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Characterization and improvement of oxygen transfer in pilot plant external air-lift bioreactor for mycelial biomass production

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Summary

The oxygen transfer dynamics in a pilot plant external air-lift bioreactor (EALB) during the cultivation of mycelial biomass were characterized with respect to hydrodynamic parameters of gas holdup (ε), oxygen transfer coefficient (K_La) and superficial gas velocity (U_g), and dissolved oxygen (DO). An increased flow rate of air supply was required to meet the increased oxygen demand with mycelial biomass growth. Consequently, an increase in air flow rate led to an increase in ε, K_La and the DO level. The enhancement of oxygen transfer rate in the cultivated broth system, however, was limited with highly increased viscosity of the mycelial broth. An increase in air flow rate from 1.25 to 2.00 v/v/m resulted in a low increment of oxygen transfer. The newly designed pilot plant EALB with two air spargers significantly improved processing reliability, aeration rate and K_La. The pilot plant EALB process, operated under a top pressure from 0 to 1.0 bars, also demonstrated a significant improvement of oxygenation efficiency by more than 20% in DO and K_La. The performance of the two sparger EALB process under top pressure demonstrated an efficient and economical aerobic system with fast mycelial growth and high biomass productivity in mycelial biomass production and wastewater treatment.

Introduction

Aerobic cultivation and wastewater treatment processes require systems which maximize the rate of oxygen transfer while minimizing the power requirement. The cultivation of mycelial microorganisms is an exothermic multiphase reaction. The mass transfer, in particular, the oxygen transfer between the culture medium and the microorganisms are important variables in the design of bioreactor (Liefke *et al.* 1990; Russell *et al.* 1994; Merchuk *et al.* 1996). The high demand for oxygen with carbohydrate substrates and the rising cost of energy and capital investment made it apparent that economical bioreactor design could have a significant impact on the cost of the process. When considering the design of a bioreactor, one of the most important requirements is to provide an adequate supply of nutrients and oxygen to the cells. To maintain microorganism growth, the total oxygen transfer rate must exceed or at least match the total oxygen consumption rate by the cells under equilibrium conditions.

The oxygen transfer rate is a function of the volumetric mass transfer coefficient, which mainly depends on the specific interfacial area of the gas bubbles. The driving force of the oxygen transfer depends on the gas, solids and liquid recirculation rates (Bugarski *et al.* 1989; Merchuk *et al.* 1994). To improve the oxygen transfer efficiency and maintain a sufficient oxygen level in the cultivation broth is extremely critical for industrial biotechnological and wastewater treatment processes, particularly if the process is conducted in a cultivated mycelium broth with highly viscous pseudoplastic suspensions. Evaluation of the local variation of the hydrodynamic parameters leads to a better understanding of these processes and improvement of reactor performance.

In recent decades, air-lift reactors (ALRs) have been received increased attention in chemical and biotechnological industries. ALRs exhibit advantages of simple construction and low energy consumption associated with high mass, momentum and heat transfer rate (Paca *et al.* 1976; Kiese *et al.* 1980; Onken & Weiland 1983; Chisti *et al.* 1986; Merchuk & Siegel 1988). The external air-lift reactor (EALR) has been recognized as a suitable bioreactor for large scale aerobic cultivation and wastewater treatment processes, particularly for processing a mycelial cultivation broth with high viscosity, and high mass and heat transfer rates required (McManamey *et al.* 1984; Weiland 1984; Gluszcz & Michalski 1994;

Jin *et al*. 1999). There have been extensive experimental studies attempting to characterize the hydrodynamics of the gas flow rate, velocity and holdup, liquid velocity, and reactor geometry for air/water systems with varying liquid properties (Barker *et al*. 1981; Jones 1984; Kawase & Moo-Young 1986; Chisti & Moo-Young 1987). However, there are very few studies dealing with the behaviour of highly viscous gas, solid and liquid systems in flow processes (Malfait *et al*. 1981; Jin *et al*. 1999). On the other hand, researchers who have attempted to quantify the significance of rheology in bioreactor design have used model media of homogeneous polymer solutions, e.g. carboxymethy cellulose (CMC), to simulate the flow behaviour of a heterogeneous cultivation broth. There is considerable question as to whether the results obtained with such fluids can be translated to an actual cultivation system. It is well known that many industrially significant bioprocesses involve rheologically complex cultivation broths (pseudoplastic suspensions). In addition, most previous investigations were conducted in laboratory scale ALRs.

More work is clearly needed on the hydrodynamics and mass transfer aspects of pilot plant ALRs with actual non-Newtonian fluids, such as mycelial cultivation broth. The aim of the present work was to characterize the oxygen transfer and improve oxygenation efficiency in an external air-lift bioreactor (EALB) of a pilot plant process. The pilot plant process was operated for mycelial biomass protein (MBP) production and wastewater treatment from starch-processing wastewater (SPW).

Materials and Methods

Mycelium strain

Fungus *Rhizopus oligosporus* DAR 2458 obtained from Division of Food Science & Technology, CSIRO in Sydney, Australia was used in this investigation and maintained on potato dextrose agar (PDA) slants at 4 °C and recultured bimonthly.

Media and inoculum

The seed culture medium contained: soluble starch, 10 g; polypeptone, 5 g; yeast extract, 5 g; K_2HPO_4, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.2 g in a litre of tap water and was autoclaved at 121 °C for 20 min. Phialospore suspensions were prepared from PDA slants on Petri dishes. The slants were incubated at 28 °C for 4 days. Spores were harvested from the surface of each slant into 10 ml of sterile water. This suspension, containing approximately 1×10^7–1×10^8 spores per ml determined by haemocytometer counts, was used as inoculum. Better results were obtained when the inoculum was prepared 1 day prior to use.

The SPW was used as the cultivation medium in the process of MBP production and SPW treatment process, and derived from the commercial production of starch and gluten from wheat and corn in Weston Bioproducts, Melbourne, Australia. As the production medium, SPW contained an organic loading of 16–22 g/l COD, 3.2–4.7 g/l suspended solids and 2.1–3.1 g starch/l at pH 5.22–5.88 and temperature around 38 °C.

Liquid properties

The fluids used for the measurement of hydrodynamic parameters in this investigation were mycelial culture broths during the course of MBP production and wastewater treatment. These fluids contained suspended mycelial particles and showed non-Newtonian flow behaviour. Table 1 shows the typical properties of the liquids at an operating temperature of 25 °C.

External air-lift bioreactor

The EALB with nominal working volume of 160 litres consisted of three major sections: riser, downcomer and gas separator (Figure 1). All sections were made of stainless steel. The gas separator had a larger diameter of 0.50 m in 0.60 m length than the rest of the riser column, which was 0.30 m in diameter and 2.5 m length. The column housed the inlet, outlet ports and electrode probes, and connected with the downcomer. The wall of the riser and downcomer was 4 mm thick. The conical bottom was to keep the particles in suspension and minimize the occurrence of dead spaces where biomass and substrate particles may become trapped. A round porous stainless steel sparger of 0.20 m diameter was located centrally in the conical bottom. The downcomer could be connected to the riser with a flexible flange connector and contained the cross-flow microscreen and an extra air distributor – a round stainless steel air sparger, fixed in the bottom of the downcomer. The cross-flow microscreen was constructed with stainless steel mesh (100 μm pore size) in the middle part of the downcomer as a cylinder. The microscreen cylinder (500 mm length) was covered with a stainless steel tube jacket. The sectional area ratio of downcomer to riser (A_d/A_r) was 0.48. There were eight view windows set up on the top, gas separator, riser and downcomer to monitor the bioprocess activities. A water jacket was built outside the riser for temperature control.

Gas holdup

The measurement of gas holdup expressed in this study was an average or overall holdup. The volume expan-

Table 1. Properties of cultivation broth.

Biomass concentration (g/l)	Density (kg m^{-3})	Viscosity (Pa s)	Surface tension (N m^{-1})
2.0	1180	6340	66,860
4.0	1470	12,400	46,400
8.0	1680	18,600	38,200

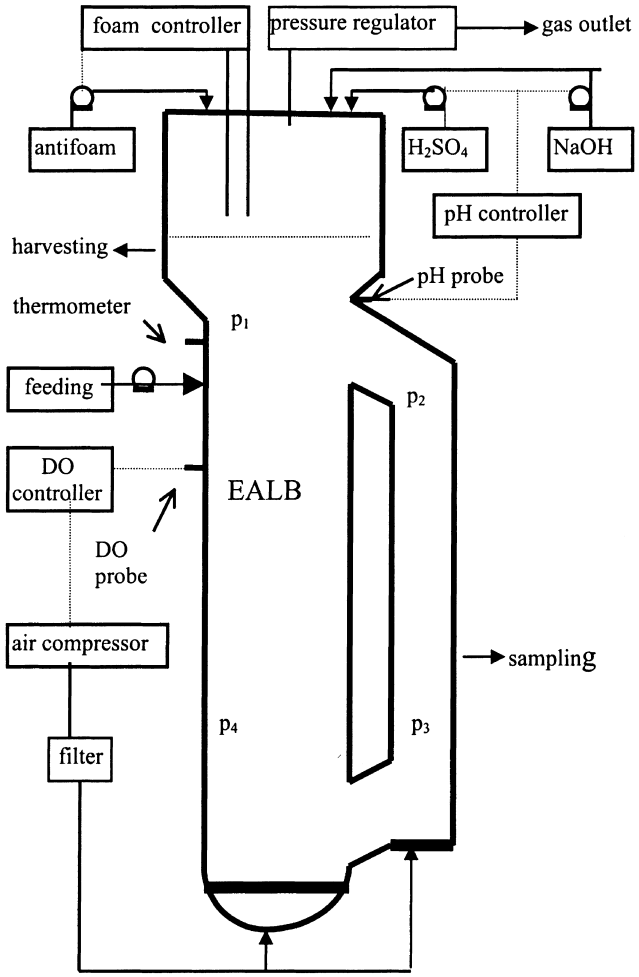

Figure 1. Schematic diagrams of the experimental set up (p_1, p_2, p_3 and p_4 – positions of pH probes for testing pulse tracer).

sion method was used. The gas holdup was estimated as the percentage increase in volume of the gassed liquid compared with ungassed liquid volume. In this specific bioreactor the variation of liquid volume can be determined by observing the height of the surface of the ungassed liquid and aerated liquid. The dispersion height was estimated by observing the position of the liquid level on a graduated stainless-steel rod suspended from the vessel top plate. At high air flow rate the liquid surface become very turbulent with the level changing erratically. In this case, a mean dispersion height was estimated.

Oxygen transfer coefficient

The volumetric oxygen transfer coefficient (K_La) is the rate of oxygen transfer across the gas–liquid interface per unit volume of the suspension and per unit of driving force. Equation (1) was used to calculate K_La for cultivation of filamentous fungi with a non-Newtonian fluid (Malfait *et al.* 1981).

$$K_La = \frac{G(y_1 - y_2)}{V\left(\frac{P_T y_1}{H} - DO\right)}, \quad (1)$$

where G is the molar air flow rate (mole/h), y_1 and y_2 the oxygen content of inlet and exit air (mole %), V the liquid volume in vessel (l), P_T the total pressure in system (atm), DO the dissolved oxygen level in liquid (mole/l) measured at top of the riser, H the Henry's law constant $= 8.345 \times 10^2$ litre·atm/mole.

Liquid, mixing time and gas velocity

The liquid velocity and mixing time were determined using a pulse tracer technique (Kennard *et al.* 1991; Russell *et al.* 1994). The tracer was a 1.0 ml injection of 4 M NaOH. The pH value was measured at four points within the vessel according to the positions indicated in Figure 1. The signal from each probe was recorded by a multichannel data logger and processed via a microcomputer. The mixing time was defined as the time for the pH response to initial peak. From the tracer outputs, the liquid velocity and circulation time could be determined. The mixing time was also determined as the time required to achieve a specific inhomogeneity (i.e. 5%) after the trace pulse had been injected. The degree of inhomogeneity (I), after the injection of a tracer pulse, was calculated from the relative deviation of the actual maximum pH (C) from the mean pH at the state of complete mixing pH (C_m).

$$I = \frac{C - C_m}{C_m}. \quad (2)$$

The characteristic mixing time (t_m) is defined as the time required to achieve an inhomogeneity (I) of 5% (Weiland 1984).

The gas velocity was determined in the riser and downcomer by noting the time taken by a visible fine bubble to traverse a marked distance on the column.

Viscosity and surface tension

Viscosity was measured with a Rheomat 15 T viscometer (Contraves AG, Switzerland). Surface tension was measured using a torsion balance (White Electrical Instrument Co. Ltd, UK).

Bubble size measurement

Bubble size distribution was determined using photographic techniques. A standard Pentax camera with close-up lens was focused on a small portion of fluid located half way up the riser. The fluid was illuminated at right angles to the camera. The photographs were analysed by an image analyser (Bild Analyzer, Kontron, Germany), and the Sauter mean diameter (D_b) was determined to describe the average diameter of the bubbles.

Mycelial biomass concentration and productivity

Mycelial biomass was harvested by filtration of 100 ml cultivation medium through a stainless steel mesh with a

pore size of 100 μm, washed twice with deionized water, followed by drying to constant weight at 105 °C for 24 h and measuring biomass weight. Biomass concentration (C_b) was expressed in grams of dry biomass per litre of culture medium, and biomass productivity was determined in grams of dry biomass per litre of medium per hour.

Cultivation system and conditions

The microbial production system comprised four main sections: microbial cultivation, controlling, monitoring and supply (Figure 1). Microbial cultivation was carried out in the EALB. The process controls encompassed pH, temperature, dissolved oxygen (DO), top pressure and foam, which were automatically controlled at predetermined levels. The growth pH was monitored and controlled by using a pH process controller (Mettler Toledo 405-50-SC-pH 7, Switzerland) activating pumps for the addition of 1 M H_2SO_4 or 4 M NaOH. The desired DO level was adjusted by using a Dissolved Oxygen Module (Mettler Toledo Inpro 6000, Switzerland) to control the air flow rate. A self-designed foam controller was manufactured in the Electronic Workshop, Weston Bioproducts, Melbourne and used to control the foam produced by adding antifoam. The operating temperature was controlled through a water bath. An adjustable pressure control unit was mounted on the top of the bioreactor. The EALB could be processed under a top pressure of 3.0 bars. The level of the cultivated medium was controlled by an electronic switch to run a butterfly valve for a continuous process. The pH, DO, temperature, and air flow rate were continuously monitored on line. Samples were taken at required intervals through sampling ports. The supply system consisted of air compressor, pumps, liquid and gas transfer pipes, liquids of antifoam, acid and alkali, and storage vessels of the medium, liquids and harvested product.

Batch cultivation process was carried out in the pilot plant EALB. The cultivation medium was inoculated with 8% (v/v) of spore suspension. Aeration rate was regulated at air flow rate of 1.0 v/v/m, unless otherwise as stated. The cultivation process was conducted at 35 °C and a growth pH of 5.0 ± 0.05.

Results and Discussion

Influence of rheological properties on hydrodynamics

Figure 2 shows that the variation of mycelial biomass concentration, i.e. broth density, follows a logarithmic growth phase. During the course of the cultivation of mycelial biomass, there was a notable variation in the visually observed bubble size distribution within the EALB. The rheological characteristics of the cultivated broth became increasingly viscous and non-Newtonian as the biomass concentration increased. Turbulence in the riser subsided considerably and the bubble size distribution changed. Even at a low biomass concentration (2 g/l) large spherical-capped bubbles (c. 4 mm in diameter) became predominant in the riser. As the broth became highly viscous, large bubbles were present in the riser along with some very small bubbles. The large bubbles rose very rapidly through the riser and disengaged at the top gas separator, while the smaller bubbles remained trapped inside the reactor. This behaviour may lead to specific hydrodynamic properties in the operation of the EALB during the cultivation of mycelial biomass fungi.

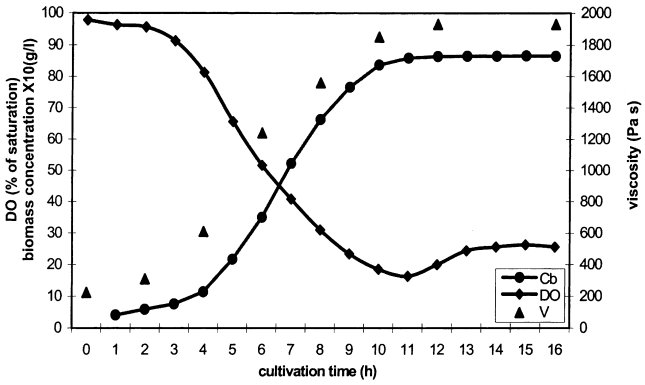

Figure 2. Profiles of mycelial biomass concentration (C_b), viscosity (V) and DO level of culture broth as a function of the cultivation time.

The DO concentration change within the broth had four phases in DO level during mycelial biomass growth: a high level, a decreasing level, an increasing level and a low level (Figure 2). During the high lag phase of the cultures the DO remained at a relatively constant high level, approaching saturation, due to no or little oxygen consumption occurring within the first 3 h of cultivation. A rapidly decreasing DO level was observed during the exponential growth phase of mycelial biomass, as the O_2 uptake by the mycelium was higher than the O_2 transfer into the medium. The DO level increased slowly as the biomass growth shifted to the biomass stationary phase. This increasing DO level, however, extended over a relatively short time, and then the DO level remained at a constant level. This behaviour reflects the dependence of mycelial biomass growth on a sufficient oxygen supply, as mycelium growth limited the O_2 transfer into the cultivated broth.

The gas holdup (ε) is an important design variable which depends on the geometry of the EALB, operating conditions and physical properties of the liquid medium. The ε value decreased significantly with the increase of broth viscosity, and a very slight increase appeared during the stationary phase of mycelial biomass growth (Figure 3). This result gave a good agreement with previous findings on non-Newtonian solutions (Yoshinori & Moo-Young 1986; Frohlich *et al.* 1990; Jin *et al.* 1999). It was interested to find that the variations of superficial gas velocity (U_g) and volumetric K_La demonstrated a similar trend during the cultivation of mycelial biomass (Figures 4 and 5). Both the U_g and

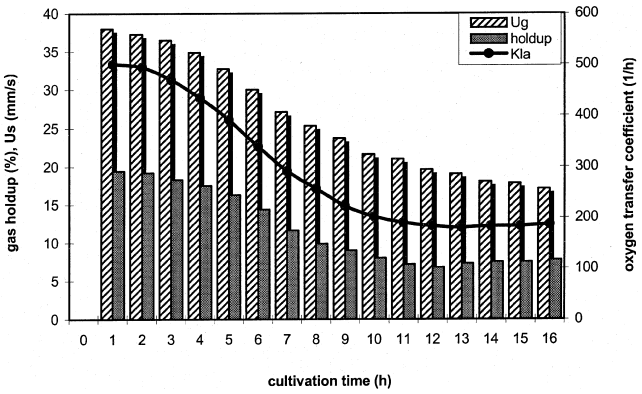

Figure 3. Hydrodynamic profiles of gas holdup, superficial gas velocity (U_g) and oxygen transfer coefficient (K_La) as a function of the cultivation time.

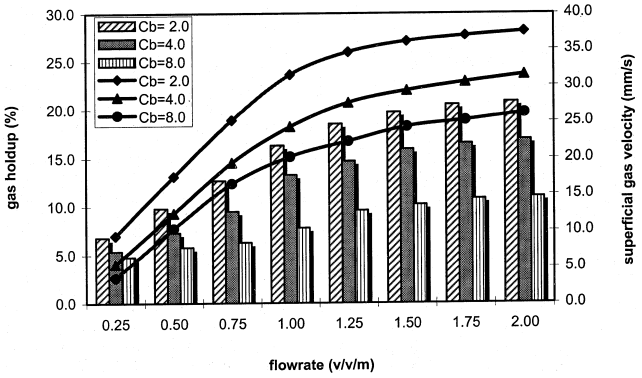

Figure 4. Effect of air flow rate and mycelial biomass concentration (C_b) on gas holdup (column) and superficial gas velocity (line).

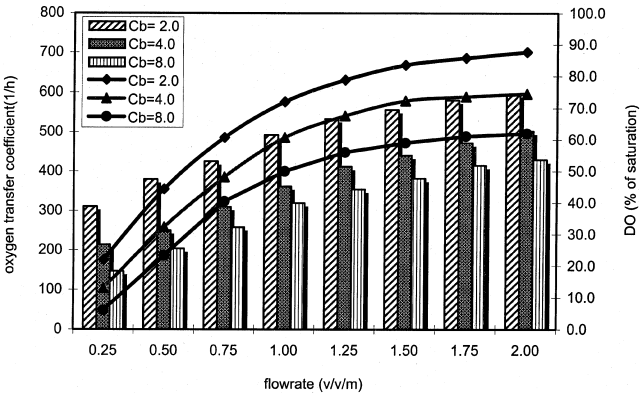

Figure 5. Effect of air flow rate and mycelial biomass concentration (C_b) on oxygen transfer coefficient (column) and DO level (line).

K_La decreased obviously with biomass growth, and remained at a constant value while mycelial biomass giving a stationary growth phase at a given air flow rate. There was no data found in literature regarding the relationship between the K_La and broth density during the course of cultivation. Konig & Shugerl (1982) stated that if a pellet suspension was formed, K_La would be influenced much less by high biomass concentration than in the case of filamentous molds.

Influence of air flow rate on hydrodynamic parameters

A fundamental relationship between air flow rate and U_g was experimentally determined as shown in Figure 4. As can be seen, the superficial gas velocity increased linearly with the air flow rate in the range from 0 to 1.00 v/v/m, while a further increase in the air flow rate led to a slow increase in U_g. In many previous investigations, the riser superficial gas velocity was represented by the aeration flow rate. However, the results presented here indicated that the relationship between the U_g and air flow rate varied with the mycelial biomass density. Although K_La and ε increased, as expected by previous investigations (Bello *et al.* 1985; Kawase & Moo-Young 1986; Kennard & Janekeh 1991; Jin *et al.* 1999), with increasing air flow rate in a low range, the influence of air flow rate was less pronounced in an increasing range of 1.25 to 2.00 v/v/m when the mycelial biomass was highly concentrated in the EALB.

It was also notable that DO level could be improved dramatically by increasing the air flow rate, but the increasing rate of DO level was decreased as the air flow rate exceeded 1.250 v/v/m in the EALB (Figure 5). On the other hand, the enhancement of the DO level by increasing air flow rate in the cultivated broth was obviously limited by the highly viscous culture broth. An increase in air flow rate from 1.25 to 2.00 v/v/m gave an increase in DO by approximately 12% of saturation at biomass concentration of 2.0 g/l, but only approximately an 8% increase in DO at C_b 4.0 g/l, and a negligible increase in DO at C_b 8.0 g/l. It appeared clearly that an increase in air flow rate at a high concentration of mycelial biomass would not achieve a desired DO level to meet a sufficient oxygen consumption for mycelial biomass growth. From the results presented in Figures 4 and 5, a general tendency for enhancing oxygen transfer in EALB process by high aeration rate was confirmed, i.e., the DO level and K_La, ε and U_g increased when the air flow rate increased. However, there were little changes in DO, K_La and ε when the air flow rate increased from 1.25 to 2.00 v/v/m, in particular, when the mycelial biomass density was high in the EALB cultivation process. This phenomenon occurred in the EALB may indicate that the mycelial biomass with increases in concentration and size caused formation of large spherical cap bubbles and a large number of tiny occluded bubbles in the non-Newtonian fluids, resulting in reducing the bubble rise velocity. The results also implied that it would be difficult to achieve a sufficient DO level in a highly viscous broth by a higher aeration rate.

Improvement of oxygen transfer by double sparger air supply

Because of the low solubility of oxygen in the cultivation media, dissolved oxygen would be consumed within a few seconds under normal fermentation conditions if fresh oxygen was not continuously transferred from the gas to the liquid phase (Onken & Weiland 1983). The supply of

oxygen to the mycelial microbes is therefore a very important aspect of the bioreactor design for aerobic fermentation. The air sparger plays an important role in improving the oxygen transfer efficiency in the EALB performance. The new pilot plant EALB equipped with two spargers was designed subsequently. In the EALB performance at an air flow rate of 1.0 v/v/m, approximately 10% of air was distributed directly into the downcomer through the small sparger, while approximately 90% of air was supplied into the riser through the large sparger, which was the main source of aeration and the driving force in circulation of the bioreactor contents.

In the EALB with a single sparger, the riser was a section of aeration and circulation driving force, and the downcomer was unaerated. However, involving a sparger in the downcomer would create a fully aerated environment within the bioreactor without changing the circulation direction due to the limited air through the sparger in the downcomer. Since aeration coefficient was improved, as expected, using double spargers in the EALB led to an increase in gas holdup by 23.5% and K_La by 20% at a given flow rate (Table 2). It seemed that the U_g was slightly decreased by involving the sparger in the downcomer, while producing a small air flow in the opposite direction to the main circulation, leading to a slight increase in mixing time. This phenomenon, however, did not affect the mixing process significantly for the EALB performance. These findings coincided with the results demonstrated in laboratory scale EALB investigation (Jin *et al.* 1999).

Figure 6 and Table 2 reveal some interesting findings. The DO level processed in the EALB with double spargers was higher than the EALB with single sparger at flow rate 1.0 v/v/m. This was due to a better aeration occurring within both the riser and the downcomer of the EALB. The cultivation of *R. oligosporus* using the EALB with double spargers led to a faster mycelial growth, shorter generation time (t_g) and higher biomass productivity (dX/dt) compared with the single sparger EALB performance.

Improvement of oxygen transfer by top pressure

Aerobic microbial processes for MBP production have been characterized by high oxygen demand. Oxygen-

Table 2. Comparison of hydrodynamic and growth kinetic parameters in the cultivation of *R. oligosporus* 2458 using the EALR with oxygenation improvement by double spargers and top pressure operations.

Parameter	Sparger		Top pressure (bar)			
	single	double	0	0.5	1.0	1.5
ε (%)	13.6	16.8	15.8	15.2	15.1	15.1
K_La (1/h)	260	310	320	360	390	405
t_m (s)	21	28	28	30	30	31
t_g (h)	14	12	12	10	9	9
μ (1/h)	0.12	0.15	0.15	0.17	0.19	0.19
dX/dt (g/l/h)	0.61	0.71	0.71	0.85	0.94	0.94

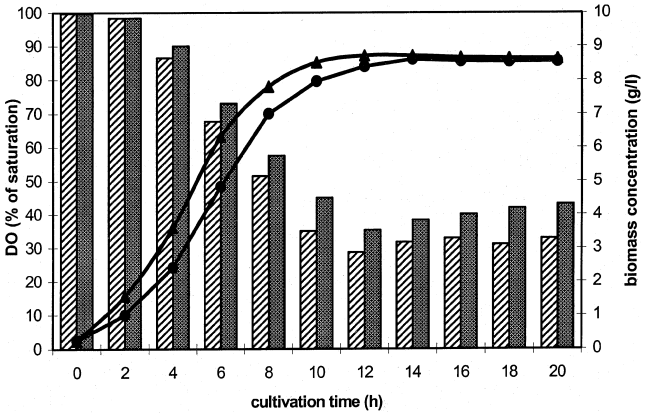

Figure 6. Profile of mycelial biomass concentration (C_b) and DO level during the cultivation of *R. oligosporus* using TEALB with single and double sparger air supply.

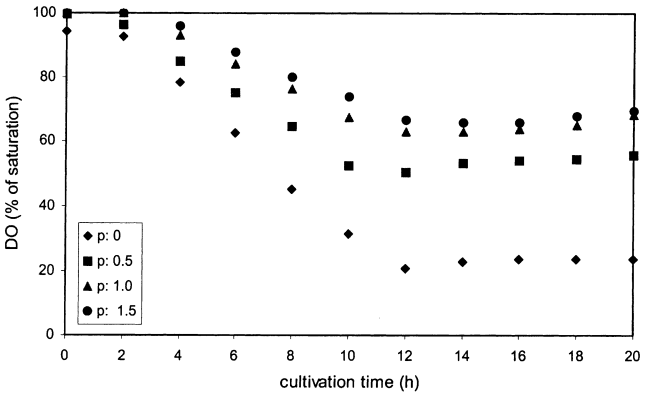

Figure 7. DO profiles during the mycelial cultivation process with top pressure (P) operations.

ation efficiency has been recognized worldwide as the key for a successful operation of aerobic ALR processes. However, our experimental results indicated that improvement of the oxygen transfer rate in mycelial biomass cultivation with highly viscous broths became much more difficult by increasing the aeration rate, due to the low solubility of oxygen. In particular, a continuous process needs to maintain a high biomass concentration over the cultivation operation. On the other hand, high aeration causes a huge energy cost operation, while resulting in a limited oxygenation increase. Furthermore, a huge amount of foam was produced in the process operation with highly sparged air flow rate and a large amount of antifoam was needed for foam control during the cultivation process. More antifoam used would not only raise the operating costs, but also adversely affect oxygen transfer within the cultivation broth. Enhancing the oxygen solubility in the mycelial broth so as to improve the DO level was experimentally investigated by changing the operating pressure. The EALB with double air spargers was conducted in the batch process under a top pressure between 0 and 1.5 bars.

The results shown in Table 2 and Figure 6 indicate that a high top pressure led to an obvious improvement

of DO level in the cultivation process, and was a stimulation of fast mycelial growth. A significant DO increase was caused by increasing the top pressure from 0 to 1.0 bar, and further increasing the top pressure up to 1.5 bars revealed little DO improvement. The hydrodynamics of ε, U_g and t_m showed unpronounced variation, but K_La increased by 21.8%. The improvement of oxygen transfer stimulated fast mycelium growth, resulting in a shorter generation time and higher mycelial biomass productivity compared with the operation without top pressure. It is remarkably interesting to find that there was no foam produced during the course of mycelial cultivation under the operation with top pressure. The results demonstrate an efficient and economical process with the operation under a top pressure. Liefke *et al.* (1990) found that microbial activities on aerobic cultivation of various *Streptomyces* spp. were improved by enhancing the oxygen partial pressure up to 2.0 bars, but growth of *Micromonspora purpurea* was inhabited in the pressured operation with an oxygen partial pressure of 1.2 bars.

Conclusions

From the results presented above, the following conclusive issues need to be addressed,

1. Superficial gas velocity had a nonlinear relationship with the flow rate of air supply in the mycelial cultivation broth with an increased viscosity. An increase in air flow rate did not give a proportional increment of aeration within the bioreactor.
2. The hydrodynamics of K_La and ε, and DO level increased with air flow rate linearly from 0 to 1.0 v/v/m, but the increase in rate in a highly viscous broth was reduced significantly at when the flow rate exceeded 1.25 v/v/m.
3. The pilot plant EALB process with two spargers of air supply created a full aeration environment in the bioreactor, which resulted in high oxygen transfer efficiency and improved mycelial biomass productivity.
4. Operation of the EALB under a top pressure led to a high efficient bioprocess with high aeration efficiency and mycelial biomass productivity, and an economical system with low operating costs by lower air demand and no antifoam used during the process.

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