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Interactions of cell morphology and transport processes in the lovastatin fermentation

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Abstract. The cholesterol lowering drug, Lovastatin (Mevacor), acts as an inhibitor of HMGCoA reductase, and is produced from an Aspergillus terreus fermentation.

Pilot scale studies were carried out in 800 liter fermenters to determine the effects of cell morphology on the oxygen transport properties of this fermentation. Specifically, parallel fermentations giving (*i*) filamentous mycelial cells, and (*ii*) discrete mycelial pellets, were quantitatively characterized in terms of broth viscosity, availability of dissolved oxygen, oxygen uptake rates and the oxygen transfer coefficient under identical operating conditions.

The growth phase of the fermentation, was operated using a cascade control strategy which automatically changed the agitation speed with the goal of maintaining dissolved oxygen at 50% saturation. Subsequently stepwise changes were made in agitation speed and aeration rate to evaluate the response of the mass transfer parameters (*DO*, *OUR*, and k_La). The results of these experiments indicate considerable potential advantages to the pellet morphology from the standpoint of oxygen transport processes.

List of symbols

DO	% sat.	Dissolved oxygen concentration
k_La	h^{-1}	Gas-liquid mass transfer coefficient
OUR	$mmol/dm^3 h$	Oxygen uptake rate
P/V	KW/m^3	Agitator power per unit volume
V_s	m/s	Superficial air velocity
μ_{app}	cP	Apparent viscosity

1 Introduction

Lovastatin (Mevacor) is a potent cholesterol-lowering agent biosynthesized as a secondary metabolite by the fungal organism Aspergillus terreus [1, 2]. The drug acts as an inhibitor of HMGCoA (hydroxymethylglutarylCoA) reductase, a key enzyme in the cholesterol biosynthetic pathway. The chemical structure of the lovastatin compound is shown in Fig. 1.

In submerged culture fermentations, Aspergillus terreus can grow into a variety of morphological forms, varying between an open filamentous network structure to closely packed, discrete pellets. This diverse morphological growth pattern is typical of fungal and actinomyces organisms in liquid fermentations, presenting a number of engineering problems in these processes. Principally, the filamentous network structure results into high non-Newtonian viscosities in the broth, which impede gas-liquid mass transport processes in the fermentation. The limitation in dissolved oxygen supply may well determine cell growth and the overall productivity of the process. On the other hand, the discrete pellet morphology is characterized by relatively lower viscosities in the liquid phase and better gas-liquid mass transfer properties. However, under some circumstances, intrapellet diffusion could become limiting to the cells in the core of the pellet, again impairing the overall productivity of the process. From this standpoint the challenge in most mycelial fermentation processes is to choose the cell morphology which best facilitates oxygen transfer to the cells.

The influence of the morphological diversity of mycelial organisms in fermentation processes has been extensively reviewed in the literature [3–5]. Martin and Waters [6] described the use of Aspergillus niger pellets in the citric acid fermentation. Konig et al. [7] evaluated the comparative performance of pellets and filamentous mycelial cells for penicillin production in airlift columns. Takahashi et al. [8] reported significant differences in viscosities between pellet and filamentous mycelial broth of Penicillium chrysogenum. These observations were confirmed by Chain et al. [9].

The adverse effects of high non-Newtonian viscosities associated with filamentous mycelial cells on gas-liquid mass transfer properties (k_La), through the broth have been noted by several researchers including Deindoerfer and Gaden [10]. It was also noted that these adverse effects on k_La were

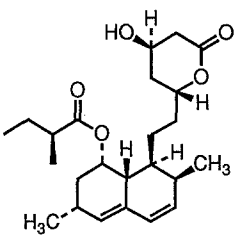

Fig. 1. Chemical structure of Lovastatin (lactone form)

diminished when the cells assume the pellet morphology [9]. This effect was demonstrated by Gbewonyo and Wang [11] using P. chrysogenum cells confined to celite beads as discrete growth particles, resulting in a two-fold improvement in $k_L a$ over the filamentous mycelial fermentations. The problem of intrapellet mass transfer limitations in mycelial pellets has been extensively studied [12, 13]. The influence of pellet size and density on the severity of intrapellet diffusion effects were noted by various researchers [14, 15].

This study characterizes the influence of cell morphology on the oxygen transfer properties of the lovastatin fermentation, specifically, to define the interactions between morphology, broth viscosities, oxygen transfer rates, and availability of dissolved oxygen under operating conditions imposed by agitation and aeration.

2 Materials and methods

A mutant culture of Aspergillus terreus was used in these studies. By careful manipulation of the inoculum and seed conditions it was possible to direct the growth of the cells into either the filamentous or the pellet morphological forms.

The fermentations were carried out at the pilot plant scale in 200 gallon fermenters, with a working volume of 500 liters. The fermenters were fitted with two Rushton flat-bladed impellers of size one-third of the tank diameter. A 7.5 horsepower motor coupled with a variable speed drive provides agitation. The agitation during the growth period of the fermentations was operated through a cascade control loop interactive with the dissolved oxygen control loop as described schematically in Fig. 2. At a later stage of the fermentation (50–75 hours), the agitation speed was independently set at specified levels for short period of time (3 hours), to determine the real time response of the mass transfer parameters. Then the agitation speed was held constant while aeration rates were changed at specified levels to determine the response in the mass transfer parameters. Other operating conditions, pressure and temperature, were held constant during these experiments.

Online mass spectrometric analysis of fermenter exhaust gases enabled the calculation of oxygen uptake rates and mass transfer coefficients by material balancing techniques as described previously [16]. Viscosities were measured on a Brookfield rotating bob viscometer. The torque generated in 400 ml of broth contained in a beaker was measured with the LVT spindle 2, at the rotation speed of 30 rpm, equivalent to a shear rate of 0.17 sec^{-1}.

Cell samples were fixed in glutaraldehyde for scanning electron microscopy (Microscopy Labs, Redbank, N.J.).

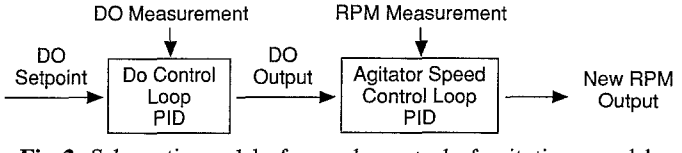

Fig. 2. Schematic model of cascade control of agitation speed by dissolved oxygen concentration DO

3 Results

3.1 *Morphological characteristics and viscosity*

Parallel fermentations were setup with the filamentous and the pellet morphological characteristics as shown in the scanning electron micrographs in Figs. 3 and 4. The pellets comprised closely packed hyphal filaments ranging in size 100–300 microns. Besides scanning electron microscopy, we have not attempted to characterize the morphological differences any further, due to the paucity of suitable quantitative methods for characterizing mycelial morphology. Nonetheless, distinct differences in viscosity were associated with the morphological variations in the fermentations. The apparent viscosity profiles shown in Fig. 5 demonstrate that the filamentous mycelial fermentation increased well over 700 cP compared to peak viscosities around 300 cP for the pellet fermentations.

3.2 *Cascade control regime*

During the growth phase (0–50 hours), the fermentations were run with cascade control of the agitation speed to maintain dissolved oxygen concentration at a setpoint of 50% saturation. This was intended to maximize cell growth during this period without constraints of dissolved oxygen supply. Both fermentations showed identical oxygen uptake rate profiles (Fig. 6) during this period with a peak of 20 m$M/l \cdot h$, though the pellet fermentation showed a slightly

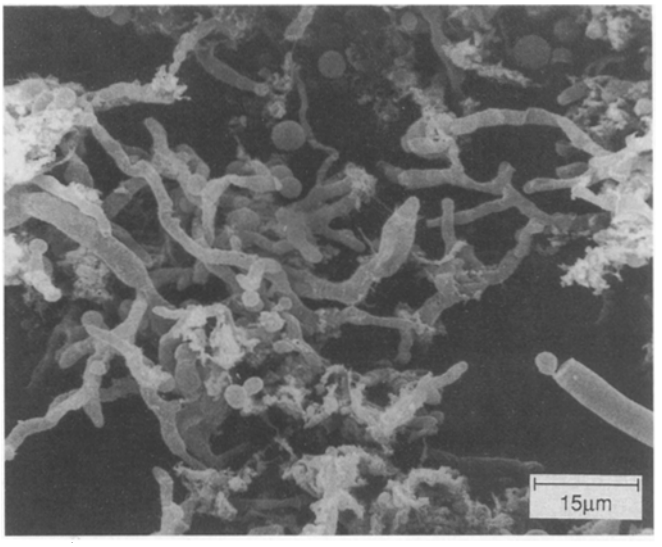

Fig. 3. Scanning electron micrograph of filamentous mycelial cells of Aspergillus terreus

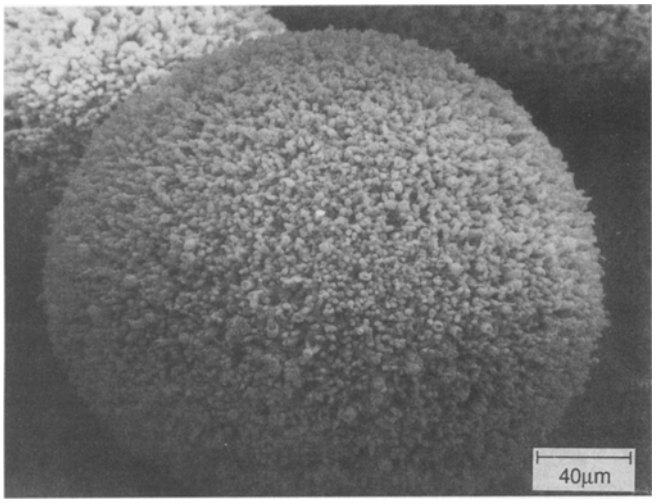

Fig. 4. Scanning electron micrograph of pellet of Aspergillus terreus

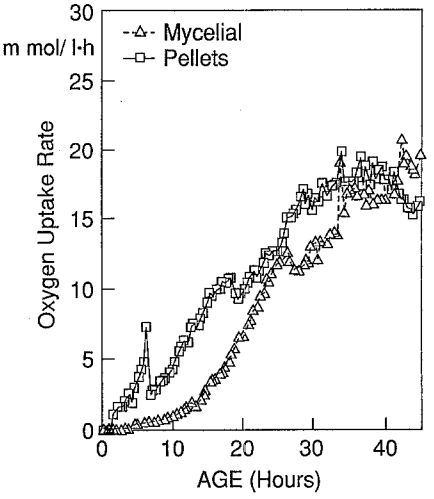

Fig. 6. Oxygen uptake profiles of fermentations during growth phase (0–45 hours)

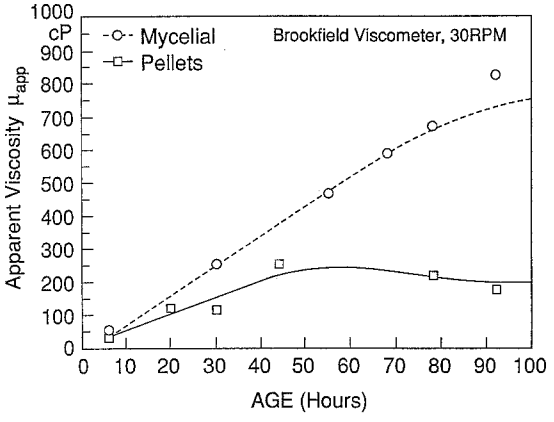

Fig. 5. Viscosity profiles of mycelial and pellet fermentations

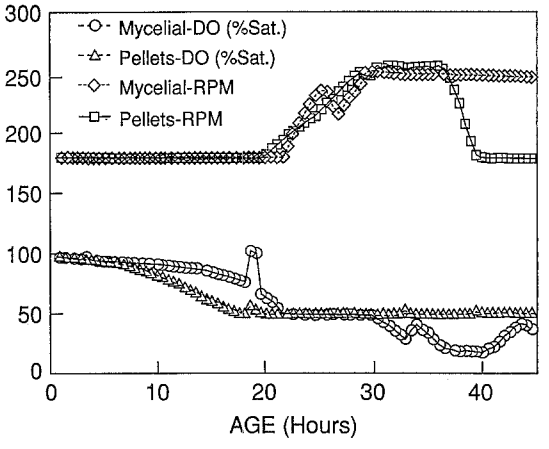

Fig. 7. Agitation speed (RPM) and dissolved oxygen concentration (% saturation) profiles of fermentations during growth phase, (0–45 hours)

faster initial rate of oxygen uptake possibly due to differences in the state of the inoculum.

The identical peak oxygen uptake rates obtained in the fermentations indicate that the biomass concentrations attained during the growth period are similar for both the pellet and mycelial fermentations. It was not possible to measure cell concentrations directly due to the presence of insoluble medium ingredients.

The agitation profiles for the pellet fermentation (Fig. 7) reached the maximum permissible speed at 300 rpm between 10–20 hrs, then the agitation speed decreased gradually afterwards to maintain the dissolved oxygen level at the set point value of 50% saturation. The agitation profile obtained in the mycelial fermentation showed a remarkably different pattern, requiring a more prolonged period of maximum speed (300 rpm), from 15 hours till 48 hours when cascade control was terminated. Even under these maximum agitation conditions, it was not possible to keep the dissolved oxygen concentration above the setpoint value. The dissolved oxygen concentration went down as low as 25% saturation.

3.3 *Response to changes in agitation speed*

After 48 hours of the fermentation, cascade control of agitation speed was discontinued and agitation speed was kept at specified levels to determine the response in dissolved oxygen concentration, oxygen uptake rates and mass transfer coefficients. The fermentations were allowed to stabilize for 3 hours at each agitation speed in order to get reliable response data. As shown in Fig. 8 the mycelial fermentation remained oxygen limited, with practically zero dissolved oxygen concentration, through the entire range of RPM changes (100–250 RPM). In contrast the dissolved oxygen levels in the pellet fermentation responded nicely to the RPM changes, varying between 60% saturation at high

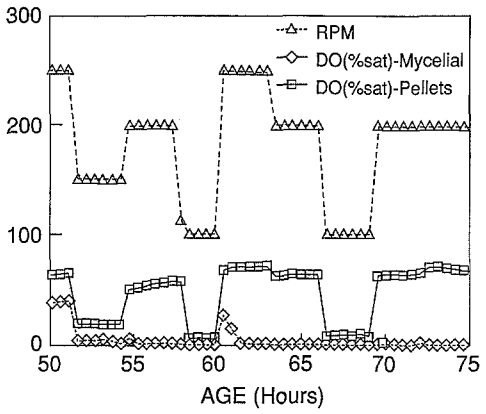

Fig. 8. Response of dissolved oxygen concentration (% saturation) to agitation speed (RPM) changes in fermentations, (50–75 hours)

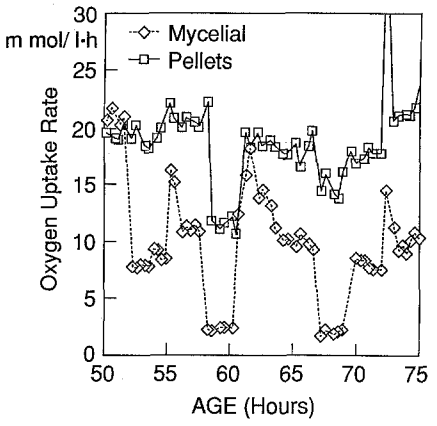

Fig. 9. Response of oxygen uptake rates to changes in agitation speed (RPM) of fermentations, (50–75 hours)

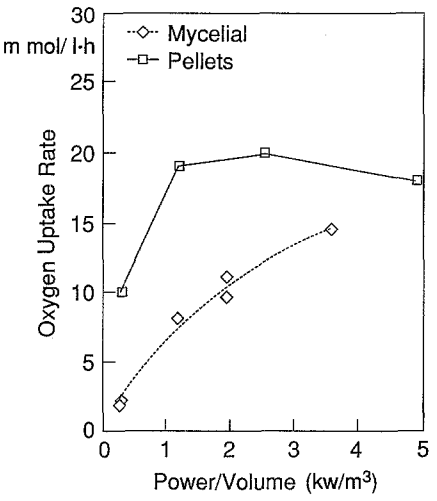

Fig. 10. Analysis of effect of power input on oxygen uptake rates in fermentations (50–75 hours)

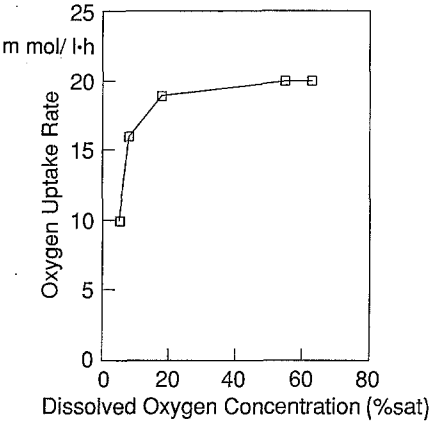

Fig. 11. Kinetics of oxygen uptake rates with respect of dissolved oxygen concentrations in fermentations, (50–75 hours)

RPMs to about 5% saturation at the low RPM. It is interesting to note that the dissolved oxygen responses obtained were reproducible as RPM was returned to the same level during its cycle of changes.

The response in the rates of oxygen uptake by the cells to the changes in RPM during this period are shown in Fig. 9. The oxygen uptake rates in the pellet fermentation were insensitive to the changes in RPM except for a noticeable decrease from 20 mM/l·h to 10 mM/l·h at 100 RPM.

The oxygen uptakes rates in the mycelial fermentations were more sensitive to RPM changes varying between 15 mM/l·h at 250 RPM to below 5 mM/l·h at 100 RPM. Again the physiological response was reproducible through the cycle of RPM changes, indicating that the cells recovered from short periods of exposure to low oxygen tension.

The data is presented in Fig. 10 to show the difference in response of oxygen uptake rates in the two fermentations with varying agitation power inputs. The mycelial fermentation shows a steady increase in oxygen uptake rates with power input, symptomatic of the dissolved oxygen-limited conditions in the broth. The pellet fermentation maintained significantly higher oxygen uptake rates with respect to power input except at the low power regime (below 1 kW/1000 l) when the oxygen uptake rates showed a noticeable decrease.

This effect is more clearly illustrated when the oxygen uptake rates for the pellet fermentation are plotted with respect to the dissolved oxygen concentrations as shown in Fig. 11. A saturation kinetics relationship is obtained with decreasing oxygen uptake rates below 20% (sat.) dissolved oxygen concentration, but beyond this value the oxygen uptake rate is insensitive to any further increases in dissolved oxygen, power input or RPM changes.

Below the critical dissolved oxygen concentration of 20% saturation the oxygen uptake rates become impaired as imperfect mixing conditions arise due to the low power input. As such the local dissolved oxygen concentrations the cells experience may actually be lower than measured by the dissolved oxygen probe, these effects are exacerbated in the viscous conditions in the mycelial fermentation [17]. Since the dissolved oxygen concentration remained consistently at zero in the mycelial fermentation despite agitation speed

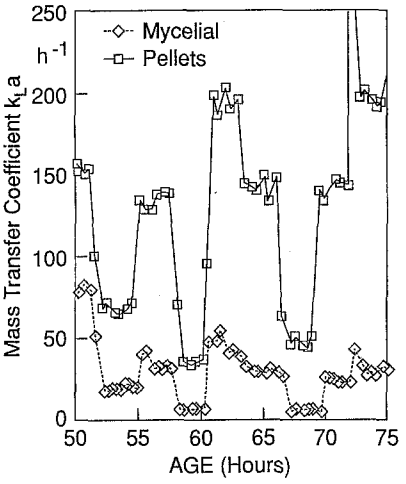

Fig.12. Response of overall mass transform coefficient, k_La, to agitation speed (RPM) changes in fermentations, (50–75 hours)

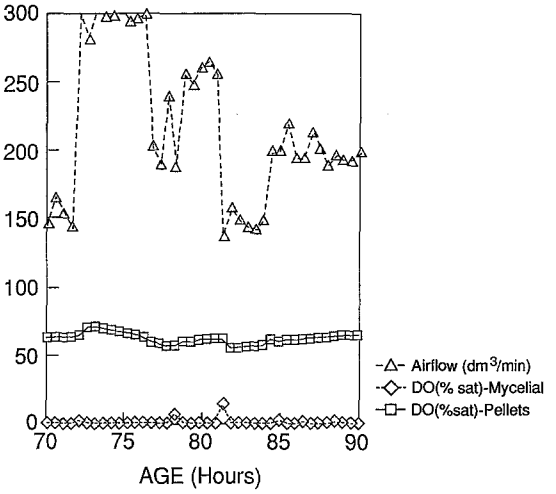

Fig. 14. Response of dissolved oxygen concentration to changes in airflow rate in fermentations, (70–90 hours)

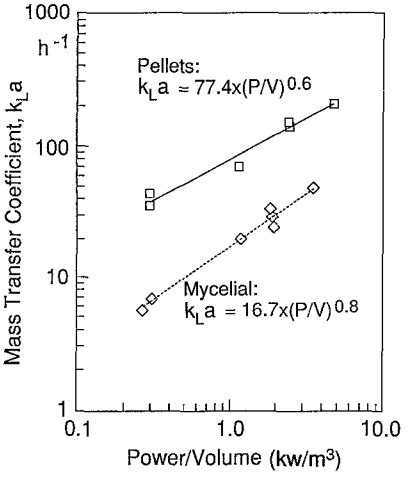

Fig. 13. Analysis of effect of power input on mass transfer coefficient, k_La, obtained in fermentations, (50–75 hours)

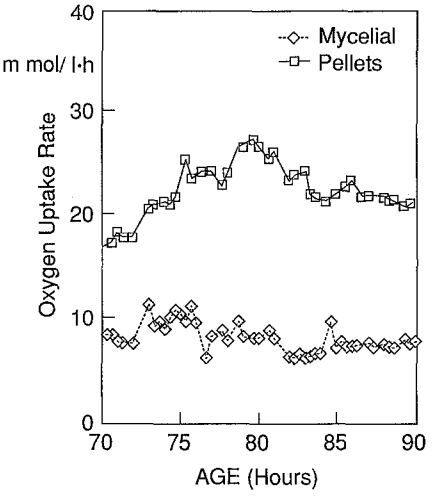

Fig. 15. Response of oxygen uptake rates to changes in airflow rate in fermentations, (70–90 hours)

changes, it is not possible to obtain a comparative kinetic plot of oxygen uptake rate and dissolved oxygen.

The overall mass transfer coefficients, k_La, determined in the pellet fermentation broth was consistently higher than the values determined in the mycelial fermentation broth at any given RPM. As shown in Fig. 12, the k_La exhibited a predictable response to changes in RPM in both fermentations, increasing at high RPMs and decreasing as RPM is reduced. Fig. 13 shows correlations of the k_La to agitator power input obtained by changing RPM. The correlations indicate a significant advantage in the magnitude of k_La for the pellet fermentation over the mycelial fermentations. For instance, at a power input of 3 kW/1000 l, the oxygen transfer coefficient in the pellet fermentation broth is about three times higher than in the mycelial fermentation broth.

3.4 *Effect of changes in aeration rates*

Between 70–90 hours the agitation speed was maintained constant at 200 RPM, while changes were made in the airflow rates as shown in Fig. 14. In the mycelial fermentation, the dissolved oxygen concentration remained practically at zero even at high airflow rates (300 l/min). The pellet fermentation maintained levels of dissolved oxygen about 60% saturation, without any significant response to the changes in the airflow rate.

The effects of aeration changes on oxygen uptake rates were also not as pronounced as the responses to agitation speed changes (Fig. 15). Nevertheless, a noticeable drift in oxygen uptake rates can be seen as aeration was increased to 300 l/min, with the pellet fermentation rising from

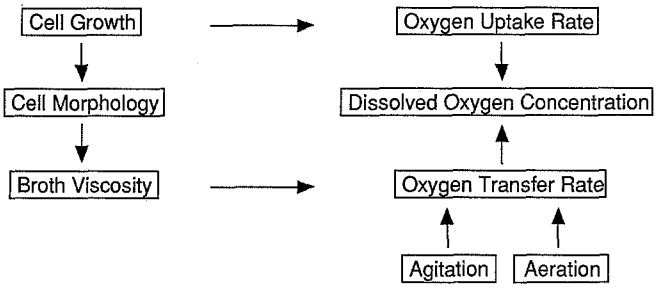

Fig. 16. Schematic model of dynamics of interactive factors controlling oxygen transfer in fermentations

20 mM/l·h to a peak of 25 mM/l·h and decreasing back to 20 mM/l·h as airflow is decreased. Similarly, the oxygen uptake rate in the mycelial fermentation drifted up from 8 mM/l·h to 10 mM/l·h in response to the increased airflow.

4 Discussion

The dynamic interplay of the biological and engineering factors in the fermentation which account for the results reported in these experiments is outlined in the schematic model in Fig. 16. Cell growth is generally accompanied by increases in oxygen demand which is met by the manipulation of agitation and aeration to promote gas-liquid mass transfer rates to the fermentation. However our results demonstrate that the cell morphology has a profound effect on the ability to promote mass transfer to the fermentation. In the filamentous mycelial growth form the effectiveness of agitation and aeration to promote oxygen transfer is considerably diminished leading to dissolved oxygen limitations. In contrast when cell growth is committed to the pellet morphology, much lower broth viscosities were obtained and accordingly, significant improvements in oxygen transfer rates were noted under identical agitation and aeration conditions.

Our results underscore the predominant influence of mycelial morphology and broth viscosity on the effectiveness of power utilization for mass transfer. The quantitative correlations obtained collaborate the trends reported in earlier studies on the impact of mycelial broth viscosities on the empirical relationships between agitator power input and gas-liquid mass transfer coefficient. Taguchi et al. [18] in a classical study of mycelial fermentations pointed out significant differences in the mass transfer correlations observed in viscous non-Newtonian fermentations from those reported for Newtonian systems:

$k_L a = K(P/V)^{0.33}(V_s)^{0.56}$, Endomyces mycelial broth, Taguchi [18]

$k_L a = K(P/V)^{0.95} V_s^{0.67}$. Air-water Newtonian system, Cooper et al. [19]

Here the dampening effect of viscosity on power utilization for mass transfer is denoted by the decrease in the exponent on the power input term.

Consequently, Ryu and Humphrey [20] proposed inclusion of an apparent viscosity term in the correlation to highlight the effects observed in non-Newtonian mycelial fermentation broth. Using data from the penicillin fermentation they demonstrated a logarithmic decrease in $k_L a$ with apparent viscosity as shown below:

$$k_L a \propto (P/V)^{\alpha} (V_s)^{\beta} (\mu_{app})^{-\omega}$$

It should be noted that the actual values for the parameters in the correlations obtained by various investigators using this framework of analysis tend to vary considerably due to differences in the experimental system employed i.e. scale of operation, impeller and tank geometry, fermentation broth characteristics and type (or strain) of micro-organism used.

In our system, the influence of viscosity differences between pellet and filamentous mycelial fermentations is manifested by as much as 80% decrease in the value of the correlation constant between $k_L a$ and power input.

5 Conclusions

With the benefit of online gas analysis we have extensively characterized the differences in gas-liquid oxygen transfer properties between pellet and filamentous mycelial fermentations. Our results demonstrate the need for a wide range of flexibility in the design and operating regimes of the mass transfer capabilities of bioreactors in order to handle the morphological diversity of mycelial fermentations. Thus morphological considerations should play a central role in the scale-up of these processes. From a practical standpoint, it would be prudent to minimize the morphological variations through process development efforts, so as to define a more realistic range of oxygen transfer requirements for the scale-up of these processes.

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