3 Introduction to Solid-State Fermentation Bioreactors

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3.1 Introduction

This book is about the design and operation of bioreactors for SSF. The current chapter briefly introduces the various bioreactor types, which will be described in more detail in Chaps. 6 to 11. Chapter 4 considers the heat and mass transfer phenomena that occur within bioreactors in a qualitative manner, while Chap. 5 shows how these heat and mass transfer phenomena are intimately linked to the question of how to design a large-scale SSF bioreactor; this discussion will highlight the need for mathematical models as tools in the scale-up process (Fig. 3.1).

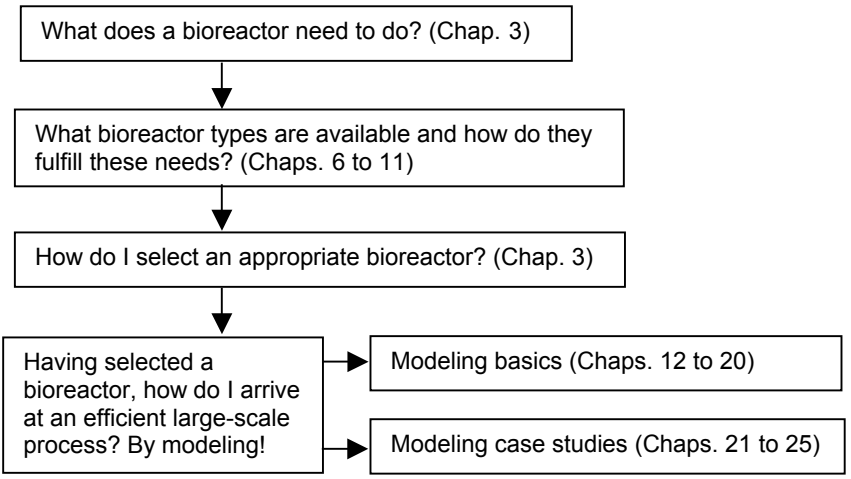

Fig. 3.1. The manner in which this book addresses the bioreactor design task

This book is written assuming that you are doing laboratory studies on a particular application of SSF, and the results give you confidence that you can develop a commercial process, for which you require a large-scale bioreactor. After undertaking the necessary kinetic characterization studies outlined in Chaps. 14 to 17, your task is to choose an appropriate bioreactor type, to design and build it, and then to operate it optimally. Clearly, the principles developed in this book are also appropriate for the situation in which you already have a process operating in a large-scale bioreactor, but in which the bioreactor was not designed based on engineering principles, and therefore you need to optimize its operation.

Note that in this book we are concentrating on the bioreactor itself. In all bioreactors there are various auxiliary operations and equipment. We do not cover bioreactor loading, unloading, and sterilization, but given the importance of the aeration system, Chap. 29 considers how it is possible to provide air at a desired flow rate, temperature, and humidity, and to change these conditions during the process. Chapters 26 and 28 consider the monitoring of the bioreactor and implementation of control schemes.

3.2 Bioreactor Selection and Design: General Questions

In taking a process that operates well in the laboratory and establishing a commercial process, the first step will be to identify what type of bioreactor will be suitable at large scale. Many of the laboratory experiments may have been undertaken in erlenmeyer flasks or thin packed-beds, but larger versions of these will probably not be appropriate for large-scale production.

Many considerations must be kept in mind when selecting a bioreactor, but a key question is: "What criterion do I use to compare different bioreactors and different operating conditions in order to be able to end up with the best system possible for my particular process?" Obviously the best criterion to use is the economic performance of the process. However, SSF processes have not been analyzed sufficiently to enable accurate estimates of capital and operating costs for new processes. In fact, at present the only way to compare the economic performance of bioreactors would be to build and operate a full-scale version of each bioreactor and record their capital and operating costs. It is likely to be some time before it will be possible to use economic performance as a criterion to guide bioreactor selection.

In the absence of sufficient information about the economics of SSF processes, the aim should then be to maximize the productivity of the bioreactor, in terms of product formation, which might be biomass or a metabolite. In other words, the criterion is the rate of production in kg of product per m^3 of bioreactor volume. Of course, if the substrate bed is not homogeneous, it will be necessary to calculate the productivity based on the evolution of the "volume-weighted biomass (or product)" curve. What is being sought is the combination of bioreactor operating strategy and harvesting time that will give the greatest value for:

$$Pr = \frac{X_{harvest} - X_{initial}}{t_{process} . V_{bioreactor}}, \tag{3.1}$$

where Pr is the productivity (kg h^{-1} m^{-3}), $X_{harvest}$ is the amount of biomass (or product) at the time of harvesting (kg), $X_{initial}$ is the amount of biomass (or product) at zero time (kg), $t_{process}$ is the overall process time (the time between successive harvests, h), and $V_{bioreactor}$ is the bioreactor volume (m^3).

Therefore bioreactor selection will be guided by the answers to several key questions about factors that affect the productivity. These are discussed in the following subsections.

3.2.1 The Crucial Questions

Possibly the three most important initial questions are:

- To what degree is the microorganism, or the desired form of the final product, affected deleteriously by agitation?
- How fast does the organism grow and how sensitive is it, and product formation by it, to increases in temperature?
- What are the aeration requirements of the system?

The answers to these questions will influence decisions about the type of aeration, mixing, and heat removal mechanisms that the large-scale bioreactor must have. Of course, these considerations are interconnected and affect the ability to control the macroscale variables of the process.

To what degree is the microorganism, or the desired form of the final product, affected deleteriously by agitation? Bioreactors can either be completely static, intermittently agitated, or continuously agitated. Frequent or continuous agitation would be desirable if it were tolerated, because it aids bulk transport of heat and O_2, improving the ability to control the conditions within the bed. Further, evaporative cooling of the bed can dry it out to water activities that restrict growth, meaning that it is often desirable to add water during the fermentation. It is only feasible to add water while the bed is being mixed. However, agitation can also affect the process deleteriously. It may damage hyphae in fungal-based processes, which might adversely affect growth and product formation. Conversely, it may be desired that the final product be knitted together by fungal hyphae, such as in the production of a fermented food, and this would be prevented by agitation. Beyond this, agitation can crush substrate particles if they do not have sufficient mechanical strength or can cause sticky particles to agglomerate, in either case producing a paste in which O_2 transfer is greatly hindered. Unfortunately, the balance between positive and negative effects of agitation has not been well characterized. It will be necessary to undertake your own studies at laboratory-scale in which the performance of agitated and non-agitated fermentations is compared, with both being forcefully aerated in order to minimize transport limitations, thereby isolating agitation as the factor responsible for any differences.

How fast does the organism grow and how sensitive is it, and product formation by it, to increases in temperature? Control of the temperature of the substrate bed is one of the key difficulties in large-scale SSF processes, especially in those processes that involve fast-growing microorganisms. At large scale, it may be difficult to prevent the temperature from reaching values that are quite deleterious to the microorganism. The various bioreactors differ in the efficiency of heat removal, with the temperatures reached depending on a complex interaction between the organism and the type of bioreactor and the way in which it is operated. These considerations may determine key decisions such as maximum bed depths.

What are the aeration requirements of the system? The majority of SSF processes involve aerobic growth. There are essentially two aeration options in SSF processes. One is to circulate air around the bed, but not to blow air forcefully through it. The other is to blow air forcefully through the bed. Agitation can influence the efficiency with which fresh air is delivered to the substrate particles. Note that in forcefully aerated beds the air phase plays an important role in heat removal. In fact aeration rates are typically governed by heat removal considerations since the air flow rates required for adequate heat removal are usually more than sufficient to avoid limitations in the supply of O_2 to the particle surface.

These considerations will be crucial in determining the agitation and aeration regimes that are appropriate. The bioreactors can then be compared on the basis of their ability to provide the desired regimes. More advice on how these various factors should be weighted in selecting an appropriate bioreactor type are considered in Sect. 3.4.

3.2.2 Other Questions to Consider

Once a bioreactor giving a certain agitation and aeration regime has been selected, various considerations will affect the details of its design:

- How important is it to have aseptic operation?
- To what degree is it necessary to contain the process organism?
- Is continuous operation desirable?
- How easy is loading and unloading and how much does labor cost?
- How much substrate is to be fermented?
- Will the bioreactor also be used for one or more of the downstream processing steps?

The degree to which sterile operation is required. Some SSF processes involve fast-growing organisms growing under conditions of low moisture that give the process organism a competitive advantage over contaminants. For example, in many fungal processes, the water activity is below that which is optimal for bacteria, so there are not serious problems with growth of bacterial contaminants, although fungal contaminants might cause problems. It may be possible to operate without strict asepsis: The process organism might be given sufficient advantage

over any contaminants through cooking of the substrate, avoidance of gross contaminations, and the provision of a relatively pure and vigorous inoculum. However, in other cases the organism grows slowly and care must be taken to design the bioreactor for sterile operation and to operate it in such a manner as to prevent contamination. In this case it is necessary to sterilize the bioreactor before operation, to properly seal openings, to filter the inlet air and to add solutions to the bioreactor during the fermentation in an aseptic manner. The various bioreactors that have been used to date differ with respect to their ability to operate aseptically.

The degree to which containment of the process organism is required. In general, transgenic organisms are not used in SSF, and processes rarely involve dangerous pathogens (although some do involve opportunistic pathogens). However, many processes do involve fungi and workers can suffer from allergies or other health problems if spores are allowed to escape freely into the environment. The bioreactor may need to be enclosed, and filters may be required on the outlet air stream. Bioreactors that have been used to date differ with respect to the ease of containing the process organism.

The desirability of continuous operation. Continuous operation in a well-mixed bioreactor is not a useful option for SSF. In SLF the nutrients added to a continuous stirred tank reactor are distributed throughout the bioreactor, becoming available to all the microorganisms. In SSF, any solid particles added to the fermentation would need to be colonized, a process that would take a significant period of time. Even if the particles were inoculated at the time of addition, early growth might be expected to be slow, especially in a mixed bed, and an unduly high fraction of poorly colonized substrate particles would leave in the outflow. However, continuous operation of the "plug-flow type" certainly is an option.

The ease of loading and unloading and the cost of labor. Loading and unloading of the bioreactor are handling operations that are required for all SSF processes. Note that the type of operation can affect how loading and unloading must be done: In continuous bioreactors the loading and unloading operations must be continuous or at least semi-continuous, while in batch operation they are done at distinct times. These operations have received little attention. The general principle is that, depending on labor costs, it may be desirable to avoid bioreactor types that require manual handing in the loading and unloading steps.

The amount of substrate to be fermented. The dimensions of the bioreactor will be determined by the volume of substrate that it must hold at any one time. This will depend on the mass of substrate that it must hold and the bulk packing density of the bed. Note that the allowable height of the bed might be limited by the mechanical strength of the substrate particles.

Involvement of the bioreactor in downstream processing steps. At times, it might be desirable either to dry the substrate bed or to leach a product from it as one of the first downstream processing steps. It may be desirable to undertake such steps within the bioreactor itself. This may influence bioreactor design.

3.3 Overview of Bioreactor Types

Many different bioreactors have been used in SSF processes, and have been given different names by different authors. However, based on similarities in design and operation, SSF bioreactors can be divided into groups on the basis of how they are mixed and aerated (Fig. 3.2).

- Group I: Bioreactors in which the bed is static, or mixed only very infrequently (i.e., once or twice per day) and air is circulated around the bed, but not blown forcefully through it. These are often referred to as "tray bioreactors".
- Group II: Bioreactors in which the bed is static or mixed only very infrequently (i.e., once per day) and air is blown forcefully though the bed. These are typically referred to as "packed-bed bioreactors".
- Group III: Bioreactors in which the bed is continuously mixed or mixed intermittently with a frequency of minutes to hours, and air is circulated around the bed, but not blown forcefully through it. Two bioreactors that have this mode of operation, using different mechanisms to achieve the agitation, are "stirred-drum bioreactors" and "rotating drum bioreactors".
- Group IV: Bioreactors in which the bed is agitated and air is blown forcefully through the bed. This type of bioreactor can typically be operated in either of two modes, so it is useful to identify two subgroups. Group IVa bioreactors are mixed continuously while Group IVb bioreactors are mixed intermittently with intervals of minutes to hours between mixing events. Various designs fulfill these criteria, such as "gas-solid fluidized beds", the "rocking drum", and various "stirred-aerated bioreactors".

Note that this division is made on the basis of the manner in which the bioreactor is operated, and not on the outward appearance of the bioreactor. For example, there are bioreactors that are essentially identical with the "stirred drum", but in which the air is introduced within the substrate bed through the ends of the paddles. Such a bioreactor should then be classified as a "stirred-aerated bioreactor", although the bed will not be as efficiently aerated as when the bed receives an even aeration across its whole cross-section. Also note that the distinction is not always perfectly clear. It is an arbitrary decision as to what frequency of mixing is separates "static" and "agitated" operation. The advantage of grouping bioreactors on the basis of the manner in which they are operated is that principles derived on the basis of work with one member of a certain group of bioreactors can be applied to other bioreactors in the group.

3.3.1 Basic Design Features of the Various Bioreactor Types

This section presents basic design features of the various bioreactors types. More details are given in Chaps. 6 to 11, but sufficient information is presented here to allow a general comparison.

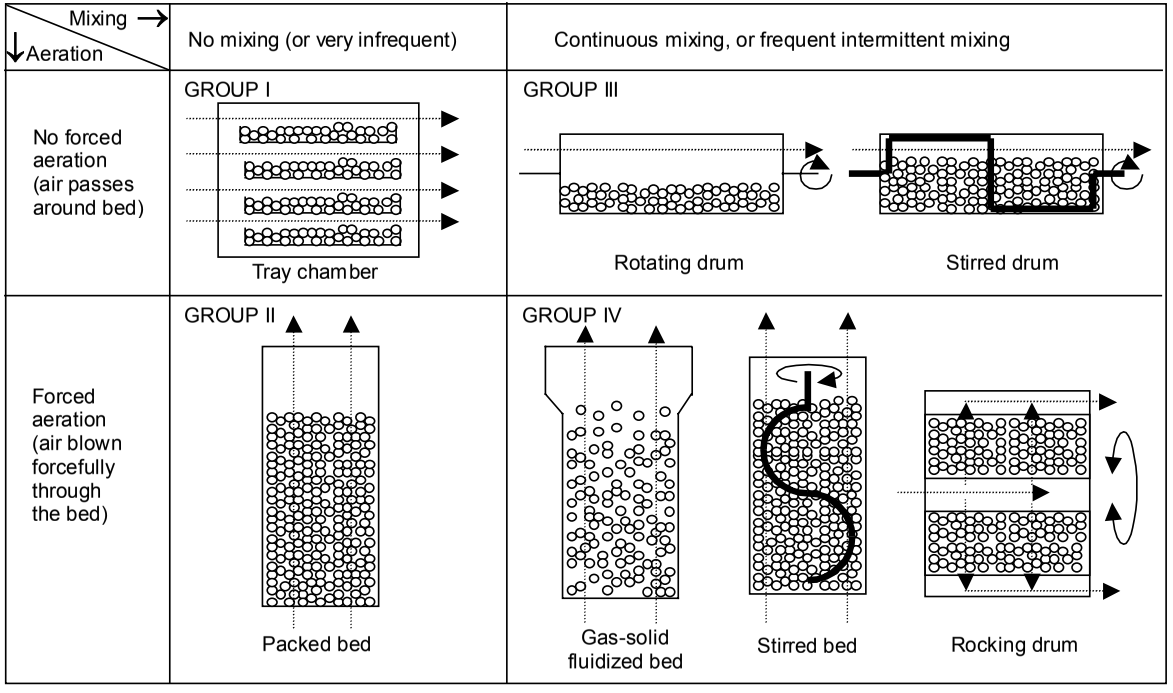

Fig. 3.2. Basic design features of the various SSF bioreactors, showing how they can be classified into four groups on the basis of how they are mixed and aerated. From Mitchell et al. (2000) with kind permission from Springer Science and Business Media

Group I bioreactors. These typically consist of a chamber containing a large number of individual trays, stacked one above the other with a gap in between (Fig. 3.2, upper left quadrant). Conditioned air (i.e., with control of humidity and temperature) is blown into the chamber and circulates around the trays. Agitation, if done, is very infrequent, and is typically done by hand. The trays themselves may be constructed of wood, bamboo, metal or plastic. They are typically open at the top and have perforated bottoms to increase the accessibility to O_2, but there are other possibilities. For example, micro-perforated plastic bags containing substrate fall within this category.

Group II bioreactors. A typical packed-bed bioreactor consists of a column of cylindrical or rectangular cross section, oriented vertically, with a perforated base plate on the bottom which supports a bed of substrate (Fig. 3.2, lower left quadrant). Air is blown up through the base plate.

Group III bioreactors. These typically consist of a drum of cylindrical cross section lying horizontally (Fig. 3.2, upper right quadrant). The drum is partially filled with a bed of substrate, and air is blown through the headspace. In rotating drums, the whole drum rotates around its central axis to mix the bed. In stirred drums, the bioreactor body remains stationary and paddles or scrapers mounted on a shaft running along the central axis of the bioreactor rotate within the drum.

Mixed and forcefully aerated bioreactors. There are several types of designs that fall into this group (Fig. 3.2, lower right quadrant). They can be operated with continuous or discontinuous mixing.

- Stirred-bed bioreactors are similar to the static packed bed in that a bed of substrate sits on a perforated base plate and air is forcefully blown through the bed, but rather than being static, an agitator is inserted and provides continuous or intermittent mixing. Such stirred beds are typically aerated from the bottom, and have the agitator inserted from the top.
- Rocking-drum bioreactors consist of three concentric cylinders - an inner perforated cylinder, an outer perforated cylinder, and an outer solid cylinder. The substrate sits in the space between the two perforated cylinders. Air is blown through into the central cylinder, passes through the substrate bed and then into the space between the outer perforated cylinder and the outer solid cylinder, before leaving through the air outlet. The two outer cylinders rotate in relation to the inner cylinder, thereby mixing the substrate bed, although not very effectively.
- Air-solid fluidized beds (ASFBs). In this bioreactor air is blown upwards through a perforated base plate at sufficient velocity to fluidize the substrate bed, which then behaves as though it were a fluid.

3.3.2 Overview of Operating Variables

Operating variables are variables that the operator can manipulate in an attempt to control the conditions within the bioreactor. The question of optimum operating

strategies for the various bioreactor types is covered in the individual bioreactor chapters (Chaps. 6 to 11) and the modeling case studies (Chaps. 21 to 25). However, it is worthwhile to make some general comments here:

- Regardless of whether the air is blown forcefully through the bed or circulated around the bed, it is possible to control the flow rate, temperature, and humidity of the air supplied at the inlet to the bioreactor or chamber. The costs of supplying air will depend on the volumetric flow rate and the pressure drop in the bed, and the need to heat or refrigerate the air. Pressure drop will be discussed in Chap. 7, which deals with packed-bed bioreactors, since its importance is greatest for this type of bioreactor.
- The conditions in the surroundings of the bioreactor can be controlled. The bioreactor may be placed in a room or other location where the air temperature, humidity, and circulation are controlled. Alternatively, the bioreactor may be fitted with a water jacket. The flow rate and temperature of the cooling water at the inlet of the jacket can be controlled. Note that if the desired air or water temperatures are different from the temperatures at which they are available, either cooling or heating will be necessary, which entails extra costs.
- Additions can be made to beds that are mixed, even if only intermittently; for example, water can be sprayed onto the bed during mixing.
- In beds that are mixed, it is possible to control the frequency, duration, and intensity (i.e., revolutions per minute of the agitator) of the mixing.

Given the difficulties in controlling the conditions in SSF bioreactors, which were mentioned in Chap. 2 and are discussed in more detail in Chap. 5, it is not a simple matter to maintain the bed conditions at the optimum values for growth and product formation by manipulating these operating variables. The aim therefore is to select combinations of operating conditions that make the best balance in:

- minimizing deviations from the optimum temperature;
- minimizing damage to the organism;
- minimizing deviations of the bed water activity from the optimum value;
- maximizing the supply of O_2 to the particle surface.

Chapters 6 to 11 will give some idea of what we already know about how to do this for the various bioreactor types. It must be stressed that, although our knowledge is increasing, it is as yet far from complete.

3.4 A Guide for Bioreactor Selection

The answers to the questions and issues raised in Sect. 3.2 will determine which of the bioreactor types shown in Fig. 3.2 is most suitable. Figure 3.3 shows how the various considerations might be used to arrive at the decision to use a particular bioreactor. For example, if the microorganism is very sensitive to shear, then a bioreactor type with a static bed must be chosen. This might cause heat removal to be a problem. If some shear can be tolerated, it is not clear which of the agitated

bioreactors is best, since shear effects during the mixing of solids in the various bioreactors are not well understood.

Figure 3.3 can give only general guidelines about bioreactor choice. The final decision comes down to bioreactor performance for a particular substrate-microorganism-product combination. However, it is not a simple matter, on the basis of laboratory-scale studies, to say which bioreactor design will perform best at large scale. Also, typically, neither large-scale nor even pilot-scale bioreactors of the various types will be available for comparative studies. Nor is the budget for the development process likely to be sufficient to build several pilot-scale bioreactors. One of the main arguments of this book is that, in the face of these limitations, mathematical modeling of bioreactor performance is a very useful tool in such scale-up tasks. Scale-up should not be done solely on the basis of experimental studies; rather it should involve a combined experimental and modeling program. This issue will be returned to in Chap. 5, after a consideration of basic heat and mass transfer principles in Chap. 4.

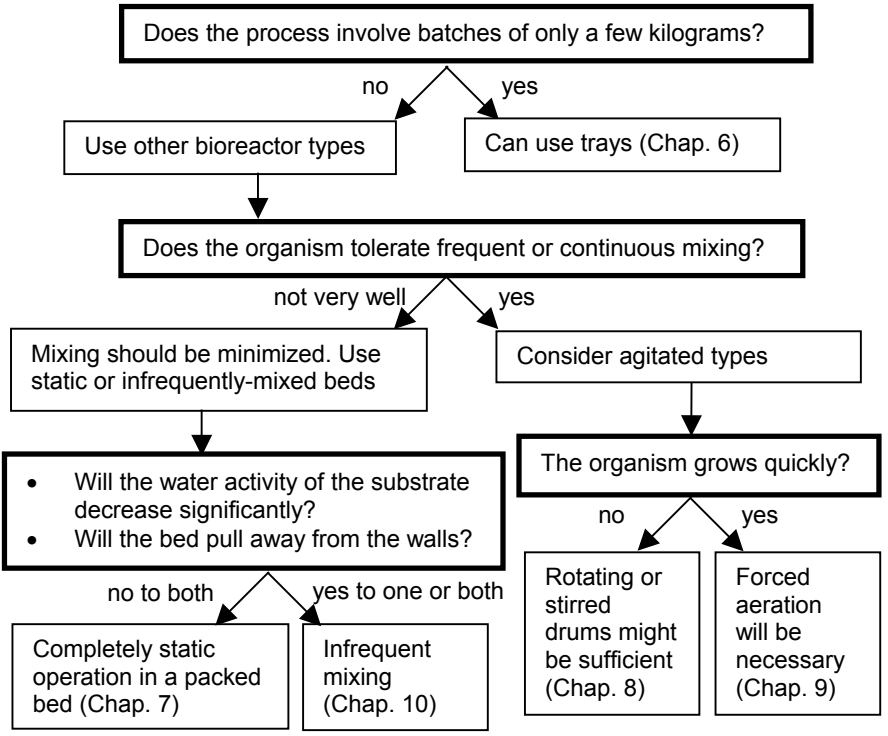

Fig. 3.3. A suggested key for SSF bioreactor selection

Further Reading

General considerations about bioreactor performance, which are relevant regardless of whether the bioreactor is used for submerged liquid fermentation or solid-state fermentation

Lübbert A, Jørgensen SB (2001) Bioreactor performance: a more scientific approach for practice. J Biotechnol 85:187–212

Economic analysis of solid-state fermentation processes, economic performance being the most important criterion in bioreactor selection

Castilho LR, Polato CMS, Baruque EA, Sant'Anna Jr GL, Freire DMG (2000) Economic analysis of lipase production by *Penicillium restrictum* in solid-state and submerged fermentations. Biochem Eng J 4:239–247

Ghildyal NP, Lonsane BK, Sreekantiah KR, Sreenivasa Murthy V (1985) Economics of submerged and solid state fermentations for the production of amyloglucosidase. J Food Sci Technol 22:171–176

General reviews of bioreactor designs for SSF

Durand A (2003) Bioreactor designs for solid state fermentation. Biochem Eng J 13:113–125

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

Fasidi IO, Isikhuemhen OS, Zadrazil F (1996). Bioreactors for solid state fermentation of lignocellulosics. J Sci Ind Res 55:450–456

Hardin MT, Mitchell DA (1998) Recent developments in the design, operation and modelling of bioreactors for solid-state fermentation. In: Kaowai F, Sasaki K (eds) Recent research developments in fermentation and bioengineering, vol. 1. Research Signpost, Trivandrum, pp 205–222

Mitchell DA, Berovic M, Krieger N (2000) Biochemical engineering aspects of solid state bioprocessing. Adv Biochem Eng/Biotechnol 68:61–138

Robinson T, Nigam P (2003) Bioreactor design for protein enrichment of agricultural residues by solid state fermentation. Biochem Eng J 13:197–203

Description of bioreactor types used in the koji industry

Mudgett RE (1986) Solid-state fermentations. In: Demain AL, Solomon NA (eds) Manual of Industrial Microbiology and Biotechnology. ASM Press, Washington DC, pp 66-83

Sato K, Sudo S (1999) Small-scale solid-state fermentations. In: Demain AL, Davies JE (eds) Manual of Industrial Microbiology and Biotechnology, 2nd edn. ASM Press, Washington DC, pp 61–79

Recent experimental and modeling studies in which various bioreactor types have been compared

Couto SR, Moldes D, Liebanas A, Sanroman A (2003) Investigation of several bioreactor configurations for laccase production by *Trametes versicolor* operating in solid-state conditions. Biochem Eng J 15:21–26

Oostra J, Tramper J, Rinzema A (2000) Model-based bioreactor selection for large-scale solid-state cultivation of *Coniothyrium minitans* spores on oats. Enzyme Microbial Technol 27:652–663