1 Solid-State Fermentation Bioreactor Fundamentals: Introduction and Overview

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1.1 What Is "Solid-State Fermentation"?

Solid-state fermentation (SSF) involves the growth of microorganisms on moist solid particles, in situations in which the spaces between the particles contain a continuous gas phase and a minimum of visible water. Although droplets of water may be present between the particles, and there may be thin films of water at the particle surface, the inter-particle water phase is discontinuous and most of the inter-particle space is filled by the gas phase. The majority of the water in the system is absorbed within the moist solid-particles (Fig. 1.1(a)). More detail about the spatial arrangement of the system components is given in Chap. 2.

In fact, here we follow the nomenclature proposed by Moo-Young et al. (1983) where the more general term "solid-substrate fermentation" is used to denote any type of fermentation process that involves solids, including suspensions of solid particles in a continuous liquid phase and even trickling filters (Fig. 1.1(b)). Therefore solid-state fermentation is classified as one type of solid-substrate fermentation. In this book we concentrate specifically on solid-state fermentation systems, in the manner that we defined them in the first paragraph.

The aim of the present section is not to give an in-depth explanation of all the characteristics of SSF systems, nor to compare SSF with submerged liquid fermentation (SLF). The further reading section at the end of this chapter gives some sources of general background information for readers who do not have much familiarity with SSF systems. Here we will give only a very broad summary of some of the main points:

- The majority of SSF processes involve filamentous fungi, although some involve bacteria and some involve yeasts.
- SSF processes may involve the pure culture of organisms, or the culture of several pure strains inoculated simultaneously or sequentially, while in some processes a "self-selected" microflora arises from the original microflora (e.g., in composting) or from a specially prepared traditional inoculum.
- The majority of SSF processes involve aerobic organisms. Note that we use the word "fermentation" in this book in the sense of its more general meaning, that

is, "the controlled cultivation of organisms" (the SSF literature uses the word fermentation in this sense).

- The substrates used in SSF processes are often products or byproducts of agriculture, forestry or food processing. Typically the source of nutrients comes from within the particle, although there are some cases in which nutrients are supplied from an external source. Usually a polymer gives the solid structure to the particle and this polymer may or may not be degraded by the microorganism during the fermentation. There are also some cases in which artificial or inert supports are used, with a nutrient solution absorbed within the matrix.

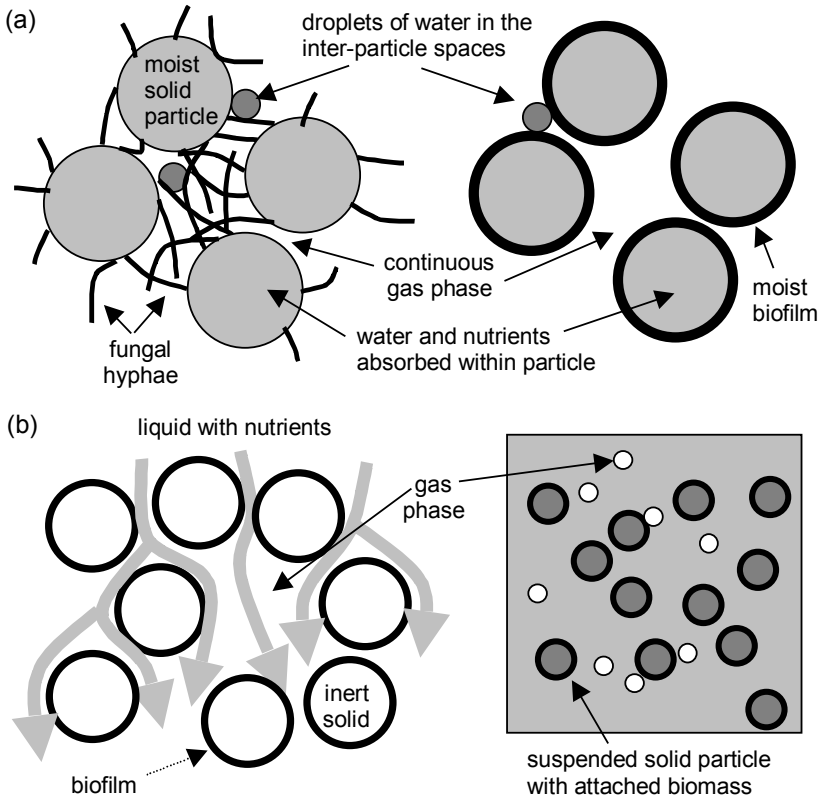

Fig. 1.1. The defining features of solid-state fermentation (SSF) systems (following the terminology of Moo-Young et al. 1983). **(a)** The arrangement of moist solid particles and the continuous gas phase in SSF systems involving a filamentous fungus (left-hand side) and a unicellular organism (right-hand side). **(b)** Other systems that involve growth on solids, but which are not defined as SSF due to the large amount of water in the inter-particle spaces. The left-hand diagram represents a trickling-filter type system while the right-hand diagram represents a suspension or slurry system

Much of this book will assume that we are working with pure cultures of aerobic filamentous fungi, to produce a specific product. In this case, there is a definite set of optimum conditions for growth of the process organism and product formation by it. Therefore this book does not consider composting, which is a specific application of SSF in which it is desirable for the temperature to vary during the process. Of course, with this and other important differences, such as the use of undefined mixed cultures, composting has its own literature, which is not directly relevant to the type of SSF process in which we are interested.

1.2 Why Should We Be Interested in SSF?

The environment that the organism experiences in SSF is different from that experienced in SLF. In SLF it is relatively easy to control the conditions to which the process organism is exposed:

- the fungal hyphae are bathed in a liquid medium and do not run the risk of desiccation;
- temperature control is typically not overly difficult, such that the organism is exposed to a constant temperature throughout its growth cycle;
- the availability of O_2 to the biomass can be controlled reasonably well at a particular level of saturation of the medium (although this can become very challenging in high density cultures);
- the availability of the nutrients to the organism can be controlled within relatively narrow limits if desired, through the feeding of nutrient solutions (at least in those processes in which soluble carbon and energy sources are provided);
- although shear forces do occur within mechanically stirred bioreactors, the nature and magnitude of these forces are well understood and it is possible to use bioreactors that provide a low-shear environment, if the organism is highly susceptible to shear damage, such as bubble columns or air lift bioreactors;
- pH control is relatively easy to provide.

In contrast, the environment in SSF can be quite stressful to the organism. For example:

- fungal hyphae are exposed to an air phase that can desiccate them;
- temperatures can rise to values that are well above the optimum for growth due to the inadequate removal of waste metabolic heat. In other words, the temperature to which the organism is exposed can vary during the growth cycle;
- O_2 is typically freely available at the surface of the particle, however, there may be severe restrictions in the supply of O_2 to a significant proportion of the biomass that is within a biofilm at the surface or penetrating into the particle;
- the availability of nutrients to the organism may be poor, even when the average nutrient concentration within the substrate particle, determined after homogenizing a sample of fermenting solid particles, is high. In other words, there tend to be large concentration gradients of nutrients within the particles;

- movement of the particles of the solid substrate can cause impact and shear damage. In the case of fungal processes the hyphae can suffer severe damage;
- it may be difficult to provide pH control.

Also, due to the different physical natures of the two systems, namely the presence of solid-air interfaces in SSF, growth morphologies of mycelial organisms, in terms of hyphal extension and branching patterns, may be quite different between SSF and SLF. This can be linked to different patterns of expression of genes, including those for several potential biotechnological products (Ishida et al. 2000).

These, and other differences, mean that SLF is an "easier" system with which to work. The ease of using SLF is greater still when substrate handling is considered. For example, it is much simpler and cheaper to pump liquids from one place to another than to move solids and it is easier to sterilize a large volume of liquid than a large volume of solids (in either batch or continuous sterilization mode). Given all these potential difficulties, for both the operator and the microorganism, it would appear that SLF should be the fermentation method of choice. In fact, in the majority of cases it is! However, there are certain instances in which, despite being more problematic, SSF may be appropriate:

- when the product needs to be in a solid form (e.g., fermented foods);
- when a particular product is only produced under the conditions of SSF or, if produced in both SLF and SSF, is produced in much higher levels in SSF. For example, certain enzymes are only induced in SSF and some fungi only sporulate when grown in SSF, in which the hyphae are exposed directly to an air phase. If it is desired to use genetically unmodified organisms in a process for the production of such a product, then SSF may be the only option;
- when the product is produced in both SLF and SSF, but the yield is much higher in SSF. For example, *Monascus* pigment and many fungal spores are produced in much higher yields in SSF;
- when socio-economic conditions mean that the fermentation process must be carried out by relatively unskilled workers. Some SSF processes can be relatively resistant to being overtaken by contaminants;
- when the product is produced in both SSF and SLF, but the product produced in SSF has desirable properties which the product produced in SLF lacks. For example, spore-based fungal biopesticides produced in SSF processes are usually more resistant to adverse conditions than those produced in SLF, and are therefore more effective when spread in the field;
- when it is imperative to use a solid waste in order to avoid the environmental impacts that would be caused by its direct disposal. This is likely to become an increasingly important consideration as the ever-increasing population puts an increasing strain on the environment.

1.3 What Are the Current and Potential Applications of SSF?

The considerations raised in the previous section have meant that SSF technology has been used for many centuries. Some examples of traditional SSF processes are:

- tempe, which involves the cultivation of the fungus *Rhizopus oligosporus* on cooked soybeans. The fungal mycelium binds the soybeans into a compact cake, which is then fried and eaten as a meat substitute. This fermented food is quite popular in Indonesia;
- the *koji* step of soy sauce manufacture, which involves the cultivation of the fungus *Aspergillus oryzae* on cooked soybeans. During the initial SSF process of 2 to 3 days, the fungal mycelium not only covers the beans but also secretes a mixture of enzymes into them. The fermented beans are then transferred into brine, in which, over a period of several months, the enzymes slowly degrade the soybeans, leaving a dark brown sauce.
- ang-kak, or "red rice", which involves the cultivation of the fungus *Monascus purpureus* on cooked rice. The fungus produces a dark red pigment. At the end of the fermentation the red fermented rice is dried and ground, with the powder being used as a coloring agent in cooking.

Beyond this, over the last three decades, there has been an upsurge in interest in SSF technology, with research being undertaken into the production of a myriad of different products, including:

- enzymes such as amylases, proteases, lipases, pectinases, tannases, cellulases, and rennet;
- pigments;
- aromas and flavor compounds;
- "small organics" such as ethanol, oxalic acid, citric acid, and lactic acid;
- gibberellic acid (a plant growth hormone);
- protein-enriched agricultural residues for use as animal feeds;
- animal feeds with reduced levels of toxins or with improved digestibility;
- antibiotics, such as penicillin and oxytetracycline;
- biological control agents, including bioinsecticides and bioherbicides;
- spore inocula (such as spore inoculum of *Penicillium roqueforti* for blue cheese production).

There is also research into the use of microorganisms growing in SSF conditions to mediate processes such as:

- decolorization of dyes;
- biobleaching;
- biopulping;
- bioremediation.

These processes commonly use waste products or byproducts of agriculture and food processing, selected as appropriate to favor growth of the producing organism and formation of the desired product. Such wastes and byproducts include wheat bran, rice bran, oil-press cakes, apple pomace, grape pomace, banana peels, citrus peels, wheat straw, rice straw, coffee pulp, citrus pulp, sugar beet pulp, coffee husk, and sugar beet molasses. Sometimes higher-value agricultural and food materials are used, such as granular milk curds, fodder beets, rice, and cassava meal. Recently there has also been some interest in the use of inert supports impregnated with nutrient solutions; at times natural inert supports such as sugar cane bagasse have been used, at other times artificial supports have been used, such as polyurethane foam cubes.

Note that the list presented above highlights only a small proportion of the overall activity in the development of SSF processes. Various reviews have been published on the applications of SSF, including details of the organisms and substrates used and the current chapter does not intend to repeat the information presented in these reviews. Readers with further interest should consult the reference section at the end of the chapter.

1.4 Why Do We Need a Book on the Fundamentals of SSF Bioreactors?

So if solid-state fermentation has such potential, why is it not a more widely used technology? Why are there relatively few "large-scale success stories" such as exemplified by the *koji* step of soy sauce production? Of course, part of the problem has already been touched upon in Sect. 1.2: Our inability to control conditions may well put a stress on the organism that causes it to produce a useful product in large quantities; however, too much stress may reduce yields and even kill the organism.

For SSF to be a more widespread technology, we need to know how to apply it, when appropriate, at both small scale (in "domestic" industries) and large scale (that is, involving large quantities in bioreactors). There is a lot of know-how related to the production of traditional fermented foods that involve SSF, which allows us to operate small-scale processes well. However, with the exception of certain success stories, SSF has not found widespread application at large scale. Why?

One of the problems is that we do not have the knowledge to translate success of one large-scale process (e.g., soy sauce *koji*) into the success of other large-scale processes. Here we are specifically talking about the question of "How do we design and operate large-scale bioreactors in such a manner as to have a profitable process". Our success with large-scale soy sauce *koji* does not necessarily translate into success with products that have lower profit margins.

Unlike SLF, for SSF we do not have a broad general theory or tools for designing and optimizing the operation of large-scale bioreactors. Of course, in both SLF and SSF each particular process can have its peculiarities, so a general theory does

not mean that technology can be directly transferred from one process to another, but such a general theory does help by allowing one to focus on those peculiarities. The "theoretical foundations of SLF technology" (by which we really mean "the application of quantitative or engineering principles") began to be established in the late 1940s, and have been continuously extended and refined since then. In comparison, the engineering principles of SSF bioreactors only began to be developed around the late 1980s. Before then, it seems that the large-scale *koji* processes must have been developed over time through trial-and-error and experience, although it is also possible that soy sauce companies do have a good fundamental engineering know-how, but do not publish it in the general literature.

The consequence of the lack of these "theoretical foundations of SSF technology" is that, despite a very large upsurge of interest since the late 1970s (as judged by the increase in the number of publications on the topic of SSF in the scientific literature), there have been relatively few process that, having shown promise in the laboratory, have managed to leave the laboratory and be established as large-scale commercial processes. These processes perform well in the laboratory, where it is a trivial problem to provide O_2 and remove heat from the bed, but when attempts are made to establish large-scale processes, it is found to be impossible to control important process parameters, such as the temperature, within acceptable limits.

However, we have now reached a stage where our understanding is sufficient for it to be appropriate to bring together the theoretical foundations of SSF technology. This is what we aim to do in this book. However, our intention is not to be comprehensive in the sense of presenting all the engineering know-how so far generated for SSF bioreactors. Rather, we aim to introduce the fundamental concepts and ideas. An understanding of these fundamentals will provide the basis for readers to progress to the more advanced principles that are currently being established and published in the literature.

Beyond bringing the fundamental principles together, the book aims to provide a guide, based on current knowledge, about how best to design and operate the various different types of SSF bioreactor. Hopefully, it will stimulate further research into the area of SSF bioreactor performance.

The main argument of this book is that we need to apply a "biochemical engineering approach" to the problem of designing and optimizing the operation of SSF bioreactors. By a "biochemical engineering approach", we mean:

- the quantitative characterization of the key phenomena responsible for controlling bioreactor performance;
- the mathematical description of these phenomena within models intended to guide bioreactor design and operation;
- undertaking this characterization and description at an appropriate level of complexity, with the appropriate level depending on the balance between the usefulness of the mathematical tools in improving process performance and the mathematical and experimental difficulty in obtaining the functioning model.

Essentially, we are saying that it is necessary to develop mathematical models of the important phenomena, and use them as tools within experimental programs for bioreactor development.

If we do in fact achieve our aim of stimulating the development of SSF bioreactor technology, we can foresee a future time in which, in the development of any particular microbial fermentation product, both SSF and SLF will be considered, and the most promising of the two will be selected. SSF will not simply be ignored due to the lack of "know-how", as is currently the case in many parts of the world, especially those that do not have traditional fermented foods that are produced using SSF.

1.5 How Is this Book Organized?

As shown by Fig. 1.2, this book can be seen as consisting of five different parts. The subsections that follow give an overview of the argument that is developed within each of these parts.

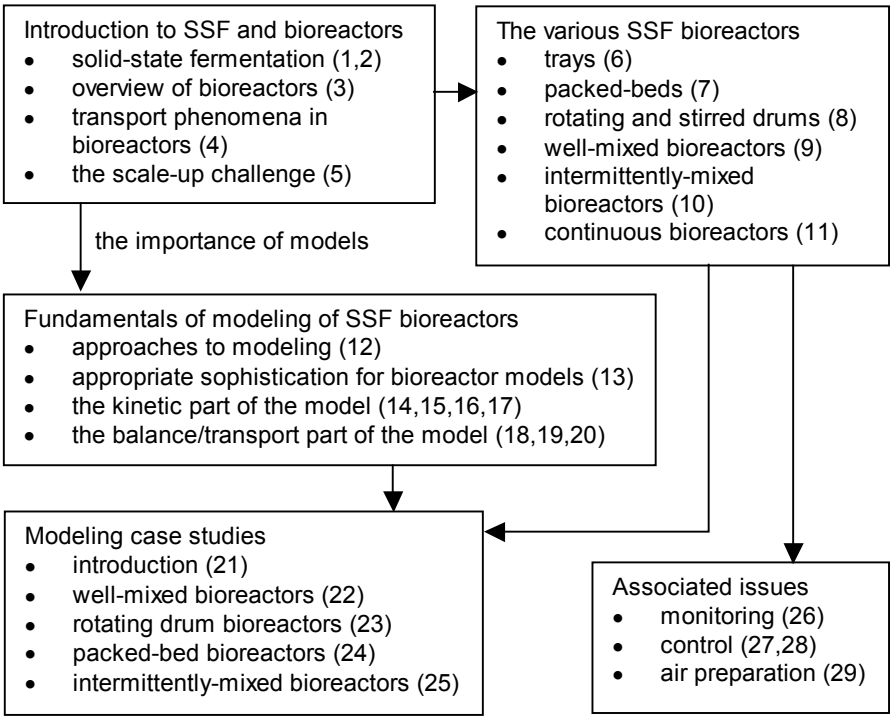

Fig. 1.2. Organization of the book. The numbers in parentheses are the chapters in which the various topics are covered

1.5.1 Introduction to Solid-State Fermentation and Bioreactors

Chapters 2 to 5 show the complexity of the task of designing efficient large-scale SSF bioreactors. Much of this complexity derives from the fact that the performance of an SSF bioreactor is the result of a complex interaction between biological and transport processes. Further, not only do the rates of these processes vary over time, but also processes such as heat and mass transfer involve several different phases within the bioreactor. Chapter 2 gives an overview of the phases present in an SSF bioreactor and the interaction between the biological and transport phenomena during the fermentation.

Chapter 3 then gives an overview of the various bioreactor types that have been used in SSF. The aim is not to give an exhaustive description of all design variations, but rather to recognize that it is useful to classify the many bioreactors into four groups, based on the manner in which they are aerated and agitated.

An understanding of the transport phenomena that occur in SSF bioreactors is essential in order to appreciate the difficulty of designing and operating efficient large-scale SSF bioreactors. Chapter 4 therefore introduces the important transport phenomena. This is done in a qualitative manner, with the quantitative aspects being covered later in the book. With this basis, Chap. 5 then explains the scale-up problem, or in other words, how limitations in mass and heat transfer mean that it is not appropriate to take a successfully operating laboratory-scale bioreactor and then simply design a geometrically identical larger version. This will not lead to a successfully operating large-scale bioreactor. Within this chapter, it becomes clear that mathematical models that combine the various biological and physical phenomena are essential as tools to guide bioreactor design and the optimization of bioreactor operation. The basic principles of these models and their application to particular bioreactors take up much of the latter part of the book.

1.5.2 Introduction to the Various Classes of SSF Bioreactors

Chapters 6 to 10 describe the various types of SSF bioreactors that have been used in batch-mode. For this purpose, they are divided into groups based on the aeration and agitation strategies. Classical bioreactors with these groups include tray bioreactors, packed-bed bioreactors, rotating drum bioreactors, and well-mixed or intermittently-mixed bioreactors with forced aeration. For each class of bioreactors the basic design and operating features are described, as well as several of the possible variations in these features. These chapters also relate information from the SSF literature about how these various bioreactors perform, highlighting the relative ease or difficulty of controlling conditions within the bioreactor and thereby of obtaining high productivity or not.

Continuous operation of SSF bioreactors is a subject that has received relatively little attention in the SSF literature. Chapter 11 describes the various ways in which SSF bioreactors can be operated in continuous mode, and also undertakes a preliminary analysis of bioreactor performance in this mode of operation. However, it will be clear that this is an area that needs much more attention.

1.5.3 Fundamentals of Modeling of SSF Bioreactors

Chapters 12 to 20 cover various aspects that are fundamental to an understanding of how to model SSF bioreactors. Chapter 12 starts with an overview of how modeling is undertaken, outlining a series of steps. The first of these steps involves making a decision about what degree of complexity is desired in the model, with more complex models potentially being more useful tools than simpler models, but also requiring much greater effort and sophistication, not only in the formulation and solution of the model equations, but also in the experimental work necessary to determine the various parameters that appear in the model. Chapter 13 then applies this question to mathematical models of SSF bioreactors, arguing that currently the best strategy is to develop and use so-called "fast-solving" models.

Chapter 12 also makes it clear that any model of an SSF bioreactor can be thought of as being comprised of two sub-models, a kinetic sub-model that describes the growth of the microorganism and a balance/transport sub-model that describes the various physical phenomena within the bioreactor. Chapter 13 argues that if a fast-solving model is desired, the kinetic sub-model should be quite simple and should not attempt to describe the dependence of the growth rate on nutrient concentrations, in order to avoid the necessity of describing simultaneous diffusion and reaction phenomena within the substrate particle.

The various steps in establishing appropriate equations for the kinetic sub-model are presented in Chaps. 14 to 17. Chapter 14 presents some basic considerations, highlighting one of the intrinsic difficulties faced in SSF systems, namely the difficulty in determining the amount of biomass in the system, which is especially problematic when the process organism is a filamentous fungus. It also presents the equations that are typically used to describe growth profiles in SSF and how the parameters of these equations can be determined by regression. Chapter 15 describes experimental systems and approaches that you can use to establish the growth profile for your own SSF system. Chapter 16 then shows how the equations should be written within the bioreactor model: Whereas the regression analysis of the growth profile is undertaken with the integral form of an equation, the equation must appear in a differential form within the kinetic sub-model. Chapter 16 also shows how the effect of the loss of dry matter from the system in the form of CO_2 can be taken into account in the kinetic sub-model. The equations developed in Chap. 16 involve various growth parameters that are in fact functions of the local conditions experienced by the microorganism, such as temperature and water activity. Chapter 17 shows how experiments can be undertaken and analyzed in order to establish appropriate correlations that give the value of the growth parameters for any given combination of local conditions. However, it will be obvious in this chapter that this is an area that needs further development.

The balance/transport sub-model is addressed in Chaps. 18 to 20. Chapter 18 introduces the concept of balance equations, showing how they include terms to describe the various transport phenomena that occur within and between subsystems within the bioreactor. The basic mathematical expressions used in these terms are presented. These expressions contain various parameters and physical

constants, the values of which must be known in order to solve the bioreactor model. Chapter 19 describes these parameters and indicates how they might be determined. The balance/transport model also contains various heat and mass transfer coefficients. Chapter 20 describes various correlations that have been used and also lists some typical values that have been reported in the SSF literature.

Note that these chapters are written at a level intended for non-engineers. This section will not teach non-engineers all the skills that are needed for writing and solving models of SSF bioreactors. However, if you are not an engineer, these chapters will help you to understand the issues involved and this will greatly enrich your interaction with engineers during the bioreactor design process.

1.5.4 Modeling Case Studies of SSF Bioreactors

After a brief introduction in Chap. 21, Chaps. 22 to 25 present case studies in which fast-solving models are used to explore the design and operation of various SSF bioreactors. These include well-mixed bioreactors with forced aeration (Chap. 22), rotating drum bioreactors (Chap. 23), packed-bed bioreactors (Chap. 24), and intermittently-mixed forcefully-aerated bioreactors (Chap. 25). The case studies ask and answer questions such as "What aeration rate will be needed in order to control the bed temperature adequately in a large-scale bioreactor?".

These models, although still needing various improvements, can already be used as useful tools in the process of designing SSF bioreactors. The programs that are used in these chapters are available to readers from a web site. Details of this site and of the use of these programs are given in the Appendix.

1.5.5 Key Issues Associated with SSF Bioreactors

The last section of the book addresses several key issues in the operation of SSF bioreactors. Chapter 26 describes various process variables that we might like to monitor during the fermentation and gives suggestions for equipment that might be used to do this. It also addresses the question of data filtering, which is essential in order to eliminate random noise from the measured data.

Of course, one of the reasons that we might like to monitor the fermentation is to be able to undertake control actions in order to maintain the conditions in the bioreactor as near as possible to the optimum conditions for growth and product formation. Process control is a complex science. Chapter 27 introduces the basic principles of process control, at a level aimed for the non-engineer, although it is impossible to do this without presenting at least a few complicated mathematical equations! Chapter 28 then describes how control schemes can be applied to SSF bioreactors. It will become clear that this is an area that is still quite rudimentary and needs much more development.

Finally, a key step in the operation of an SSF bioreactor is the supply of air at an appropriate flow rate, temperature, and humidity. Chapter 29 describes how the air preparation system can be designed to do this and various related issues such as

the selection of the air blower and the need for filtration. It will become clear that it is not an easy task to adjust the flow rate, temperature, and humidity of the air, independently, without building highly sophisticated systems. It presents a case study of the development of an air preparation system for a pilot-scale SSF bioreactor.

1.5.6 A Final Word

Solid-state fermentation bioreactor technology is still developing. We hope that this book stimulates you either to apply the principles presented to the design of a bioreactor for your own SSF process or even to contribute to development of the technology itself!

Further Reading

General features and applications of SSF

Doelle HW, Mitchell DA, Rolz CE (eds) (1992) Solid substrate cultivation. Elsevier Applied Science, London

A broad overview of solid-state fermentation

Mitchell DA, Berovic M, Krieger N (2002) Overview of solid state bioprocessing. Biotechnol Ann Rev 8:183–225

Physiological advantages that make SSF interesting for the production of certain products

Holker U, Hofer M, Lenz J (2004) Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Applied Microbiology and Biotechnology 64:175–186

Applications of SSF

Pandey A, Soccoll CR, Mitchell D (2000) New developments in solid-state fermentation: I – Bioprocesses and products. Process Biochemistry 35:1153–1169