6 Group I Bioreactors: Unaerated and Unmixed

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6.1 Basic Features, Design, and Operating Variables for Tray-type Bioreactors

Group I bioreactors, or "tray bioreactors", represent the simplest technology for SSF. They have been used for many centuries in the production of traditional fermented foods such as tempe and in the production of soy sauce *koji*. However, this chapter does not review these applications. Readers interested in traditional fermented foods should consult the reading listed at the end of the chapter. The current chapter considers tray bioreactors as candidates in the selection of bioreactors for newly-developed SSF processes. Trays may be appropriate for a new process if the product is not produced in very large quantities, if a "produced-packet" of fermented product can be sold directly, or if labor is relatively cheap.

The basic design features of tray-type bioreactors have already been presented in Chap. 3.3.1. Figure 6.1 shows these features in more detail. Some possible variations in the design include:

- the tray chamber may be relatively small, such as an incubator, or it may be a room large enough for people to enter;
- the tray may be constructed of various different materials, such as wood, bamboo, wire or plastic. In fact, a plastic bag might be used instead of a rigid tray;
- the bottom and sides of the tray may be perforated or not;
- water-cooled heat exchange surfaces might be incorporated.

The available design features for tray-type bioreactors are:

- the dimensions of the tray, namely length, width, and height;
- the positioning of the trays within the bioreactor;
- the presence of cooling surfaces within the tray chamber.

The available operating variables are:

- the temperature, humidity, and flow rate of the air entering the tray chamber and the velocity of circulation past the tray surface;
- if cooling surfaces are present, then the temperature of the cooling water.

Note that, although this type of bioreactor is nominally static, the bed may be mixed infrequently. For example, it is typical for the tray contents to be turned by hand once or twice per day.

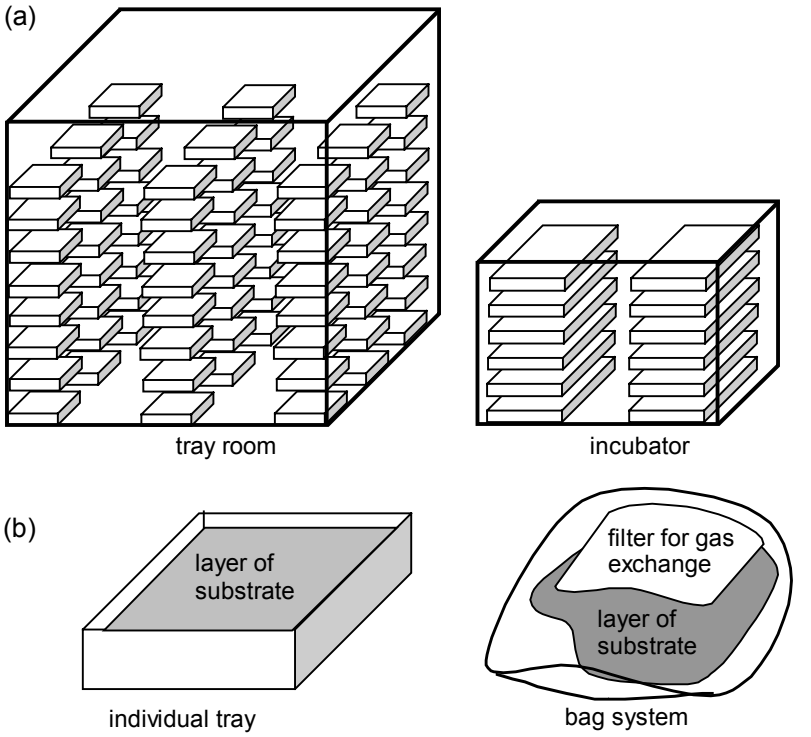

Fig. 6.1. Basic design features of tray-type bioreactors and possible design variations. **(a)** Different tray chambers, including tray rooms and incubators, in which the trays are arranged on shelves. **(b)** Different basic tray designs. The tray on the left could be made of wood, plastic, bamboo, or wire. The plastic bag on the right could be made entirely of a gas-permeable plastic, or could contain a filter insert that allows gas exchange

6.2 Use of Bag Systems in Modern Processes

Typically for newly-developed processes, plastic bags will be used, employing special plastics that allow the exchange of O_2 and CO_2, but do not allow the exchange of water, thus allowing the microorganism to respire but preventing the bed from drying out. The great advantage of this system over the traditional technology of open trays is that the plastic prevents contaminants from entering the bed. Either the whole bag may be made of this plastic, or the bag may be made of

a gas-impermeable plastic and have a "window" made of a special plastic, paper, or fabric. This technology has been used for over 20 years to produce spore inoculum for the *koji* process (Lotong and Suwanarit 1983), although of course it is not an appropriate technology for the production of soy sauce *koji* itself in modern processes, where individual batches are of the order of several tons. However, it may be appropriate if smaller volumes are produced. For example, in Australia, a biopesticide based on spores of *Metarhizium anisopliae* is produced on rice grains within "self-aerating bags" (Milner 2000). In 1999, at the commercial trial stage, 9 tons of product was produced, which corresponds to a productivity of 25 kg per day, averaged over 365 days.

Cuero et al. (1985) used micro-porous plastic bags, consisting of polypropylene with 0.4 μm pores. The bags can be autoclaved. They allow gas and water vapor exchange, but neither the release of spores nor the entry of contaminants. Due to the fact that the bags allowed water vapor exchange, the bags were incubated in a high humidity environment (95% relative humidity).

6.3 Heat and Mass Transfer in Tray Bioreactors

Depending on the situation, it may be appropriate to consider either an individual tray or the whole-tray chamber as the bioreactor. For example, it would be appropriate to treat the whole tray chamber as the bioreactor when the trays are open to free gas and water exchange with their surroundings and the temperature and humidity of the air in the tray chamber are carefully controlled.

The question about optimum design of tray chambers has received little attention. For example, quantitative information is not available about the best way to position trays in the chamber. As a result, it is not possible to state what is the best spacing to leave between trays in order to maximize volumetric productivity (that is, the amount of product produced per unit volume of tray chamber). Most attention has been given to the individual trays.

As a generalization, within an individual tray, large O_2 and temperature gradients will arise in the substrate layer during the fermentation. The following subsections outline what is known about the limitations on O_2 and heat transfer within tray bioreactors.

6.3.1 Oxygen Profiles Within Trays

Since in a tray bioreactor air is not blown forcefully through the trays, O_2 and CO_2 can only move within the bed by diffusion. Potentially, due to the temperature gradients that arise, there could be natural convection within the void spaces within the bed, although this has not been studied. This discussion will focus on O_2. Similar considerations apply to CO_2, although it will typically be diffusing in a direction opposite to that of O_2.

The limitation of O_2 movement in the bed to diffusion through the void spaces, coupled with its simultaneous uptake by the microorganisms at the particle surfaces, leads to the establishment of O_2 concentration gradients within the void spaces (Fig. 6.2). Rathbun and Shuler (1983) noted O_2 gradients within the gas phase of a bed of tempe (which involves the cultivation of the fungus *Rhizopus oligosporus* on cooked soybeans) of the order of 2% (v/v) cm^{-1}. This represents a drop equal in magnitude to 10% of the gas phase O_2 concentration in air (~21% (v/v)) over 1 cm. Of course, the exact shape of the spatial O_2 concentration profile will depend on whether the bottom of the tray is perforated or not and the rate at which O_2 is being consumed by the organism.

Ragheva Rao et al. (1993) proposed an equation to estimate the maximum depth that a tray could be in order for the O_2 concentration not to fall to zero at any part in the tray during a fermentation. They referred to this depth as the critical depth (D_c, cm):

$$D_c = \sqrt{\frac{2DCY_{XO}}{R_{XM}}}. \tag{6.1}$$

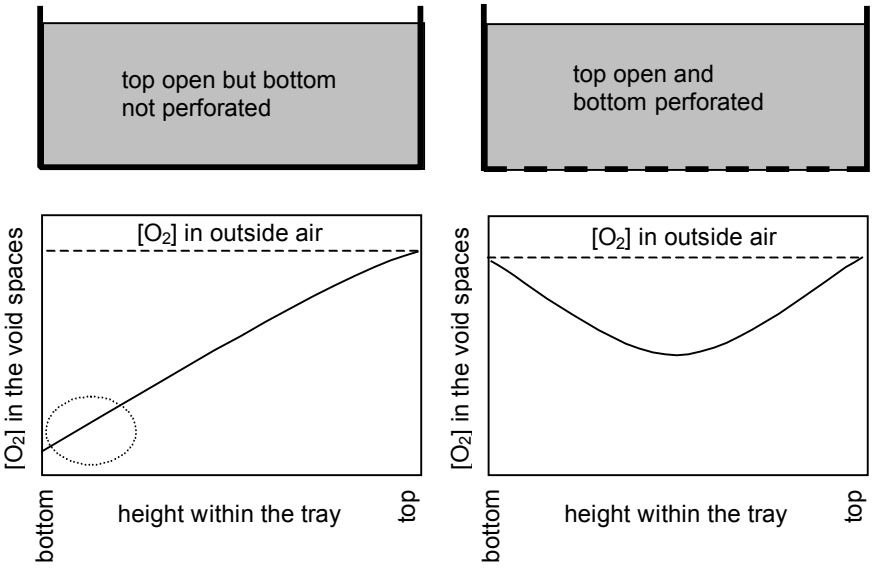

Fig. 6.2. O_2 concentration gradients within trays. Note that the spatial gradients will change over time, depending on the rate of growth of the microorganism. In the bioreactor with an unperforated bottom, O_2 limitation will be the factor that has the greatest influence on growth at the bottom of the bed (the area indicated by the dotted circle) since heat removal through the bottom of the bed will control the temperature in this region reasonably well

In Eq. (6.1) Y_{XO} is the yield coefficient of biomass from O_2 (g-dry-biomass g-O_2^{-1}), C is the O_2 concentration in the surrounding atmosphere (g cm^{-3}), D is the effective diffusivity of O_2 in the bed (cm^2 h^{-1}), and R_{XM} is the maximum growth rate (g-dry-biomass cm^{-3}-bed h^{-1}). Ragheva Rao et al. (1993) estimated D as 0.03 cm^2 s^{-1} and Y_{XO} as 1.07 g-dry-biomass g-O_2^{-1}. In dry air at 25°C and 1 atm pressure, C will be 2.7×10^{-4} g cm^{-3}. Using experimental estimates for R_{XM}, they concluded that the critical depth would be of the order of 2.4 cm. For a tray with a perforated bottom, oxygen can penetrate this distance from both the top and bottom surfaces, meaning that the bed depth in the tray can be twice the critical bed depth, namely 4.8 cm. This can be taken as a typical value for trays, although of course the exact value is influenced not only by the growth rate but also by the effective diffusivity of O_2 within the bed, which will decrease as the biomass grows into the inter-particle spaces during the fermentation.

6.3.2 Temperature Profiles Within Trays

Rathbun and Shuler (1983) found temperature gradients as high as 1.7°C cm^{-1} within a static bed of tempe, while Ikasari and Mitchell (1998) measured temperatures as high as 50°C at 5 cm depth during the cultivation of *Rhizopus oligosporus* on rice bran in a tray within a 37°C incubator.

Szewczyk (1993) derived a simplified equation that can be used to describe the temperature profile within a tray bioreactor, from the central plane ($z=0$) to the surface ($z=1$), when the top and bottom half of the tray are identical, that is, in the situation shown in Fig. 6.3(a):

$$T=\frac{(T_s+273)+\Theta+N_{Bi}(T_a+273)}{N_{Bi}+1}+\frac{N_{Bi}}{N_{Bi}+1}\left(T_s-T_a+\Theta\right)z-z^2\Theta\,, \tag{6.2}$$

where T_s and T_a are the temperatures of the bed surface and surrounding air (°C), respectively. The spatial coordinate z is expressed as a dimensionless fraction of the total bed height (Z, m). N_{Bi} is the Biot number, given by $\alpha.Z/k$ where α is the heat transfer coefficient for bed-to-air heat transfer at the top of the bed (W m^{-2} °C^{-1}), and k is the thermal conductivity of the bed (W m^{-1} °C^{-1}). Finally, the symbol Θ represents the temperature difference that would occur between the bottom of the solid bed and the tray surface if there were no heat transfer through the bottom of the tray. It is given by the following equation:

$$\Theta=\frac{R_Q Z^2}{2k}\,, \tag{6.3}$$

where R_Q is the volumetric heat production rate (W m^{-3}). It is not simple to apply Eq. (6.2), since the surface temperature of the tray needs to be known. The surface temperature depends on the value of the heat transfer coefficient α, but α also appears in Eq. (6.2), within N_{Bi}. A more complex modeling approach is needed to relate T_s and α. Szewczyk (1993) used such a model to derive the relationship

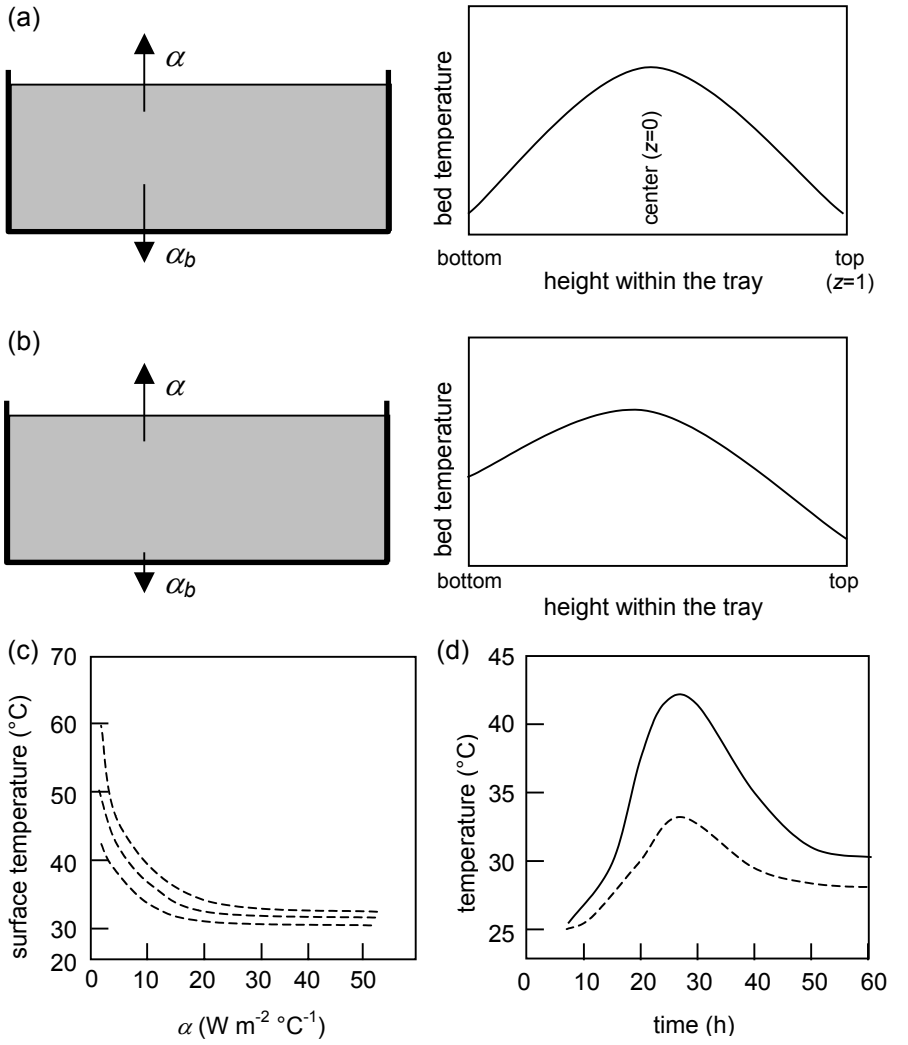

Fig. 6.3. (a) The temperature profile that would be expected when the bed-to-air heat transfer coefficients (α and α_b) at the top and bottom of the tray are identical. **(b)** The temperature profile that would be expected when the bed-to-air heat transfer coefficient at the bottom of the tray (α_b) is smaller than that at the top of the tray (α). **(c)** The effect of the bed-to-air heat transfer coefficient at the upper tray surface on the surface temperature, for three different heat generation rates (from bottom to top the curves represent 20, 40, and 60 W kg^{-1}, for a 6-cm-high bed of the type shown in Fig. 6.3(a) that is incubated in a 100% relative humidity atmosphere at 30°C. **(d)** How the temperatures at the center of the bed (——) and the bed surface (– – –) might typically be expected to vary over time, for the case where $\alpha = 10$ W m^{-2} $°C^{-1}$. This figure is based on data provided by Szewczyk (1993)

shown in Fig. 6.3(c). The temperature profile within the bed will depend on the relative values of the heat transfer coefficients at the top and bottom of the bed. If they are equal, then the profile will be symmetrical about the center plane of the bed (Fig. 6.3(a)). If not, then the profile will be asymmetrical (Fig. 6.3(b)). The surface temperature of the bed is greatly affected by the heat transfer coefficient α at values below 10 W m^{-2} $°C^{-1}$. Above this value, further increases in the heat transfer coefficient have little effect (Fig. 6.3(c)). The value of α will depend on the velocity at which air is circulated past the tray surface. Szewczyk (1993) simulated the growth of *Aspergillus niger* on wheat bran in a tray, with a value of α of 10 W m^{-2} $°C^{-1}$. At the time of peak heat generation, the center of the bed was 10°C hotter than the surface (Fig. 6.3(d)).

6.3.3 Insights from Dynamic Modeling of Trays

No modeling case study will be presented for trays in this book and therefore this section will discuss the insights that dynamic mathematical models of tray bioreactors have given into the relative importance of temperature and O_2 limitations in controlling the performance of trays.

Rajagopalan and Modak (1994) developed a model to describe heat and mass transfer in trays, which included the various processes shown in Fig. 6.4. They used their model to investigate the relative importance of high temperatures and low O_2 concentrations in determining the specific growth rate in a 6.4-cm-high tray. Since the tray was assumed to be symmetrical around the center plane it was only necessary to consider a depth of 3.2 cm from the surface to the central plane. Their results are shown in Fig. 6.5. In interpreting these results, it must be remembered that the overall growth rate (R_X, kg-dry-biomass m^{-3} h^{-1}) is a combination of the biomass density (X, kg-dry-biomass m^{-3}) and the specific growth rate according to the following equation:

$$R_X = \mu X\left(1-\frac{X}{X_{max}}\right), \tag{6.4}$$

where X_{max} (kg-dry-biomass m^{-3}) is the maximum possible value of the biomass density. The specific growth rate constant μ (h^{-1}) is affected by both temperature and the biofilm O_2 concentration according to the relationship:

$$\mu = \mu_{max}\mu_{FT}\mu_{FO}\,. \tag{6.5}$$

In this equation, μ_{max} (h^{-1}) is the maximum value that the specific growth rate constant can have, that is, its value under optimal conditions for growth. On other hand, μ_{FT} and μ_{FO} are dimensionless fractions, that is, they vary between 0 and 1. The parameter μ_{FT} describes the limitation of growth by deviations from the optimum temperature of 38°C while μ_{FO} describes the limitation of growth at low O_2 concentrations.

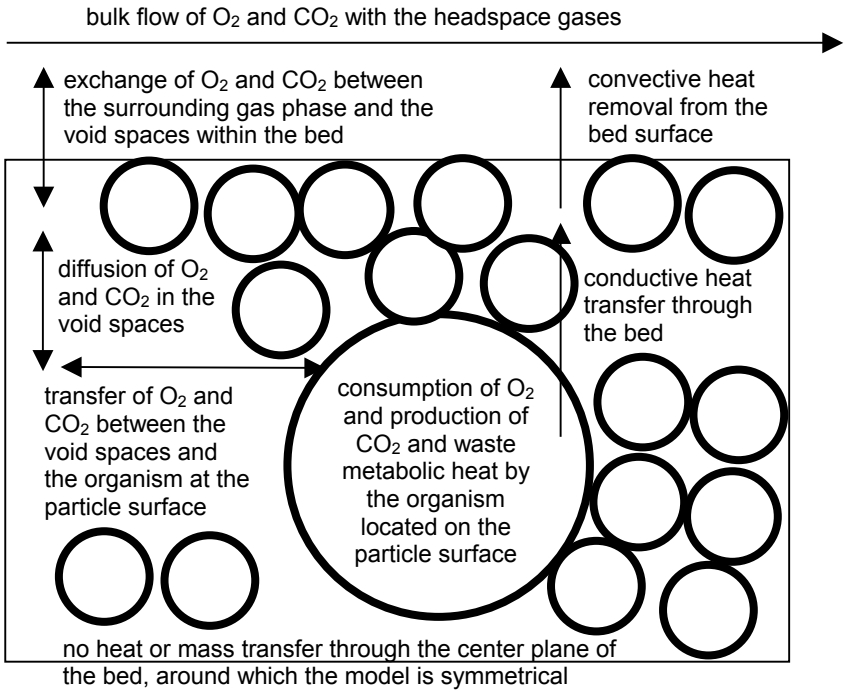

Fig. 6.4. Heat and mass transfer processes described by the model of Rajagopalan and Modak (1994). Note that for simplicity, it was assumed that the whole biofilm was at the same O_2 concentration, although in reality there would be an O_2 concentration gradient due to the simultaneous diffusion and consumption of O_2

A key prediction of this modeling work of Rajagopalan and Modak (1994) is that limitation of growth due to lack of O_2 occurs even though the gas phase O_2 concentration never falls to very low values; in their simulations the gas phase O_2 concentration was always two-thirds or more of the O_2 concentration in the surrounding atmosphere, regardless of time or depth (Fig. 6.5(b)). Since the organism within the biofilm can consume O_2 much faster than the rate at which O_2 can transfer from the gas phase to the biofilm, biofilm O_2 concentrations can fall to low levels (Fig. 6.5(d)). This occurs at the top of the bed where, due to the fact that the temperature remains near to the optimum for growth since it is effectively cooled by the surrounding atmosphere (Fig. 6.5(a)), the organism grows rapidly and consumes the O_2 in the biofilm, reducing it to levels that significantly decrease the specific growth rate. In this case, the growth of the biomass is controlled by the rate at which O_2 is transferred to the biofilm ((Figs. 6.5(e) and (f)).

The highest temperatures occur at the central plane of the bed, and these are sufficiently high to decrease the specific growth rate significantly (Fig. 6.5(c)). Indeed, due to the fact that the high temperatures cause low growth rates in this

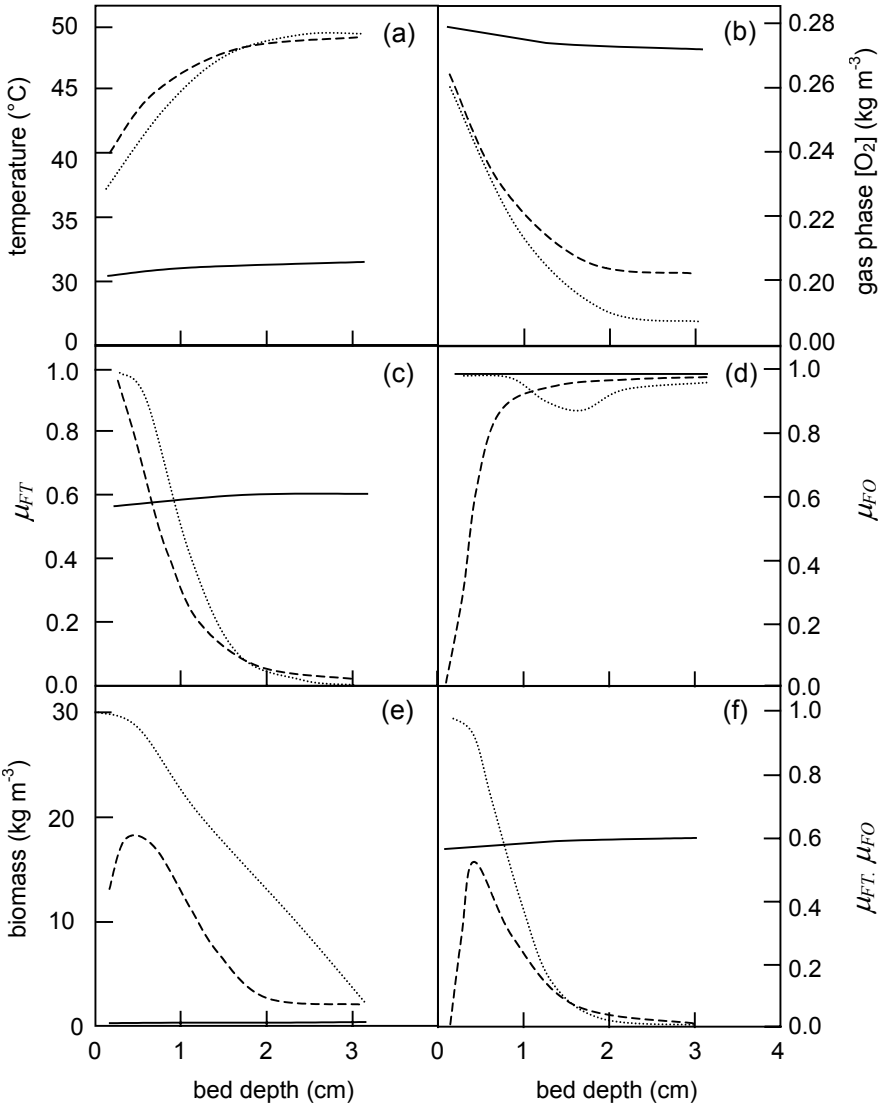

Fig. 6.5. Predictions of the model of Rajagopalan and Modak (1994). Key: (——) 20 h; (– – –) 60 h; (·····) 100 h. Note that the top of the tray is represented by zero bed depth and the center plane corresponds to a bed depth of 3.2 cm. The fractional modifiers of the specific growth rate constant (μ_{FT} and μ_{FO}) are explained in the text (see Eq. (6.5)). Adapted from Rajagopalan and Modak (1994), with kind permission from Elsevier

region, the rate of O_2 consumption in the biofilms within this region is sufficiently low that the O_2 transfer from void space to biofilm can maintain a high O_2 concentration in the biofilm. In other words, in the center of the bed, temperature is the most important factor limiting growth. In fact, the temperature limitations are so severe in the middle of the bed that even after 100 h (when the organism would easily have reached its maximum concentration if it had been growing at the maximum possible specific growth rate), much of the bed has a biomass concentration significantly lower than the maximum biomass concentration (Fig. 6.5(e)).

The work of Smits et al. (1999) confirms that O_2 levels in the inter-particle spaces will generally not be a limiting factor. They used a heat and mass transfer model to investigate how the relative importance of O_2 limitation and temperature limitation depends on the thermal conductivity of the bed and the effective diffusivity of O_2 within the pores of the bed. For the growth of *Trichoderma reesei* in a tray, their model predicted that O_2 diffusion within the inter-particle spaces would only become limiting at a 10-cm bed depth if the effective diffusivity of O_2 in the bed was less than 4×10^{-6} m^2 s^{-1} and the thermal conductivity was greater than 0.45 W m^{-1} $°C^{-1}$. The effective diffusivity of O_2 in a bed with biomass at its maximum density is actually of the order of 4×10^{-6} m^2 s^{-1} (Auria et al. 1991) meaning that O_2 supply to a 10-cm bed depth can potentially become limiting, although this will only happen if there is a combination of high biomass concentration and high growth rate.

Smits et al. (1999) also modeled the diffusion of water vapor in the void spaces of the bed. When it was assumed that the air surrounding the tray was maintained at a high humidity, then the combination of metabolic water production with the relatively slow water vapor diffusion meant that the predicted water content of the substrate remained above the initial value. Under such conditions there will be no danger of the growth rate being limited by low water activities of the solid substrate. Of course, as Smits et al. (1999) point out, water could become limiting if the trays were incubated in an environment of low relative humidity. This would complicate operation since it would be necessary to periodically spray water onto the bed and to mix it in.

Rajagopalan and Modak (1994) used their model to investigate the effect of the height of the bed and the temperature of the surroundings on the average biomass concentration in the bed after 100 h of cultivation. For bed heights of 1.6 cm and less, the average biomass content reached its maximum possible value (i.e., 30 kg-dry-biomass m^{-3}) within 100 h only when incubated at temperatures near the optimum temperature of 38°C, namely at 35°C and 40°C (Fig. 6.6). This was because with these small bed thicknesses the bed temperature remained near the incubation temperature.

At a bed height of only 3.2 cm, the maximum biomass concentration was achieved by 100 h only when the bed was incubated at temperatures below the optimum temperature (i.e., between 30-35°C). Of course this lower outside temperature, combined with the metabolic heat production, combined to maintain the whole of the bed near the optimum temperature of 38°C.

For a bed height of 6.4 cm it was impossible to maintain the majority of the bed near the optimum temperature for growth, as evidenced by the fact that the highest

value for the average biomass content at 100 h was only 16.6 kg-dry-biomass m^{-3}, obtained with incubation at 30°C. Incubation at lower temperatures controlled the temperature in the interior of the bed at values near the optimum, but cooled the surface to values at which growth was very slow. The problem of adequate temperature control became worse still at a bed height of 12.7 cm.

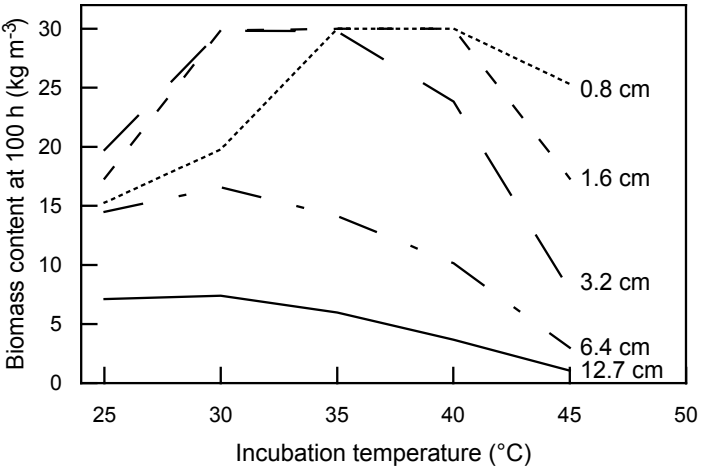

Fig. 6.6. Results obtained by Rajagopalan and Modak (1994) when they used their model to investigate the effect of the height of the bed and the temperature of the surroundings on the average biomass concentration in the bed after 100 h of cultivation. Adapted from a table presented by Rajagopalan and Modak (1994), with kind permission from Elsevier

6.4 Conclusions

The layer of substrate in trays is limited to a bed height of around 5 cm by considerations of heat and O_2 transfer within the bed. Therefore scale-up of the process cannot be achieved by increasing the bed height. The only manner to scale up a tray process to large scale is to increase the surface area of the trays, which is equivalent to saying that the large-scale process must use a large number of trays of the same size as those in which the laboratory studies were done. The use of large numbers of trays implies the necessity either for manual handling or highly sophisticated robotic systems, both of which can be inordinately expensive. However, in regions in which manual labor costs are low, such tray-type processes may find applications.

Further Reading

Traditional fermented food processes that involve tray technology

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Stanton WR, Wallbridge A (1969) Fermented food processes. Process Biochem April:45–51

Steinkraus KH (1986) Fermented foods, feeds, and beverages. Biotechnol Adv 4:219–243

An extension of the model of Rajagopalan and Modak (1994) that describes simultaneous diffusion and consumption in the expanding biofilm that develops on the particle surface

Rajagopalan S, Modak JM (1995) Modeling of heat and mass transfer for solid state fermentation process in tray bioreactor. Bioprocess Eng 13:161–169

Studies of heat and mass transfer in tray bioreactors

Ghildyal NP, Ramakrishna M, Lonsane BK, Karanth NG (1992) Gaseous concentration gradients in tray type solid state fermentors - Effect on yields and productivities Bioprocess Eng 8:67–72

Ghildyal NP, Ramakrishna M, Lonsane BK, Karanth NG, Krishnaiah MM (1993) Temperature variations and amyloglucosidase levels at different bed depths in a solid state fermentation system. Chem Eng J 51:B17–B23

Rathbun BL, Shuler ML (1983) Heat and mass transfer effects in static solid-substrate fermentations: Design of fermentation chambers. Biotechnol Bioeng 25: 929–938

Smits JP, van Sonsbeek HM, Tramper J, Knol W, Geelhoed W, Peeters M, Rinzema A (1999) Modelling fungal solid-state fermentation: the role of inactivation kinetics. Bioprocess Eng 20:391–404