Evaluation of strategies for temperature and moisture control in solid state packed bed bioreactors

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Abstract
Different control strategies of bed temperature and moisture were investigated using various inlet air temperatures and air fluxes in both the ordinary packed bed bioreactor (without cooling water in the jacket) and the bioreactor with cooling water in jacket. The experiments were carried out within a 1-L solid-state packed bed bioreactor in which *Aspergillus niger* was cultivated on wheat bran. On-line measurements of oxygen quantity in the outlet air and temperature of the bed and the inlet air flux were carried out in both types of the bioreactors. Effects of certain control strategies on fungal growth rate were compared in both the bioreactors. According to experimental results, using the bioreactor with the cooling water in the jacket is a better strategy for control of bed temperature and moisture during packed bed solid state fermentation. Cumulative oxygen consumption in this bioreactor was approximately 1.7 times higher than other control strategies used in this study.
Keywords: *Aspergillus niger*; Control Strategies; Cooling Jacket; Packed Bed Bioreactor; Solid State Fermentation (SSF).

INTRODUCTION

Solid state fermentation (SSF) involves the growth of microorganisms on moist solid substrates in the absence of free water (Cannel and Moo-Young, 1980). This cultivation technique has potential to be used at commercial scale for production of some microbial products, especially in those situations where higher yields or better product qualities are obtained in SSF than in submerged liquid fermentation (SLF) processes (Krishna, 2005; Pandey, 2003).

Although scale-up methods for SLF have been developed, these methods could not be applied directly to SSF bioreactors, due to differences in the physical structures of the systems. In the case of SSF, heat removal is a major consideration in the design of bioreactors, whereas in aerobic SLF processes the supply of O_2 is usually the major problem to be overcome (Yang *et al*., 2003; Lillo *et al*., 2001).

One of the simplest designs of SSF is the packed bed bioreactor which has potential, particularly for those fungal fermentations in which agitation is harmful (Sangsurasak and Mitchell 1998; Rojagapalan and Modak 1994; Durand, 2003). Due to the absence of free-flowing water and low thermal conductivity of solid substrates in these systems, removal of the heat produced by growing microorganisms, can be problematic and adversely affect microbial activity (Weber *et al*., 2002; Hamidi-Esfahani *et al*., 2004). Cooling of the bed by forced aeration, due to evaporation, is the main effective way to overcome temperature and moisture gradients in the bed during static operation (Mitchell *et al*., 2000a; Nagel *et al*., 2001; Von Meien *et al*., 2004). Although tray bioreactors can be used as static versions, packed beds are more appropriate because the forced aeration allows one to have some control over fermentation parameters. This can be

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achieved through manipulation of the flow rate and temperature of the air used during fermentation. However, forced aeration results in evaporation of water and desiccation of the substrate. Even with water saturated inlet air, evaporation still occurs because the increase in air temperature between the inlet and the outlet air increases the water-holding capacity of the air (Mitchell *et al*., 1999). Desiccation of the substrate can lead to an unfavorably low water activity resulting in poor microbial activity, and subsequent channeling in the bed (Weber *et al*., 2002). Using cooling water in the jacket of the small diameter packed bed bioreactor increases the effects of conduction and thus is another way of controlling operation during SSF (Saucedo-Castaneda *et al*., 1990). However, for a large-scale process it is not practical to use small diameter cylindrical beds. In the rectangular Zymotis large scale packed-bed bioreactor, heat removal by radial conduction is promoted by the insertion of closely-spaced internal heat transfer plates into the bed (Roussos *et al*., 1993; Mitchell and Von Meien 2000b; Durand, 2003).

Many SSF processes involve filamentous fungi, which bind tightly to the substrate. Therefore, microbial biomass during SSF is estimated using indirect measurements of growth, such as O_2 consumption or glucosamine content. Oxygen uptake rate (OUR) or carbon dioxide production rate (CPR) offer the advantage of a fast response time and are directly linked to the metabolism of microorganisms (Mitchell *et al*., 2004, 2006).

The available operating variables for control of the bed temperature and water content of the bed are temperature, air flow rate and humidity of the inlet air and moisture of the substrate at the beginning of the operation (Lillo *et al*., 2001; Von Meien *et al*., 2004; Ashley *et al*., 1999). There are no reports on simultaneous measurements of the bed temperature, moisture and microbial growth and also comparison between different control strategies during operation in the packed bed bioreactors.

In the current research, simultaneous measurements of bed temperature, moisture and OUR (as an indirect indication of biomass concentration) for *Aspergillus niger*, a fungi growing actively on wheat bran, were carried out. These measurements were implemented in both ordinary packed bed bioreactor (OPB) and packed bioreactor with cooling water in the jacket (PBCW). Also, certain strategies on applicability of air flow rate for control of the bed temperature and moisture in OPB were proposed and compared to operation in the PBCW.

MATERIALS AND METHODS

Inoculum preparation: *Aspergillus niger* CBS 122.49 was obtained from the Central Bureau voor Schimmelcultures, The Netherlands The fungus was grown on potato dextrose agar (PDA) at 25ºC for one week. A stock suspension of spores was prepared by flooding the cultures with sterile peptone physiological salt solution (1 g of bacteriological peptone, 8.5 g of NaCl and 0.5 g of Tween-80, in 1 L of distilled water), followed by gently scraping the colony surface with a sterile spatula. The resulting spore suspension was poured into a vessel containing sterile glycerol giving a final glycerol concentration of 23% (w/v). The spore suspension was stored at -80ºC (Weber *et al*., 1999 and 2002).

Substrate preparation and inoculation: 149 g of wheat bran was mixed with distilled water to obtain a final moisture of 55% (w/w), on a wet basis. The pH of the wet substrate was adjusted to 4.5 by 1 N HCl. The substrate was sterilized at 121ºC for 20 minutes and then cooled to ambient temperature. The sterile wheat bran was inoculated with the spore stock solution to give 2×10^6 spores per gram of wet substrate.

Packed bed bioreactor: A 1 L jacketed cylindrical glass bioreactor, with an internal diameter of 5 cm and a height of 60 cm was used for all of experiments (Fig. 1). The cooling water in the jacket of the bioreactor

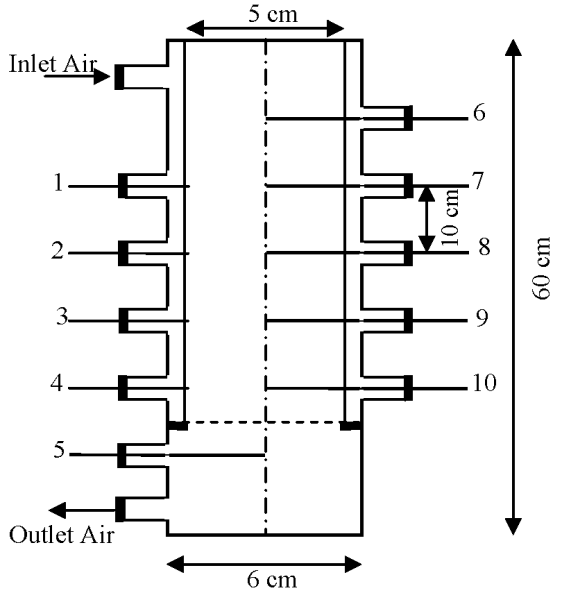

Figure 1. Schematic diagram of the solid state packed bed bioreactor and temperature sensors in: (1-4) wall fermentor, (7-10) centre of fermentor and (5, 6) the outlet and inlet air temperature.

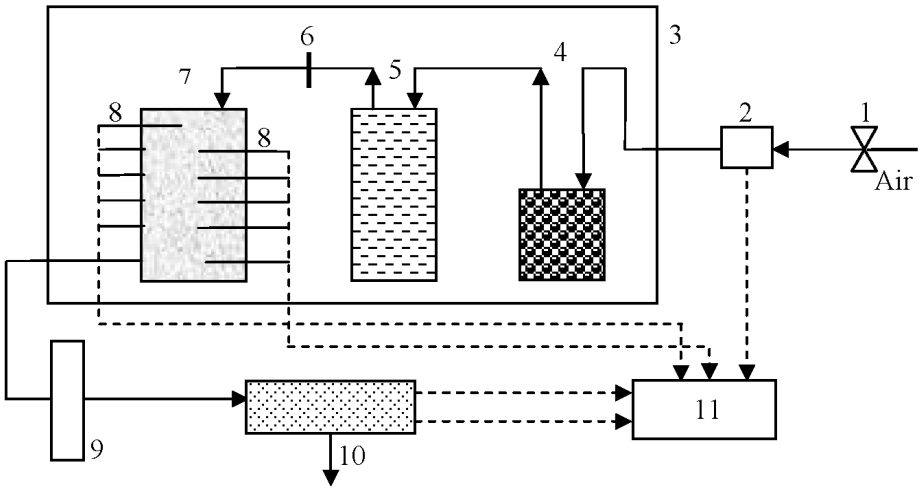

Figure 2. Schematic setup of the solid state fermentation system. (1) pressure control, (2) mass flow controller, (3) temperature controlled cabinet, (4) CO_2 absorber, (5) humidifier, (6) air filter, (7) packed bed bioreactor, (8) temperature sensors, (9) condenser, (10) gas analyzer, (11) computer. The dashed lines indicate on-line computer measurements.

was used in some experiments to remove heat from the bioreactor wall by convection. The bioreactor was autoclaved for 30 minutes at 121ºC, prior to operation. After cooling to room temperature it was filled with sterile wet substrate in a laminar flow cabinet under sterile conditions. The bed height at the beginning of the fermentation was approximately 38 cm.

Experimental arrangement: A schematic diagram of the experimental setup consisting of the packed bed bioreactor with aeration, control and on-line monitoring systems is shown in Figure 2. The pressure of inlet air was set at 1 atm using a pressure controller. During cultivation, aeration (from top to bottom of the bioreactor) was controlled by a mass flow controller (Brooks Instrument BV, The Netherlands).

The incoming air was de-carbonized in a 1-litter column filled with 1 N NaOH solution. It was then humidified at 30-35ºC in a jacketed column (12 cm i.d.× 60 cm in height) filled with distilled water and Rasching rings (Rasching AG, Germany). After filtration of humidified air (0.2 µm PFTE-membrane filter, PolyVent 1000, Whatman Inc., Ann Arbor, MI USA), it was passed through the bioreactor to provide required O_2 for fermentation, remove CO_2 produced during fermentation and to dissipate heat. In order to prevent water condensation in the tubes and filter, the fermentor, the humidifier and CO_2 absorber were maintained in a temperature controlled cabinet at 30ºC, as shown in Figure 2. A fraction of the outlet gas was dehumidified in a glass condenser at 5ºC and pumped to the gas analyzer at a rate of 100 ml min^{-1}. For indirect measurement of microbial biomass, the O_2 concentration of the bioreactor outlet gas was measured by a paramagnetic analyzer (Xentra 4100, Servomex, The Netherlands).

The bed temperature in the centre and at the wall of the fermentor was measured at different heights and in the input and output air using temperature sensors (Pt100 Ω-Sensors, Tempcontrol, The Netherlands).

The cultivation process was monitored and controlled on-line by a personal computer using Field Point hardware and LabVIEW software (National Instruments, The Netherlands). On-line measured variables included inlet air flow rate, inlet and outlet air temperature, O_2 concentration (%v/v) in the outlet gas and bed temperature at various points of bed height (Fig. 2).

Analysis: The moisture content of the solid substrate at the beginning and end of cultivation was determined by the gravimetric method, at 80ºC (Oostra *et al.*, 2000; Weber, 2002). The samples of fermented substrate were taken from various positions along the radius and height of bed. The pH of the substrate was measured after mixing 1 g of the sample in 10 ml of distilled water. Oxygen uptake rate as an indirect measurement of microbial growth rate was expressed as mole O_2 per kg initial dry substrate (IDW) per hour. Fungal growth distribution based on aerial hyphal growth was investigated visually during fermentation.

RESULTS

Ordinary packed bed bioreactor (OPB): The average results of temperature in the center and at the wall, at different heights of the OPB under the conditions of 30ºC (temperature of controlled cabinet), 55 %(w/w) bed moisture, pH 4.5, inlet air temperature of 30ºC and inlet air flux of N=0.216 mole/m^2.s are shown in Figure 3.

Control strategies of bed temperature and moisture in OPB: In order to investigate the effects of air flow rate on the bed temperature and moisture during fermentation, some control strategies for biomass concentration were carried out in OPB as follows:

- *Use of high air velocity* with an inlet air temperature of 37ºC (approximate optimal growth temperature) and molar air of flux of 2.5 mole/m^2.s (Fig.4).

- *Control by aeration velocity* with inlet air temperature of 35ºC, using a proportional-differential (PID) controller in which the central bed temperature at 32.5 cm from the top of the bed was 41ºC (Figs. 5 and 7).

- *Control by aeration velocity with a high initial bed moisture* 65 % (w/w).

Packed bed bioreactor with cooling water in the jacket (PBCW): The results of axial bed temperature gradients in the PBCW with cooling temperature and aeration at 35ºC, initial bed moisture of 55% (w/w) and inlet air flux of 0.145 mole/m^2.s, at both the center and bioreactor wall are shown in Figure 6.

DISCUSSION

Cultivation in OPB: According to Figure 3, during the first 12 h of fermentation, the bed temperature profile was approximately constant, but a few hours later with an increase of growth rate, a rapid increase in the bed temperature to approximately 45ºC (after 30 h) occurred. At maximum heat production (Fig. 3), average temperature gradients in the axial direction on the wall, at the center of the fermentor and in the radial direction were 0.4, 0.6 and 2ºC cm^{-1}, respectively.

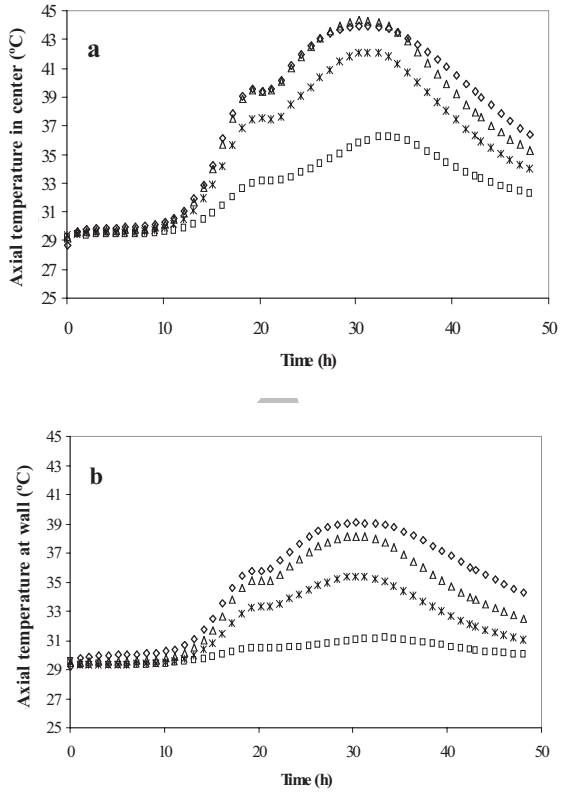

Figure 3. Axial gradient temperature in the OPB at heights of 2.5 cm (□), 12.5 cm (∗), 22.5 cm (△) and 32.5 cm (◇) from the air inlet, at 30 ºC and N=0.216 mole/m^2.s (a) in the center, (b) and at the wall of fermentor.

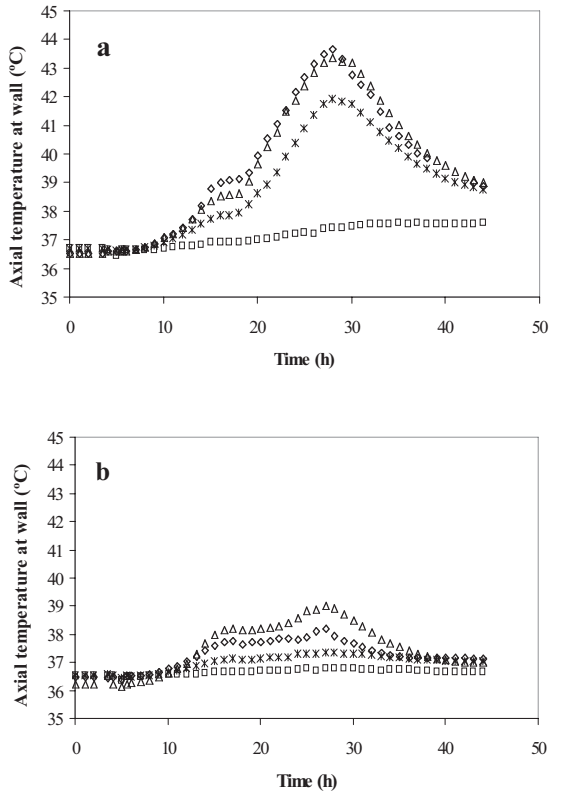

Figure 4. Axial gradient temperature with first control strategy in the OPB at heights of 2.5 cm (□), 12.5 cm (∗), 22.5 cm (△) and 32.5 cm (◇) from the air inlet at 37ºC and N=2.5 mole/m^2.s (a) in the center, (b) and at the wall of fermentor.

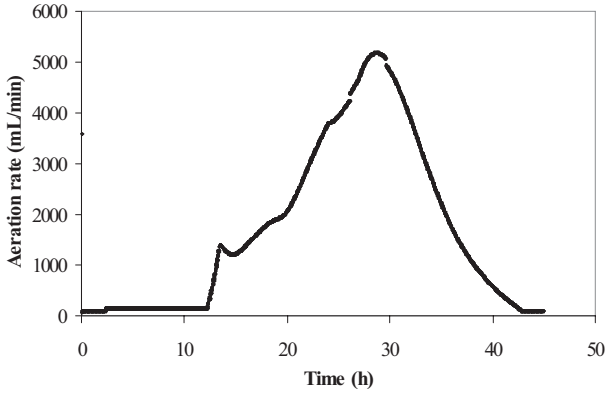

Figure 5. Variation of bioreactor aeration velocity with fermentation time using proportional-differential controller based on constancy of maximum recorded bed temperature in the OPB.

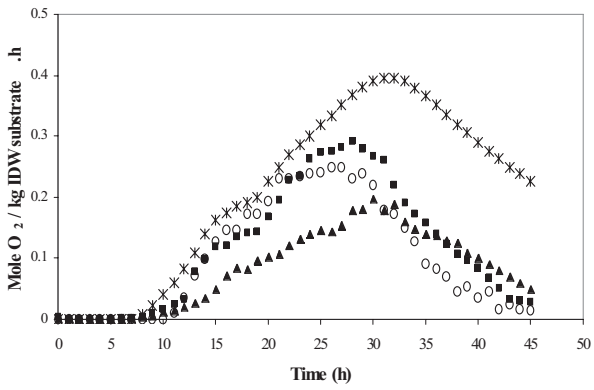

Figure 7. Indirect microbial growth rate of PBCW and three control strategies with respect to oxygen uptake rate (OUR): (∗) PBCW; (○) high and constant air velocity; (■) controlled aeration velocity; (▲) controlled aeration velocity with high initial bed moisture.

After 30 hours, three different fungal growth zones appeared along the bed. There was no fungal growth at the first zone;a distance of about 7 cm of the bed from the aeration inlet that is related to both the low inlet air temperature and high air flow rate. The second zone appeared at 20 cm from the middle of bed showing uniform fungal growth. In the third zone at the bottom of the bed with high temperature, small amount of fungal growth was observed.

With radial conduction, the wall temperature was less than the optimum growth temperature (35-42ºC) (Fig. 3b), so fungal growth rate at the wall was less than the axial growth rate at the center. On the other hand, heat transfer at the center of the fermentor was low. So, by moving towards the bottom of the fermentor from 2.5 to 22.5 cm, temperature gradient and fungal growth rate increased. But at the bottom of fermentor, with a temperature higher than the optimum growth conditions, fungal growth decreased.

Reduction of total bed weight was approximately 13% (w/w) during 48 h of fermentation. Because of radial heat transfer by conduction, the bed moisture at the center of the OPB was less 40-55% (w/w) than it was at the fermentor wall 60-74% (w/w). Also, the axial temperature revealed that in the middle zone of the bed, uniform fungal growth was observed under optimal temperature and moisture conditions.

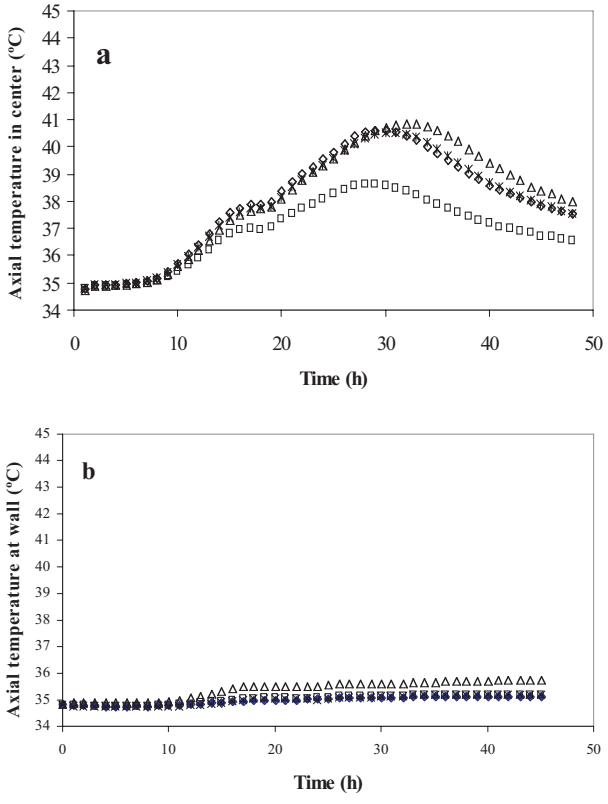

Figure 6. Axial gradient temperature in the PBCW at heights of 2.5 cm (□), 12.5 cm (∗), 22.5 cm (△) and 32.5 cm (◇) from the air inlet at 35ºC and N=0.145 mole/m^2.s (a) in the center, (b) and at the wall of fermentor.

Control strategies in OPB

Use of high air velocity: In order to reduce the bed temperature gradient, the first control strategy that included a high air velocity of 2.5 mole/m^2.s and an inlet air temperature of 37ºC was carried out. Although a uniform fungal growth was observed in the OPB, fungal growth (Fig. 7) and bed moisture reduced after 25 h. Bed temperature was near the optimal growth temperature both at the center and at the wall (Fig. 4), while total bed weight reduction was approximately 40% after 48 h and bed moisture at the center and wall length of bioreactor were 32% (w/w) and 37-59% (w/w), respectively. At the end of

fungal growth, the average bed moisture about 39% (w/w) was lower than the minimum condition for fungal growth 40% (w/w) bed moisture (Hamidi-Esfahani *et al*., 2004).

Control by aeration velocity: During this cultivation, the central bed temperature at 32.5 cm from top of the bioreactor was kept at 41ºC by means of a PID controller that continuously monitored the temperature and adjusted the air flow rate. Under this condition, despite the uniform fungal growth, total bed weight reduced by approximately 37% after 48 h and bed moisture reduced from the initial 55% (w/w) to 45% (w/w) at the end of fermentation. Bed moisture at the center of the OPB remained constant approximately 37% (w/w), but there was a high gradient bed moisture on the wall (41% (w/w) to 73% (w/w) from top to bottom of the fermentor). Maximum evaporation of bed moisture occurred at 30 h of fermentation, during which aeration rate, control temperature and oxygen consumption rates were maximum (Figs. 5 and 7).

Control by aeration velocity with high initial bed moisture: In order to prevent bed moisture reduction in the OPB, initial moisture of substrate was adjusted to 65% (w/w). Again a uniform fungal growth was observed, but total bed weight reduction was 32% and average bed moisture was 61% (w/w) at the end of the fermentation. After 48 h of fermentation, bed moisture at the center of the fermentor was 49-66% (w/w) and at the wall was approximately 51-70% (w/w). Bed porosity reduction and consequently less oxygen availability are the reasons for biomass decrease compared to those observed in previous experiments (Fig. 7).

Cultivation in PBCW: Despite the uniform fungal growth in the OPB using all of the above mentioned control strategies, low biomass concentration was observed due to high aeration flux and low bed moisture (Fig. 7). The results of preliminary experiments in the PBCW under low aeration flux also showed uniform growth. Figure 6 shows gradients of bed temperature at the center and wall of the bioreactor. The axial bed temperature gradients from 12.5 to 32.5 cm at the center and near the wall of the bioreactor are negligible and maximum gradient of bed temperature is 2ºC.

Reduction of total bed weight was approximately 5% (w/w) during 48 h of fermentation. Bed moisture at the center of the PBCW along the bed reduced (39-47%) but on the wall it was approximately 74%.

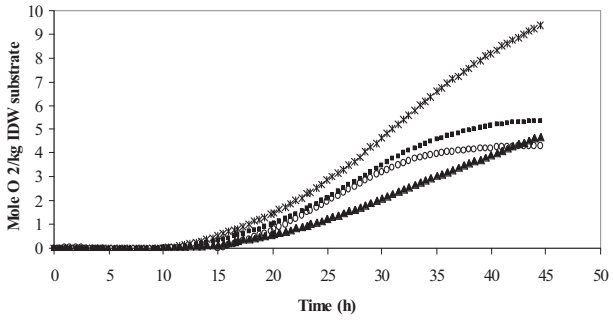

Figure 8. Comparison of three control strategies and PBCW with respect to cumulative oxygen consumption: (∗) PBCW; (○) high and constant air velocity; (■) controlled aeration velocity; (▲) controlled aeration velocity with high initial bed moisture.

Despite the high aeration temperature with low aeration flux, low bed temperature gradient (near optimal growth temperature), high biomass concentrations (without apparent gradient) and also lower reductions in the bed moisture were observed.

Comparison of the bioreactors: Figure 8 compars of the effects of three control strategies of the bed temperature and moisture in the OPB with PBCW on accumulative oxygen consumption (indirect measurement of biomass) during fermentation.

As shown in Figure 8, cumulative oxygen consumption for bed with higher initial moisture content controlled by variation of air velocity (as the third strategy) is lower than other control strategies. Reduction of the bed void fraction and contact area for oxygen adsorption can be the reason for this phenomenon. In the presence of both the first control options, the maximum axial temperature gradients were about 10ºC (Fig. 4), while it was about 15ºC in the center of the OPB without control (Fig. 3). However, the use of the second control strategy will economically be the most interesting, as less energy will be required for the aeration. However, the axial temperature gradient in this system is constantly high. The highest oxygen consumption is observed in the PBCW due to low bed temperature and moisture gradients. Axial temperature gradients can be reduced by radial conduction heat transfer more effectively than high air flow rate.

In all of the control strategies, although the bed moisture was decreased during fermentation, temperature remained near the optimal for growth and uniform growth was observed in all cases. So, it may be con-

cluded that uniform growth in the fermentor is more dependent on bed temperature than on bed moisture.

CONCLUSION

According to the results obtained in this study it can be concluded that changing the inlet air temperature and velocity during the simultaneous control of bed temperature and moisture of the ordinary solid state bioreactor may not be an appropriate method. Use of cooling water in the wall of the solid state bioreactor is a suitable strategy for reduction of bed temperature and moisture gradient, in order to obtain uniform fungal growth with high biomass production.

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