10 Group IVb: Intermittently-Mixed Forcefully-Aerated Bioreactors

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10.1 Introduction

This chapter concerns the design and operation of SSF bioreactors under conditions where forced aeration is used and the substrate bed is mixed intermittently. These will be referred to as intermittently-mixed, forcefully-aerated bioreactors or, more simply, as intermittently-mixed bioreactors. As explained in Chap. 3, this mode of operation is appropriate for those SSF processes in which continuous mixing is not tolerated well by the microorganism, but intermittent mixing events do not have unduly deleterious effects. For much of the fermentation the bioreactor operates as a packed-bed bioreactor. The advantage is that the mixing event prevents the pressure drop from becoming too high within the bed and that water can be added to the bed, in a reasonably uniform manner, during the mixing event.

10.2 Basic Features of Group IVb Bioreactors

The basic design features of intermittently mixed bioreactors are similar to those of the various continuously mixed designs (Chap. 9), the difference being in the mode of operation. Since the mixing is only intermittent and the bioreactor spends periods in the static mode of operation, designs should be preferred that give a uniform aeration of the bed when it is static. Forced aeration may or may not be applied during the mixing period, depending on the design. Figure 10.1 shows possible basic designs for intermittently mixed bioreactors.

Intermittently mixed bioreactors have the same design and operating variables as packed-bed bioreactors (Sect. 7.2), which affect the performance during the periods of static operation. In addition to this, the type of agitation is an extra design variable for intermittently mixed bioreactors. The bed may be mixed by a mechanical stirrer, by rotation of the whole bioreactor or by the air flow.

In addition to having the operating variables for packed-bed bioreactors, intermittently mixed bioreactors have several extra operating variables available.

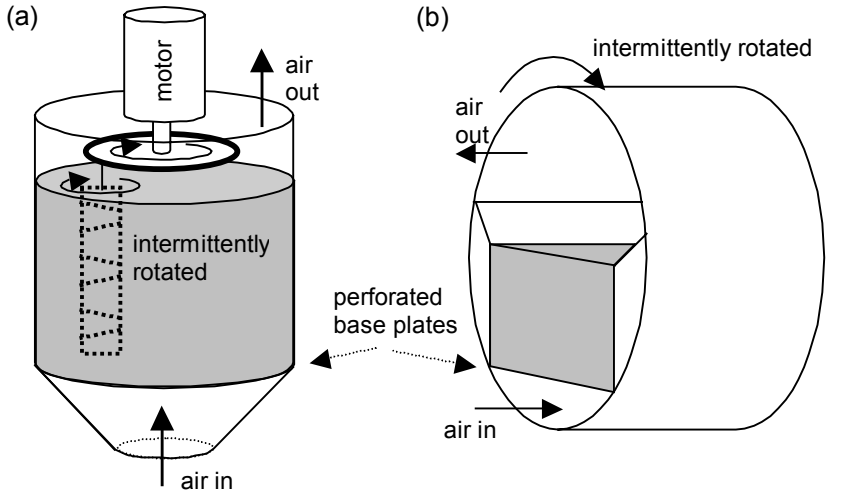

Fig. 10.1. Two basic options for mixing of intermittently mixed bioreactors. **(a)** The bed may be agitated by a mechanical agitator inserted into the substrate bed. In this case forced aeration can be applied during the mixing period. The agitator may simply rotate around its axis, in which case it will need to be almost as wide as the bioreactor, it may rotate with a planetary action (as shown) or it may travel from side to side across the bioreactor. **(b)** The bed may be agitated through rotation of the bioreactor around its central axis (Toyama 1976). There is no mechanical structure within the bed itself. In this case it is not practical to aerate the bed forcefully during the mixing period

Firstly, there is the strategy for initiating mixing events, which will determine the frequency of the mixing events. Secondly, the duration and intensity of mixing events can be varied. Thirdly, unlike packed beds, the relative humidity of the inlet air is potentially available as an operating variable. Since water can be added to the bed in a reasonably uniform manner during the mixing events, unsaturated air can be used to aerate the bed in order to promote evaporative cooling.

The values selected for these extra design and operating variables will be most affected by:

- the temperatures reached in the bed during static operation (e.g., mixing could be triggered by high temperatures at the outlet end of the bed);
- the water activities reached in the bed during static operation (e.g., mixing could be triggered when the outlet-air relative humidity falls below a set point);
- the pressure drop through the bed (e.g., mixing could be triggered when the pressure drop reaches unacceptably high values);
- the sensitivity of the organism to damage during mixing events, which will affect the frequency, intensity, and duration of mixing events.

Considerations affecting the selection of appropriate values of other operating variables, such as the air flow rate and temperature, will be similar to those for packed-beds (Sect. 7.2).

Channeling should be less of a problem for intermittently mixed beds than for static packed beds. The mixing events will tend to break up the bed so that the particles remain separate and these will tend to settle as the bed shrinks, rather than being knitted together and pulled away from the wall as happens with packed beds. However, channeling might be caused by an imperfect bed structure at the end of the mixing event. For example, in the case that the agitator stays in the bed during the static periods, it may leave a hole behind or around it as it comes to a stop. Alternatively, in the case that it is withdrawn from the bed, it may leave a hole as it leaves. In either case, if nothing is done to close the hole, the air will flow preferentially through it during the period of packed-bed operation.

This chapter explains what is known, on the basis of experimental studies, about how these design and operating variables influence bioreactor operation. Later, Chap. 25 will show how mathematical models can be used to explore further the design and operation of intermittently mixed bioreactors.

10.3 Experimental Insights into the Performance of Group IVb Bioreactors

This section presents and discusses the knowledge that experimental work has given into the phenomena that occur within intermittently mixed bioreactors and into the operability of this type of bioreactor.

10.3.1 Large-Scale Intermittently-Mixed Bioreactors

10.3.1.1 The Koji Industry

Intermittently agitated designs have been used in the *koji* industry. Sato and Sudo (1999) report a bioreactor with a capacity of 15 tons of rice *koji* on a 12-m diameter disk (Fig. 10.2). The inoculated substrate is placed in the upper chamber, where it remains for one day. After this period the screw mixer is used to transfer the substrate to the bottom chamber, where it is mixed intermittently. The bioreactor is computer controlled. However, Sato and Sudo (1999) give no further details. For example, it is not clear exactly how often the mixing is carried out.

Interestingly, Sato and Sudo (1999) note that, even for an industry with much experience, the maximum height of the substrate bed is of the order of 20 cm. This means that large-scale bioreactors will occupy a large area. The 15-ton capacity bioreactor has disks of 12 m diameter. In comparison, an SLF bioreactor would have a diameter of about 5 m to hold the same working volume, assuming that the solid bed has a packing density of 400 kg m^{-3} and therefore a volume of 37.5 m^3 and that the SLF bioreactor has a height to diameter ratio of 2:1.

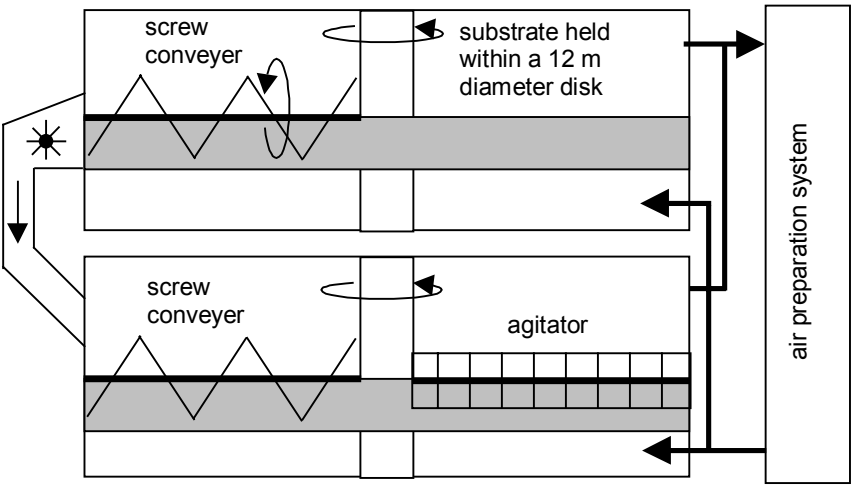

Fig. 10.2. Intermittently-mixed bioreactor of the type used in the *koji* industry for soy sauce production by Nagata Brewing Industry Co Ltd., Takarazuka, Japan. It has a 12 m diameter bed and a capacity for 15 tons of substrate. After one day the upper disk is rotated, with the upper screw conveyer transferring the substrate to the lower disk, where it can be agitated intermittently. Note that the mixers rotate in place and the whole circular bed moves to bring the substrate to the mixing point. This is a simplified version of a diagram presented by Sato and Sudo (1999)

10.3.1.2 The Bioreactor of INRA-Dijon

Durand and Chereau (1988) developed an intermittently mixed bioreactor at INRA, in Dijon, France. It is 2 m long, 0.8 m wide and has an overall height of 2.3 m, with a working bed height of 1 m. This gives a working volume of approximately 1.8 m^3, sufficient to hold a bed of approximately 1 ton of moist material. The mixing is provided by a number of screw augers (i.e., designed to lift the substrate as they turn) that are mounted on a carriage on top of the bioreactor (Fig. 10.3). This carriage travels from one end to the other at a top speed of 6.5 cm min^{-1}, meaning that it takes 35 minutes to traverse the bioreactor for one end to the other. The screws rotate at a top speed 22 rpm. The agitation regime, in terms of the frequency and duration of mixing events, can be varied according to the needs of the process, as determined by the particular microorganism and substrate used. Further, if necessary, different agitators such as hollow screws or helicoid screws, can be fitted, depending on the mixing behavior of the solid medium to be used in the fermentation. The carriage has spray nozzles fitted onto its underside, allowing the addition of inoculum, water, nutrient or pH-correcting solutions during mixing. The aeration system has a maximum capacity of 1500 m^3 h^{-1}, which means that it is possible to aerate with over 13 volumes of air per bed volume per minute (i.e., over 13 vvm).

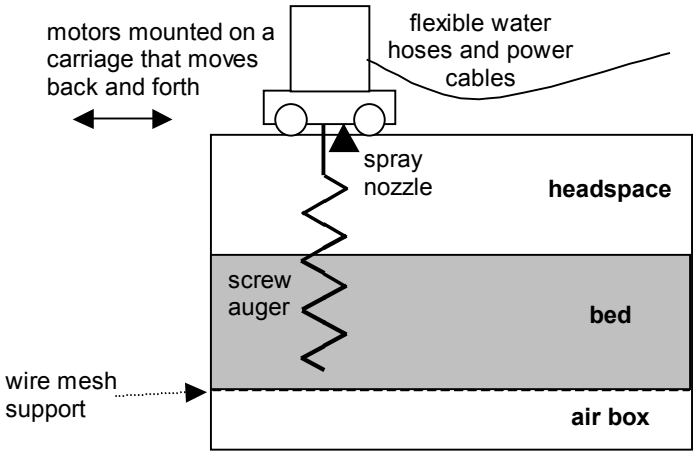

Fig. 10.3. Intermittently-mixed bioreactor of the type used by Durand and Chereau (1988). There are three motors/agitators mounted across the width of the bioreactor (behind the one shown in the side view given here). The bioreactor dimensions are given in the text. Adapted from Durand and Chereau (1988) with kind permission of John Wiley & Sons.

Durand and Chereau (1988) cultivated *Trichoderma*, which grows optimally at temperatures around 28°C, on a sugar beet pulp medium for the production of single cell protein. It was possible to maintain the temperature and water content of the bed within acceptable limits by maintaining the relative humidity of the inlet air constant at 90%, and by manipulating the flow rate and temperature of the air supplied to the bioreactor. They describe the operating regime as follows:

- three to four "turnings" (i.e., mixing events) during the 48 h cultivation;
- for the first 10 h, an air flow rate of 750 $m^3 h^{-1}$ (i.e., about 7 vvm) at 29°C;
- as the growth rate accelerates, an increase of the air flow rate to 1000 $m^3 h^{-1}$ (i.e., about 9 vvm) at 26°C.

With this operating regime, during exponential growth the temperatures at 85-cm depth in the bed and at 20 cm depth in the bed ranged from 26.5 to 29.0°C.

Since it was first reported, the use of this bioreactor has been extended successfully to the production of enzymes and biopesticides (Durand 2003).

Xue et al. (1992) adapted the bioreactor of Durand and Chereau (1988) to build a much larger scale process for the production of microbial protein from sugar beet pulp by *Aspergillus tamarii*. The bioreactor is built from concrete, having a length of 17.6 m, a breadth of 3.6 m, and an overall height of 2.0 m. A perforated stainless steel plate, designed to support the bed, is fixed at a height of 0.6 m. The actual bed height used is 0.7 m, leaving a headspace of 0.7 m, and giving a bed volume of 44 m^3. This corresponds to 25 tons of moist substrate, which, given a water content of 80% (wet basis), gives 5 tons of dry substrate. The carriage holding the screw mixers has a linear speed of 30 cm min^{-1} and rotates the screws at 13.3 rpm. The facility has two such bioreactors. The air system has a maximum capacity of 60,000 $m^3 h^{-1}$. Divided over two bioreactors, this is 11 vvm.

Very little performance data was provided. They used two turning cycles during the first 48 h of the 72 h process. The aeration rate used was 220 m^3-wet-air min^{-1}, at a relative humidity of 88% and at a temperature that ranged from 32 to 34°C. However, it was not specified whether this aeration rate was for one or both bioreactors. The outlet air was reported to have "operational parameters" of 100% relative humidity and 33°C, although it is not clear whether this was achieved.

10.3.1.3 The Bioreactor of PUC-Chile

Pérez-Correa and Agosin (1999) built a bioreactor with a capacity for a bed of 200 kg. The bioreactor has three sections (Fig. 10.4). The bottom section, which remains stationary, is simply the air box. The 150 cm diameter bed is held by the second section, which is rotated in its entirety by a motor. The top of the bioreactor represents a third section, which is stationary, and on which the agitators are mounted. The thermocouples can be withdrawn from the bed into the headspace during the mixing event. The bioreactor was designed to enable a bed height of 80 cm, although in practice the bed height was kept at 60 cm or below.

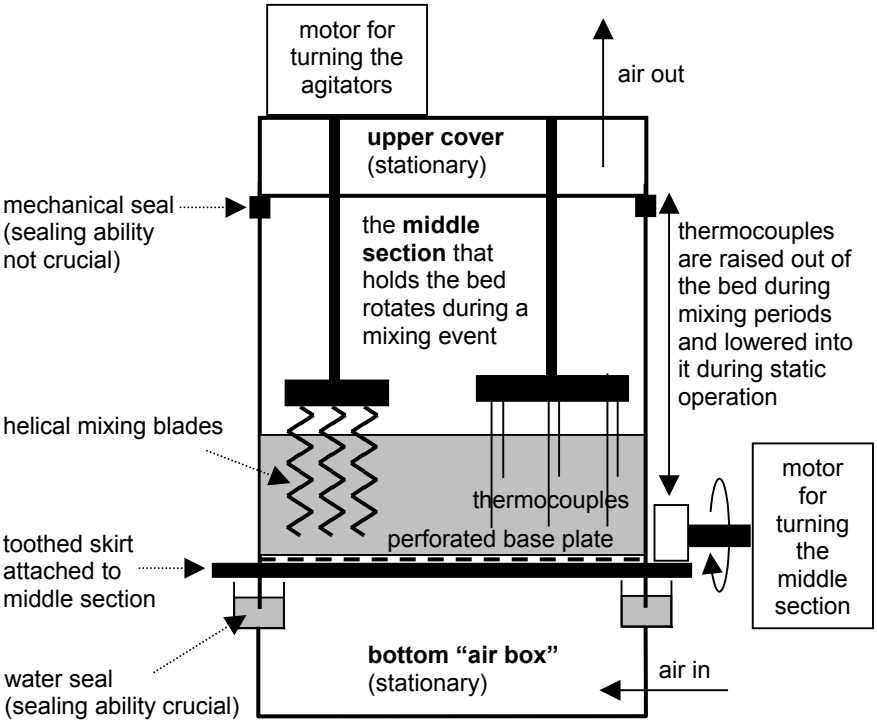

Fig. 10.4. The 200-kg capacity intermittently-mixed bioreactor used by Pérez-Correa and Agosin (1999). The upper cover and air box are maintained stationary by an outer frame while the middle section is rotated by the motor

The advantage of holding the agitators stationary and rotating the bed is that this simplifies the design of the agitator device. However, it also brings a disadvantage: The seal between the bottom and middle sections of the bioreactor, which move relative to one another, must not allow air to escape, otherwise air will leave the bioreactor without passing through the bed. The bioreactor shown in Fig. 10.4 has a water seal. However, since the depth of water in the seal is only 10 cm, this means that the pressure drop across the bed cannot be more than the equivalent of 10 cm of water; otherwise the air will simply bubble through the water in the seal and leave the bioreactor without passing through the bed. This limits the height of the bed that can be used and means that often mixing events are necessary simply to prevent the pressure drop from becoming too high, rather than being prompted by a need for water replenishment or temperature control.

Figure 10.5 shows typical data obtained from this bioreactor, for the growth of *Gibberella fujikuroi* on extruded wheat bran granules for the production of gibberellic acid. A bed height of 40 cm was used. It was possible to control most of the bed within the range of 25 to 30°C most of the time. However, there were hotspots formed, in which the bed temperature exceeded the temperature of the outlet air (compare Fig. 10.5(b) with Fig. 10.5(c)); these hotspots most likely represent regions that were receiving poor aeration due to channeling. The CO_2 production rate peaked at around 40 h (Fig. 10.5(c)). In an attempt to control the temperature in the bed at 28°C, the temperature (Fig. 10.5(c)), humidity (Fig. 10.5(e)), and flow rate (Fig. 10.5(f)) of the inlet air were manipulated. The pressure drop was kept well below 10 cm of water by the mixing events (Fig. 10.5(g)). These 30-min-long mixing events occurred, on average during a fermentation, once every 6 to 10 h, although during periods of high heat production they were as frequent as once every 4 h. Water needed to be replenished to replace evaporated water (Fig. 10.5(h)). This was done over the 30-min period of the mixing event, with the amount of water necessary being calculated from a set of mass balance equations.

10.3.2 Pilot-Scale Intermittently-Mixed Bioreactors

Pérez-Correa and Agosin (1999) also developed a bioreactor with a capacity for 50 kg of moist substrate (Fig. 10.6). This bioreactor used a similar strategy to that used in their larger scale bioreactor in the sense that mixing was achieved through movement of the bed past a number of fixed mixing blades. The bed was held within a perforated basket, 1.15 m in diameter and 0.28 m high, that was rotated when mixing was desired. It was necessary to have a seal between the basket and the body of the bioreactor to make sure that the air flowed through the basket and not around its sides. This bioreactor was capable of being operated aseptically. The whole lid could be raised to give access to the interior, but was hermetically sealed during the fermentation. This bioreactor was used for the production of gibberellic acid by *Gibberella fujikoroi* (Pérez-Correa and Agosin 1999) and for the production of *Trichoderma* (Agosin and Aguilera 1998).

In the 50-L bioreactor of Chamielec et al. (1994) and Bandelier et al. (1997), which is designed for sterile operation, the substrate bed is supported on a wire

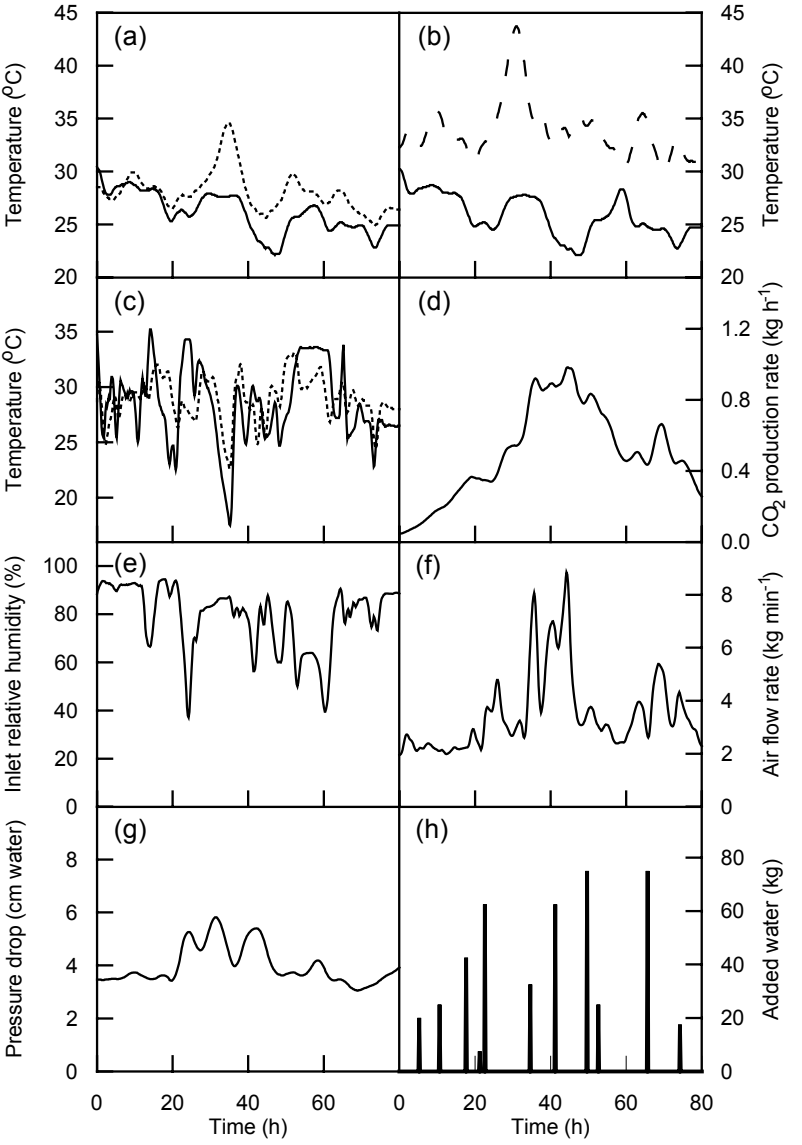

Fig. 10.5. Typical results from the 200-kg capacity bioreactor of Pérez-Correa and Agosin (1999). **(a)** Temperatures 10 cm from the wall at (——) 5 cm bed height and (- - -) 20 cm bed height; **(b)** Temperatures 10 cm from the center at (——) 5 cm bed height and (- - -) 20 cm bed height; **(c)** Air temperature at the (——) inlet and (- - -) outlet; **(d)** Rate of CO_2 production from the bioreactor, as an indicator of the overall growth rate; **(e)** Relative humidity of the inlet gas (controlled by the controller); **(f)** Flow rate of air into the bioreactor (controlled by the controller); **(g)** Pressure drop through the bed; **(h)** Addition of water during the fermentation (added over a 30 minute period during mixing events)

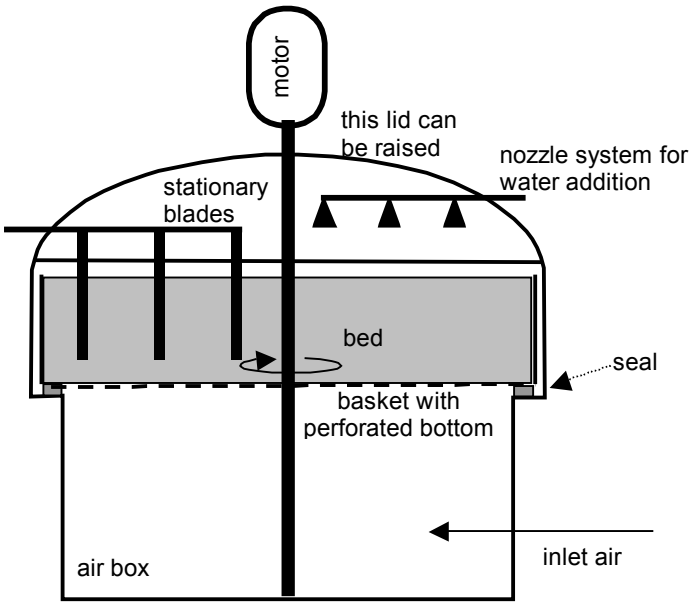

Fig. 10.6. The 50-kg capacity bioreactor of Pérez-Correa and Agosin (1999). Mixing is achieved by rotating the basket that holds the bed while maintaining the mixing blades stationary. The bed dimensions are given in the text

mesh, and is mixed by an agitator that undergoes a "planetary motion", that is, the agitator rotates around its central axis while this central axis simultaneously rotates around the central axis of the bioreactor (as in Fig. 10.1(a)). The bioreactor is fitted with a water jacket. It was used successfully in the production of gibberellic acid by *Gibberella fujikoroi*. The bioreactor contained 12 kg of moist wheat bran at a moisture content of 50% (wet basis). The bed was mixed for 10 s every 2 h, but this can be adapted as necessary according to process requirements. The air flow rate was 15 L min^{-1} kg-dry-$matter^{-1}$, which corresponds to a flow rate of 90 L min^{-1}. Since *Gibberella fujikoroi* is a relatively slow-growing organism, with the process taking 11 days, the major challenge was aseptic operation of the bioreactor. Given the low heat generation rate, temperature control was not difficult. The bed temperature was maintained within 1.3°C of the desired temperature of 28.5°C by maintaining the inlet air temperature at 28°C until 50 h and then reducing it progressively to 22°C at the end of the process (250 h).

The 50-L solids mixer of Schutyser et al. (2003b) presented in Sect. 9.3.1 could be used with intermittent mixing although use of this bioreactor in such fermentations has not yet been reported. Note that if the bioreactor were to be operated in the intermittent mixing mode, then aeration should be from top to bottom in order to ensure that the sides of the bed would be well aerated during the periods of static operation. Introducing air at the bottom of the bioreactor would tend to aerate only the central axis.

10.3.3 Laboratory-Scale Intermittently-Mixed Bioreactors

There are in fact very few reports about the use of intermittent mixing in forcefully-aerated bioreactors at pilot scale. The 7.6 cm diameter spouted-bed of Silva and Yang (1998) (see Fig. 9.5(b)) could be operated in either continuous- or intermittently-spouted mode. As noted in Sect. 9.3.2, intermittent spouting gave better results, presumably due to the lesser shear damage caused to the organism when compared to continuous spouting.

10.4 Insights into Mixing and Transport Phenomena in Group IVb Bioreactors

Intermittently-mixed bioreactors are typically static for most of the fermentation and therefore the principles of heat and mass transfer in them have many similarities to those of packed-bed bioreactors, or namely, axial and possibly radial temperature gradients will be established, the magnitude of which will depend on the combination of bed height, superficial air velocity and microbial growth rate (see Sect. 7.3). As pointed out in Sect. 10.2, the operating variables that intermittently mixed bioreactors have in addition to those of packed-bed bioreactors include the humidity of the inlet air, the strategy for initiating mixing events (which affects their frequency), and the duration and intensity of mixing events.

Little work has been done to characterize quantitatively the damage that intermittent mixing causes to the microorganism and the speed of recuperation, or not, of the microorganism after mixing. Schutyser et al. (2003a) reported a decrease of about 10% in the O_2 consumption rate immediately after mixing events in their intermittently agitated bioreactor, although they did not actually show the results.

Schutyser et al. (2003a) also investigated the timing of the first agitation event, concluding, at least in the case for fungi that produce significant amounts of aerial hyphae, that an early mixing event should be scheduled to prevent the formation of bound aggregates of substrate particles. If such aggregates are allowed to form, then they will be difficult to break apart in subsequent mixing events and O_2 supply to the particle surfaces within the aggregates will be greatly restricted. They showed that for *Aspergillus oryzae* growing on wheat, this "hyphae-disrupting" mixing event will be needed before it is necessary to make the first water addition, even if evaporation is the sole cooling mechanism.

There has been little effort to characterize experimentally the heat and mass transfer phenomena associated with the intermittent mixing mode of operation, although the modeling study of Ashley et al. (1999) suggests that this mode of operation can potentially lead to temperatures being reached that are higher than those that would be obtained in completely static (i.e., packed bed) operation (Fig. 10.7(a)). Immediately before a mixing event, the temperature profile in the bioreactor is identical to that which would be expected for packed-bed operation. In this situation the rate of heat removal is uniform at the different heights within the bed.

Immediately after a mixing event, due to the absence of an axial temperature gradient, the cooling effect is concentrated at the bottom of the bioreactor. As a result there is significant heat transfer to the air, warming it up to such a degree that it is ineffective in cooling the top of the bed. This allows the top of the bioreactor to heat up since in this region the metabolic heat is not being removed as fast as it is produced. The cooling effect travels up the bioreactor like a "wave-front" (indicated by the region within the dotted ellipse in Fig. 10.7(b)). Under the conditions simulated, it takes around 20 min for the cooling effect to reach the top of the bioreactor, during which time the temperature has risen to a value over 2 °C higher than the value for packed-bed operation. Once this cooling "wave-front" arrives, the temperature returns to the value for packed-bed operation.

Pressure drops will typically not be a crucial problem in intermittently-mixed, forcefully-aerated bioreactors, since the intermittent mixing will tend to disrupt the inter-particle hyphae that develop during static periods and squash aerial hyphae onto the surface of the substrate particles. After a mixing event the pressure drop through the bed will typically be significantly smaller than the pressure drop before the mixing event. In some cases the mixing event has been triggered exactly for this reason, that is, to reduce the magnitude of the pressure drop across the bed.

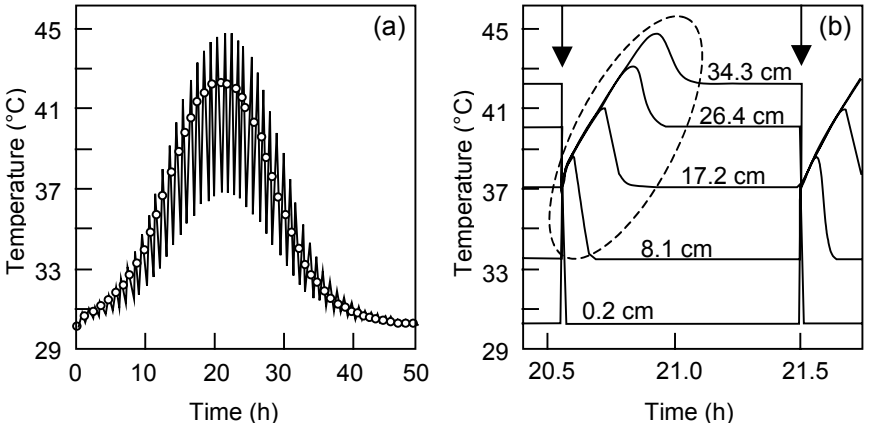

Fig. 10.7. Predictions of the modeling study of Ashley et al. (1999) about the temperatures reached in the intermittent-mixing mode of operation in a bioreactor or 34.5 cm height with a superficial air velocity of 0.0236 m s^{-1}. **(a)** Temperature profile predicted for a bioreactor mixed approximately every hour. At each mixing time the sensible energy in the bed is distributed evenly amongst the bed contents. The *hollow symbols* (o) represent the temperature profile expected for the absence of mixing events, that is, for simple packed-bed operation. **(b)** More detail of the temperature profiles at different heights in the bed, showing why the maximum bed temperature exceeds the value expected for packed-bed operation, which in this case is the value of 40.7 °C at the top of the bed immediately before the mixing event. The arrows mark the timings of the mixing events. The *dashed oval* shows how the "cooling wave-front" moves up the bed after a mixing event. Adapted from Ashley et al. (1999), with kind permission of Elsevier

10.5 Conclusions on Group IVb Bioreactors

Intermittently-mixed, forcefully-aerated bioreactors appear to have some potential, judging by the fact that several processes involving bioreactors that operate in this mode have been demonstrated at a reasonably large scale. They appear to offer some benefits in control of the conditions within the bed, while minimizing the deleterious effects that continuous mixing can have, at least for fungal processes.

Based on what is known to date, it would seem that the best strategy is not to try to use mixing of the bed directly as a temperature control strategy. For fungal fermentations such a strategy would lead to intolerably frequent mixing events. Rather, the mixing events should be used to:

- prevent undue aggregation of substrate particles, unduly high pressure drops, and the appearance of cracks and channels in the bed;
- replenish water in the bed in order to prevent low water activities in the bed from being one of the factors that limit growth.

Attempts to control the temperature in such bioreactors therefore should be focused on manipulation of the temperature, humidity, and flow rate of the inlet air. These have not been explored to any great extent, but Chap. 25 will present a mathematical model of an intermittently-mixed, forcefully-aerated bioreactor that can be used to explore the question of how best to operate such bioreactors in order to control the temperature.

Further Reading

Studies regarding the effect of intermittent mixing on aggregation of the substrate by fungal hyphae

Schutyser MAI, de Pagter P, Weber FJ, Briels WJ, Boom RM, Rinzema A (2003) Substrate aggregation due to aerial hyphae during discontinuously mixed solid-state fermentation with *Aspergillus oryzae*: Experiments and modeling. Biotechnol Bioeng 83:503–513

More detailed descriptions of the INRA Dijon and PUC Chile bioreactors

Agosin E, Perez-Correa R, Fernandez M, Solar I, Chiang L (1997) An aseptic pilot bioreactor for solid substrate cultivation processes. In: Wise DL (ed) Global environmental biotechnology. Kluwer Academic Publishers, Dordrecht, pp 233–243

Durand A, Chereau D (1988) A new pilot reactor for solid-state fermentation: Application to the protein enrichment of sugar beet pulp. Biotechnol Bioeng 31:476–486

Durand A, Renaud R, Maratray J, Almanza S, Diez M (1996) INRA-Dijon reactors for solid-state fermentation: Designs and applications. J Sci Ind Res 55:317–332

Fernandez M, Perez-Correa JR, Solar I, Agosin E (1996) Automation of a solid substrate cultivation pilot reactor. Bioprocess Eng 16:1–4