# **29 Design of the Air Preparation System for SSF Bioreactors**

*Oscar F. von Meien, Luiz F.L. Luz Jr, J. Ricardo Pérez-Correa, and David A. Mitchell* 

#### **29.1 Introduction**

All solid-state fermentation (SSF) bioreactor types potentially need an air preparation system. The need is most obvious for forcefully aerated bioreactors, since the conditions of the air at the inlet have a strong influence on the heat and mass transfer phenomena within the bed. However, even those bioreactor types that are not forcefully aerated can benefit from an air preparation system. For example, although it is possible to operate tray bioreactors by circulating air taken directly from the surroundings, it is likely that the process will operate better if conditioned air is circulated through the headspace.

The considerations guiding decisions about the air preparation system are different for SSF and submerged liquid fermentation (SLF). In SLF the rate at which air is blown into the bioreactor is calculated based on the  $O_2$  demand of the microorganism; heat removal considerations do not influence decisions about the aeration rate. In other words, in SLF good temperature control can be attained by circulating hot or cold water through water jackets and cooling coils, without any need to supply or remove heat in the air stream. This is possible due to the diluted nature and favorable heat transfer conditions within the liquid fermentation medium: the medium is typically well agitated and has a high thermal diffusivity.

In contrast, in SSF few alternatives are available for heating or cooling of the substrate bed other than manipulating the temperature, flow rate, and humidity of the inlet air. At first glance, it does not seem to be a difficult task, especially given the high heat removal capacity of evaporative cooling; however, we may be restricted in terms of the values that we can use for these two operating variables, even in a bioreactor operated in the intermittently-mixed mode. For example, if we supply air that is not saturated with water vapor to the bioreactor, this will improve evaporative heat removal but will also accelerate the drying of the bed, increasing the frequency with which water must be added. However, frequent water addition can be undesirable. If the bed is not mixed during the addition of water, then it will be almost impossible to ensure uniform distribution of the water, leading to flooded and dry regions within the bioreactor. Addition of water as a spray

while the bed is being agitated may allow uniform distribution of water, however, frequent agitation will typically be deleterious to the performance of processes that involve fungi due to the mechanical damage caused to fungal hyphae by shear and impact forces within the bed. In order to minimize this damage, the frequency of mixing and water addition events should be minimized. However, this means that we should use saturated air at the air inlet in order to minimize the evaporation rate. Therefore the operating strategy must seek to find those conditions that give a reasonable rate of heat removal without causing undue damage to the microorganism. Note that even if saturated air is to be used, it is not necessarily easy to keep the air saturated if one intends to vary its temperature.

In short, in SSF the air stream has a role that goes beyond the supply of  $O_2$ . It plays a fundamental role in heat removal and the design of an adequate air conditioning system is essential for good operation and control of an SSF bioreactor.

#### **29.2 An Overview of the Options Available**

The air stream not only must be supplied at proper conditions of temperature and humidity as addressed above, it also must overcome the pressure drop caused by the bed, piping, and other accessories. All these aims have to be achieved at low cost, since many SSF processes have low profit margins. At this point, we can examine some alternatives for air preparation, starting with the very simple alternative presented in Fig. 29.1.

The system presented in Fig. 29.1 will only be appropriate if aseptic conditions are not required, since it has only a simple dust collector, like those found in home air conditioning units. A porous plate is necessary for efficient air distribution in the humidifier. Porous stainless steel plates with high permeability are available in the market; they are very efficient but are not cheap. Sintered ceramic plates are also available; they are cheaper than metallic plates, however, they require a mechanical support in order to withstand the mass of the water in the tank. In this system the temperature of the air leaving the humidification tank will be close to the water temperature, therefore it is desirable to control the water temperature, which can be done using electrical resistance heaters and an ordinary thermostat.



**Fig. 29.1.** A simple configuration for an air preparation system in which the air is bubbled through a humidification tank

This simple system has three important features. Firstly, it is not practical to obtain air temperatures below the ambient temperature since no cooling unit is included in the system. Secondly, it will not allow a fast change of the temperature of the air fed into the bioreactor due to the high thermal inertia of the water mass. Thirdly, high air flow rates will cause the evaporation of large amounts of water and therefore water must be replenished periodically in order to prevent the tank from drying out.

Since the system presented in Fig. 29.1 does not allow a rapid manipulation of the air temperature, another possible arrangement is presented in Fig. 29.2.

Aseptic cultivation will be easier to achieve with this arrangement. Firstly, the cooling of the air before filtering will eliminate part of the microorganisms with the purged water. Secondly, the micro-porous filter will not allow particles larger than  $0.3 \mu$ m to pass, which is sufficient to remove fungal spores, bacterial cells, and any larger organisms.

In this system a pair of valves and two sets of electrical resistances can be used to control the air temperature. Typically the process will require saturated air at temperatures higher than the ambient during the lag phase while during the rapid growth phase it will be necessary to supply air at a temperature below the optimum temperature for growth in order to promote heat removal. The highest rates of heat removal will be obtained by supplying cold, dry air in order to promote evaporative cooling, however, this will also promote drying of the bed. It is possible to inject steam into the cold air; but it is not easy to generate steam at temperatures around say 20°C. Finally, it is important to note that it is not a simple matter to produce saturated air by direct mixing of steam and dry air, since it is not easy to design a mixing device that does not produce condensation.



**Fig. 29.2.** A configuration for an air preparation system that allows control of the temperature and humidity of the air supplied to the bioreactor

A third alternative is presented in Fig. 29.3, where a humidification column replaces the steam-air mixer. A well-designed humidification column will guarantee saturated air. The heater for producing cooler dry air that appeared in Fig. 29.2 is not present since only saturated air will be provided by this system. In this system the temperature of the outlet air will be very close to the water temperature, as was the case for the system presented in Fig. 29.1. It is therefore interesting to work with two reservoirs, one with hot water and other with cold water. A set of synchronized solenoid valves can be used to change from circulation of hot water to circulation of cold water and vice-versa, proving saturated air at a higher temperature at the beginning of the fermentation and saturated air at lower temperatures for cooling the reactor during the rapid growth period. It is important to remember that even with the use of saturated air the bed will still dry out, since the air is heated as it passes through the bed and therefore its capacity to carry water increases (see Fig. 4.3). In other words, the use of saturated air will reduce the frequency with which water must be replenished but water replenishment will still be necessary. Note that aseptic operation is unlikely to be feasible due to the difficulty in operating the entire humidification system (reservoirs and column) in an aseptic manner.

Of course, combinations and variations of the alternatives presented in this section can be worked out. The suggested configurations demonstrate the advantages and disadvantages of selecting a particular combination of devices. The decision for a particular arrangement must be based on criteria of economic performance of the process, which will depend on the capital cost of the selected devices and operating costs related to energy consumption for the various unit operations such as blowing the air, heating or cooling the air, producing steam, and heating water. In fact, great care should be taken in computing energy costs, since they can compromise the feasibility of the project, particularly when working with products that have low profit margins. The following sections give some further advice about various aspects of the design of the air preparation system.



**Fig. 29.3.** A configuration for an air preparation system that provides saturated air at either a hot or a cold temperature

#### **29.3 Blower/Compressor Selection and Flow Rate Control**

The air blower is likely to be responsible for a large proportion of the energy consumed in bioreactor operation and therefore selection of an appropriate blower is very important. In general, SSF bioreactors need high air flow-rates at low pressures. If the pressure drop of the whole system, including piping, accessories, and bioreactor, is equal to or less than 35 cm of water, a fan is the best device. The power consumed by a fan (*P*, kW) can be calculated as follows:

$$
P = 2.72 \times 10^5 F p,\tag{29.1}
$$

where *F* is the air flow-rate  $(m^3 h^{-1})$  and *p* is the operating pressure of the fan (cm-H<sub>2</sub>O). Manufacturers usually supply operational curves and installation details.

For higher pressure-drops, a centrifugal compressor would be the best choice, however, the energy consumption would be prohibitive in many cases. If possible, it is advisable to design and operate the bioreactor and air preparation system in such a manner as to minimize the pressure drop, such that it is possible to use a fan, rather than to work with a compressor.

Usually fans work at fixed velocity whereas the aeration requirements change over time. Therefore a flow control valve (FCV), placed in the air line between the blower and the bioreactor, is required. For air at low pressure a butterfly valve is the best choice. Centrifugal compressors can be operated in a similar manner or, alternatively, their velocity can be controlled with inverter drives.

Both fans and centrifugal compressors work under a "characteristic curve" that gives the flow rate provided by the equipment as a function of the pressure in the air line. Note that the pressure in the air line depends on the pressure drop suffered by the air as it flows from the blower, through the system, to the air outlet (that is, the pressure at the air inlet must be at least equal to the sum of the pressure at the air outlet and the pressure drop within the system). Both these types of blowers will provide larger flow rates at lower pressure, with the flow rate reducing as the pressure in the air line increases. The characteristic curve depends on the type and the design of the blower and should be provided by the manufacturer.

The required air flow rate for the bioreactor will depend on heat removal needs, as was clearly demonstrated in the various modeling case studies presented in Chaps. 22 to 25. The blower must be capable of producing the flow rate required at the time of maximum heat production within the substrate bed. Obviously, models of the type presented in these chapters are useful tools in deciding on the requirements of the blower.

Note that the pressure drop that the system must be capable of overcoming is that which is present in the system at the time of maximum heat production. Potentially, the pressure drop as air flows through the bed can make a significant contribution to the overall pressure drop in the aeration system in those bioreactors in which the bed is forcefully aerated. The pressure drop across the bed depends on the substrate, the microorganism, and their interaction during the process and, due to our relatively poor understanding of these phenomena, it is not possible to use a set of theoretical calculations to predict the magnitude of the pressure drop

that can be expected. Therefore, some experimental assays at laboratory scale will be necessary in order to estimate the pressure drops for a particular system. In systems in which the bed is agitated, a maximum allowable pressure drop can be set as a parameter for triggering mixing events, and this should prevent the pressure drop across the bed from reaching high values.

The best strategy is to select the equipment that provides the largest pressure range for the maximum required flow-rate, this flow-rate being deduced from the energy balance model. It is then necessary to check whether the equipment will operate economically in terms of energy consumption at the required combination of pressure and flow rate. If energy consumption is too high then possibly an inferior blower will need to be selected. This may not be capable of meeting the aeration needs during the periods of peak heat generation, so the performance of the process may be deleteriously affected. As stated above, many of the products obtained by SSF have low profit margins; in this case the energy consumption of the aeration system is a crucial factor in determining process profitability.

#### **29.4 Piping and Connections**

The specifications for the piping used in the air line will be affected by the sterility requirements of the process. If a high degree of sterility is required then the piping will need to be able to withstand either steam or chemical sterilization before each fermentation: For example, it may be necessary to use stainless steel. If sterility is not a crucial issue, then less resistant materials can be used.

Also, given that saturated air will typically be supplied to the bioreactor, it is possible that condensation will occur within the air line. It is advisable to have strategically placed drains (or "purges") in the air line in order to remove this water intermittently during the process. Rotation of the bioreactor can complicate the aeration system. If the bioreactor rotates while air is introduced into it, then a rotating seal will be necessary between the air line and the bioreactor body. If the inlet and outlet air lines are removed before rotation of the bioreactor, then it is necessary to have a connection that is fully airtight when connected, but simple to remove and re-attach. Also, manually operated butterfly valves may be necessary on the air inlet and air outlet of the bioreactor in order to prevent substrate from flowing out of the bioreactor as it is rotated.

#### **29.5 Air Sterilization**

The selection of an appropriate filter type will be based on considerations of minimizing the pressure drop for a desired air flow rate while removing very small particles. It may also be influenced by the types of filters that local suppliers actually have available.

At laboratory scale, contamination is typically somewhat easier to control while at large scale sterilization and prevention of contamination is more complicated. In fact, contamination may be one of the most frequent operational problems for those processes that require aseptic operation of the bioreactor. During the production of gibberellic acid by *Gibberella fujikuroi* in the 200-kg capacity pilot scale bioreactor of PUC, Chile (see Sect. 10.3.1.3 and Fig. 10.4) there were frequent contamination problems when the air system contained only a pre-filter and an absolute filter with a cut-off of  $0.3 \mu$ m. These contamination problems were reduced significantly by (1) the installation of UV lamps in the air duct between the filter system and the bioreactor and (2) chemical sterilization of the air duct system prior to each fermentation run.

In designing an appropriate system, several factors will need to be considered, such as capital and operating costs, the effectiveness of removal of microorganisms, the potential for failures in the system (such as the rupturing of filters), and the pressure drop contributed by the air sterilization system. It is also important to consider at what stage of the air preparation system the sterilization should occur. If the air is dry at the time of sterilization, then there should be no problem of wetting of filters, however, any subsequent humidification steps will need to be done aseptically if aseptic bioreactor operation is required for the particular process in question.

#### **29.6 Humidification Columns**

Usually, it is hard to find a supplier for humidification columns such as that shown in Fig. 29.3, so they are typically custom-made from a design supplied by the purchaser. Within the interior of humidification columns the water flows downwards through a bed of packing that ensures a high superficial area of contact between the air and water, in order to guarantee saturation at the air outlet.

The column diameter is chosen obeying the criteria of minimum pressure drop and avoidance of flooding. Flooding, namely the accumulation of water at the top of the column, happens in packed humidification columns when the air velocity through the column becomes large enough to stop the liquid from flowing down the bed. The height of the column necessary to ensure saturation can be calculated from mass and energy balances. Once the dimensions are determined then the pressure drop across the column can also be calculated.

Sources of advice on how to design humidification columns are given in the Further Reading section at the end of the chapter.

## **29.7 Case Study: An Air Preparation System for a Pilot-Scale Bioreactor**

The air preparation system presented in Fig. 29.3 represents a compromise between technical specifications and costs in the sense that, while it does not allow as flexible a control of the conditions of the air supplied to the bioreactor as the system shown in Fig. 29.2, it will be much cheaper to build and operate than that system and it will allow better control than the system presented in Fig. 29.1. On the basis of these considerations, the system shown in Fig. 29.4 was recently constructed for a pilot-scale SSF bioreactor with a 200-L substrate bed. Although the bioreactor has not yet gone into operation, it is worthwhile to describe briefly the calculations that were used to design the system.

**Maximum air flow rate requirement.** The maximum air flow rate that would be needed was calculated on the basis of heat removal considerations. Assuming logistic growth kinetics, the maximum heat generation rate  $(R<sub>O</sub>, kJ h<sup>-1</sup>)$  is given by (Mitchell et al. 1999):

$$
R_Q = \rho_b X_{\text{max}} Y_q \mu_{\text{max}} V_b / 4. \qquad (29.2)
$$

The substrate packing density  $(\rho_b)$  was estimated as 350 kg-dry-substrate m<sup>-3</sup>, the maximum biomass content  $(X_{max})$  as 0.125 kg-dry-biomass kg-dry-substrate<sup>-1</sup>, the metabolic heat yield coefficient  $(Y_q)$  as  $10^7$  J kg-dry-biomass<sup>-1</sup>, the maximum value of the specific growth rate constant  $(\mu_{max})$  as 0.324 h<sup>-1</sup>, and the bed volume  $(V_b)$  as 0.2 m<sup>3</sup>. The calculation gave a value of  $R_Q$  of 7.1 MJ h<sup>-1</sup>.

A conservative estimate of the capacity of the air to remove heat from the bed was made as  $Q_{rem} = 5$  kJ kg-air<sup>-1</sup> °C<sup>-1</sup>. This represents the sum of the heat capacity of humid air  $(-1.0 \text{ kJ kg-dry-air}^{-1}$ <sup>o</sup>C<sup>-1</sup>) and the contribution of evaporative cooling of " $\lambda$ .( $dH_{sat}/dT$ )" where  $\lambda$  is the enthalpy of evaporation of water (2500 kJ kgwater<sup>-1</sup>) and  $dH_{sat}/dT$  (kg-vapor kg-dry-air<sup>-1</sup> °C<sup>-1</sup>) is the change in the watercarrying capacity of air with a change in temperature (see Sects. 18.5.2.2 and 19.4.1). Use of Eq. (19.20) shows that  $dH_{sat}/dT$  varies from 0.0016 kg-vapor kg- $\frac{d}{dx}$  dry-air<sup>-1</sup> °C<sup>-1</sup>at 30°C to 0.0048 kg-vapor kg-dry-air<sup>-1</sup> °C<sup>-1</sup> at 50°C. Using the value of *dHsat*/*dT* at 30°C therefore leads to a more conservative estimate and with this value " $\lambda$ .( $dH_{\text{sat}}/dT$ )" is calculated as 4 kJ kg-air<sup>-1</sup> °C<sup>-1</sup>.

The mass flow rate of air required ( $W_{air}$ , kg h<sup>-1</sup>) was then calculated as:

$$
W_{air} = \frac{R_Q}{Q_{rem} \Delta T},\tag{29.3}
$$

where  $\Delta T$  is the maximum allowable rise in temperature of the air as it flows through the bed. This was taken as 5°C. Substituting the values of  $R<sub>O</sub>$  and  $Q<sub>rem</sub>$ ,  $W_{air}$  was calculated as 283.5 kg h<sup>-1</sup> (235 m<sup>3</sup> h<sup>-1</sup> at 15°C and 1 atm). Since a conservative value was used for  $dH_{\text{sat}}/dT$ , this is probably an overestimate, but will therefore allow a reasonable margin for error.



**Fig. 29.4.** Configuration used for an air preparation system for a 200-L bed capacity pilotscale SSF bioreactor

**Filter.** The filter selected is a HEPA (High Efficiency Particulate Air filter), with a minimum efficiency of 99.97% for particles larger than  $0.3 \mu$ m. The dimensions of the filter cartridge are 305 mm by 305 mm, with an overall thickness of 78 mm. The pressure drop caused by this filter is equal to a 25 mm water column. In order to protect the filter, a dust pre-filter is included in the cartridge.

**Reservoirs.** The reservoirs of warm and cool water are large  $(1 \text{ m}^3 \text{ each})$  in order to make the regulation of their temperature easier, that is, the reservoirs have a large thermal inertia. Note that the intention is to maintain the water temperature of each reservoir constant at the desired set point during the whole fermentation; manipulation of the reservoir temperatures is not part of the control strategy. For the saturation, at 40°C, of 283.5 kg-air h<sup>-1</sup>, the evaporation rate will be 7.7 kg-H<sub>2</sub>O  $h^{-1}$  (an inlet relative humidity of 80% was assumed, based on local weather information; obviously it can vary significantly with location). If the make-up water is provided at this rate but at 10°C, then heat must be added at the rate of 270 W in order to maintain the temperature of the water in the warm reservoir at 42°C. By placing a resistance heater of 1000 W in the warm water tank and one of 700 W in the cool water tank, the water temperature can be controlled easily (this can be assured even without making specific calculations about heat losses since the reservoirs are made of polypropylene, which has insulating properties, and also the tanks are covered to prevent evaporation to the surrounding air). This extra capacity also allows a faster warm-up of the reservoir at the beginning of a fermentation.

**Humidification column.** A computer program was used to determine the minimum design necessities for the humidification column. For a column diameter of 40 cm, a water flow rate through the column of 1.5  $m<sup>3</sup> h<sup>-1</sup>$ , a packing consisting of 25 mm Raschig rings, an inlet water temperature of 42ºC and an inlet air temperature of 20°C, Fig. 29.5 shows the resulting predictions for the air and water temperature and the air humidity as functions of height within the column.

A 35 cm high column is sufficient to saturate the air at 40°C. For this height the pressure drop is equivalent to 2 mm of water. The air temperature can be manipulated by changing the water temperature or flow rate, although any such change will be done between different fermentation runs and not during a given fermentation (temperature change during a run is impractical due to the large thermal inertia of the reservoir). With this particular set of parameters, the air velocity through the column is  $0.45 \text{ m s}^{-1}$ , far below the air velocity that would cause flooding (1.5) m s<sup>-1</sup>). Our column was designed with a packing height of 70 cm in order to guarantee saturation of the air even if we use different operating conditions.



**Fig. 29.5.** Predicted performance of the humidification column operating under the conditions given in the text. **(a)** Temperatures of the  $(-)$  air and  $(- -)$  water as a function of height within the column; **(b)** Humidity of the air as a function of height in the column. (- - -) saturation humidity, which changes due to the change in air temperature as the air passes through the column;  $(\rightarrow)$  actual humidity of the air. This graph is used to decide on the height of the bed in the humidification column

### **Further Reading**

*Humidification*

- Coulson JM, Richardson JF (1990) Chemical engineering, 4th edn, Vol. 1, Pergamon, Oxford, Chap 13, pp 621-672
- McCabe WL Smith JC (1976) Unit operations of chemical engineering, 3rd edn, McGraw-Hill, New York, Chap 19, pp 596-621