor types and scale, a very important factor is the difference in composition of the fluids, namely that the "real" fungal culture fluid is highly heterogeneous in nature, containing biomass in a range of morphologies with attached biopolymer and biopolymer in solution, whereas the simulated systems are homogeneous, simpler systems. Thus, data based on such model systems may not be a good guide to what the hydrodynamic and mixing behaviour of real fungal cultures will be in reactors.

The correlation equations obtained from our present work can be used to predict the effect of  $U_g$  and K on gas hold-up, liquid



Figure 8 The effect of K on mixing time.

Hydrodynamic characteristics X Kang et al

circulation velocity and mixing time in real filamentous fungal systems in ALR-IL. It can be applied to describe systems from largely Newtonian to highly non-Newtonian fluids. The models can aid in interpreting some of the phenomena observed and the underlying mechanisms. The equations obtained using data from the real fermentation systems give much more precise predictions of fermentation process than those obtained using data from Newtonian fluids or simulated high-viscosity systems. Meanwhile, the results can be used as valuable references for ALR-IL design. Further researches in to the behaviour of real bioprocess fluids with heterogeneous non-Newtonian character are clearly needed.

# Conclusions

Hydrodynamic and mixing characteristics were studied in an ALR-IL fermenter with a highly viscous filamentous fungal (S. glucanicum) culture fluid. The culture fluid containing high concentrations of biomass and scleroglucan exhibited strong non-Newtonian pseudoplastic behaviour. With a fixed reactor configuration,  $\varepsilon_{g}$ ,  $U_{ld}$  and  $t_{m}$  are strongly influenced by superficial gas velocity and the viscosity of the fluid. The descriptions of culture fluid behaviour developed are more accurate than those obtained from other correlations based on Newtonian and broth-simulated non-Newtonian fluids in ALR-IL.

# Nomenclature

- $A_{\rm d}$ downcomer cross-sectional area  $(m^2)$
- $A_{\rm r}$ riser cross-sectional area  $(m^2)$
- С tracer concentration  $(kg/m^3)$
- $C_{\infty}$ tracer concentration on perfect mixing (kg/m<sup>3</sup>)
- h degree of inhomogeneity (-)
- K fluid consistency coefficient (Pa  $s^n$ )
- L length (m)
- п flow behaviour index (-)
- time (s)  $t_{\rm a}$
- mixing time (s)
- $t_{\rm m}$  $U_{\rm g}$  $U_{\rm gr}$ superficial gas velocity in bubble zone (m/s)
- superficial gas velocity in riser (m/s)
- $U_{\rm ld}$ liquid circulation velocity in downcomer or annular space (m/s)
- $U_{
  m lr}$ liquid circulation velocity in riser (m/s)
- Vt aeration volume (m<sup>3</sup>)
- $V_1$ liquid volume  $(m^3)$
- $\varepsilon_{\,\rm g}$ total mean gas hold-up
- effective viscosity (Pa s)  $\eta_{\rm eff}$

#### References

- 1 Allen DG and CW Robinson. 1989. Hydrodynamics and mass transfer in Aspergillus niger fermentations in bubble column and loop bioreactors. Biotechnol Bioeng 34: 731-740.
- 2 Blenk H. 1985. Biochemical loop reactors. In: Rehm H-J and G Reed (Eds.), Biotechnology, Vol. 2. Weinheim, New York, pp. 465-517.
- 3 Cai Z, S Yang, R Kang, X Sun, W Liu, G Li and Y Wang. 1992. Studies on the performance of bioreactors used for the fermentation process with highly viscous broth. In: Furusaki S, I Endo and R Matsuno (Eds.), Proceedings of Asia-Pacific Biochemical Engineering Conference. Springer-Verlag, Tokyo, pp. 429-432.
- 4 Chisti Y. 1998. Pneumatically agitated bioreactors in industrial and environmental bioprocessing: Hydrodynamics, hydraulics, and transport phenomena. Appl Mech Rev 51 (1): 33-112.
- Fumitake Y. 1982. Aeration and mixing in fermentation. Ann Rep Ferment Process 5: 1-27.
- 6 Lehnet J. 1972. Berechung von mischvorgangen in schlanken schlaufenappreten. Dissertation. University of Stuttgart, Germany.
- 7 Lin CH. 1976. Oxygen transfer and mixing in a tower cycling fermenter. Biotechnol Bioeng 18: 1557-1572.
- 8 Merchuk JC and MH Siegel. 1988. Airlift reactors in chemical and biological technology. J Chem Technol Biotechnol 41: 105-120.
- 9 Misra TK and SM Barnett. 1987. Evaluation of a novel foam fermenter in the production of xanthan gum. In: Ho CS and JY Oldshue (Eds.), Biotechnology Processes: Scale-up and Mixing. American Institute of Chemical Engineering, New York, pp. 227-237.
- 10 Popovic M and CW Robinson. 1987. The specific interfacial area in external circulation loop airlifts and bubble column: II. Carboxymethyl cellulose/sulphite solution. Chem Eng Sci 42 (12): 2825-2832
- 11 Popovic M and CW Robinson. 1988. External circulation loop airlife bioreactors: study of the liquid circulation velocity in highly viscous non-Newtonian liquids. Biotechnol Bioeng 32: 301-312.
- 12 Popovic MK and CW Robinson. 1993. Mixing characteristics of external loop airlift: non-Newtonian systems. Chem Eng Sci 48 (8): 1405-1413.
- 13 Rau U, E Gura, E Olszewski and F Wagner. 1992. Enhanced glucan formation of filamentous fungi by effective mixing, oxygen limitation and fed-batch processing. Ind Microbiol 9: 19-26.
- 14 Schugerl K. 1990. Comparison of the performance of stirred tank and air-lift tower loop reactors. J Biotechnol 13: 251-256.
- 15 Scragg AH. 1991. Bioreactors in Biotechnology A Practical Approach. Ellis Horwood, New York, pp. 112-125.
- 16 Wang Y and B McNeil. 1992. A study of gas hold-up, liquid velocity and mixing time in a complex high viscosity fermentation fluid in an air-lift bioreactor. Chem Eng Technol 19 (2): 143-153.
- 17 Wang Y and B McNeil. 1995. pH effects on exopolysaccharide and oxalic production in cultures of Sclerotium glucanicum. Enzyme Microb Technol 17: 124-130.
- 18 Wang Y and B McNeil. 1996. A study of gas hold up, liquid velocity and mixing time in a complex high viscosity fermentation fluid in an airlift reactor. Chem Eng Technol 19: 143-153.

(ÎI)

214