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Environmental Solid-State Cultivation Processes and Bioreactors

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Abstract Solid-state cultivation involves the growth of microorganisms in beds of moist solid particles that have a minimum of free water between the particles. The chapter describes environmentally-related solid-state cultivation processes. For example, some processes use substrates that are residues of agriculture, forestry, or food-processing, thereby reducing

From: *Handbook of Environmental Engineering, Volume 10: Environmental Biotechnology*
Edited by: L. K. Wang et al., DOI: 10.1007/978-1-60327-140-0_7 © Springer Science + Business Media, LLC 2010

the environmental impact of the residue. Other processes do not use residues, but produce products that have environmental applications. Still other processes use environmental-friendly biotransformations that have the potential to replace current industrial processes. Finally, some solid-state cultivation processes can be used to remove pollutants from soil or waste streams. Typically, environmental applications of solid-state cultivation involve large-scale processing of organic solids. The current chapter addresses the design and operation of bioreactors for these processes. It shows how the various bioreactor types can be classified according to the aeration strategy, namely whether the bed of solids is forcefully aerated or not, and according to the agitation strategy, namely the frequency of mixing of the bed of solids. It discusses the current state-of-the-art in optimizing the design and operation of the various bioreactor types, showing how mathematical models that combine microbial growth kinetics and heat and mass transfer phenomena are the most powerful tools that we have available for this task. The chapter concludes by highlighting the necessity to convert current mathematical models into user-friendly computer programs that can guide design and operation decisions for large-scale solid-state cultivation bioreactors.

Key Words Bioreactor • solid state cultivation • SSF • solid organic residues • mathematical models • heat and mass transfer.

1. DEFINITION OF SOLID-STATE CULTIVATION PROCESSES

This chapter explores the applications of solid-state cultivation processes within the area of environmental biotechnology. The term solid-state cultivation is used to denote cultivation processes that involve the growth of microorganisms on particles of moist solid substrate particles, with a minimum of free water between the substrate particles. Figure 7.1 shows the essential features of solid-state cultivation systems and how this method of cultivation differs from various other cultivation processes.

Note that in solid-state cultivation systems, the inter-particle spaces (void spaces) may contain thin films and droplets of water, but the majority of water in the system is adsorbed within the moist substrate particles (1). The gas phase within the bed is continuous, with all solid surfaces that are not in contact with each other being exposed to this gas phase. This is the only cultivation method that offers such intimate contact between the microbial biomass, which develops at the surface of the substrate particles, and air. In this respect, it is quite different from various other cultivation systems (Figure 7.1b):

- *Trickling filters*: A liquid containing nutrients flows downwards through a bed of inert solids, with an upward flow of air. A microbial biofilm develops attached to the solid surfaces. Such systems are sometimes used in the aerobic treatment of wastewater. The gas phase may be largely continuous, but there is a significant amount of liquid in the spaces between the solid particles, with many solid surfaces in direct contact with a flowing liquid phase.
- *Submerged cultivation of suspended solids*: Solid particles containing nutrients are suspended in a continuous liquid phase. The liquid phase may already contain soluble nutrients, while other soluble nutrients may leach out of the solid phase into the liquid, and the microbial biomass will typically grow both in the liquid phase and as biofilms at the surface of the solid particles. The gas phase within the culture medium is discontinuous (i.e., present in the form of bubbles). Such systems include high-solids anaerobic digestion.

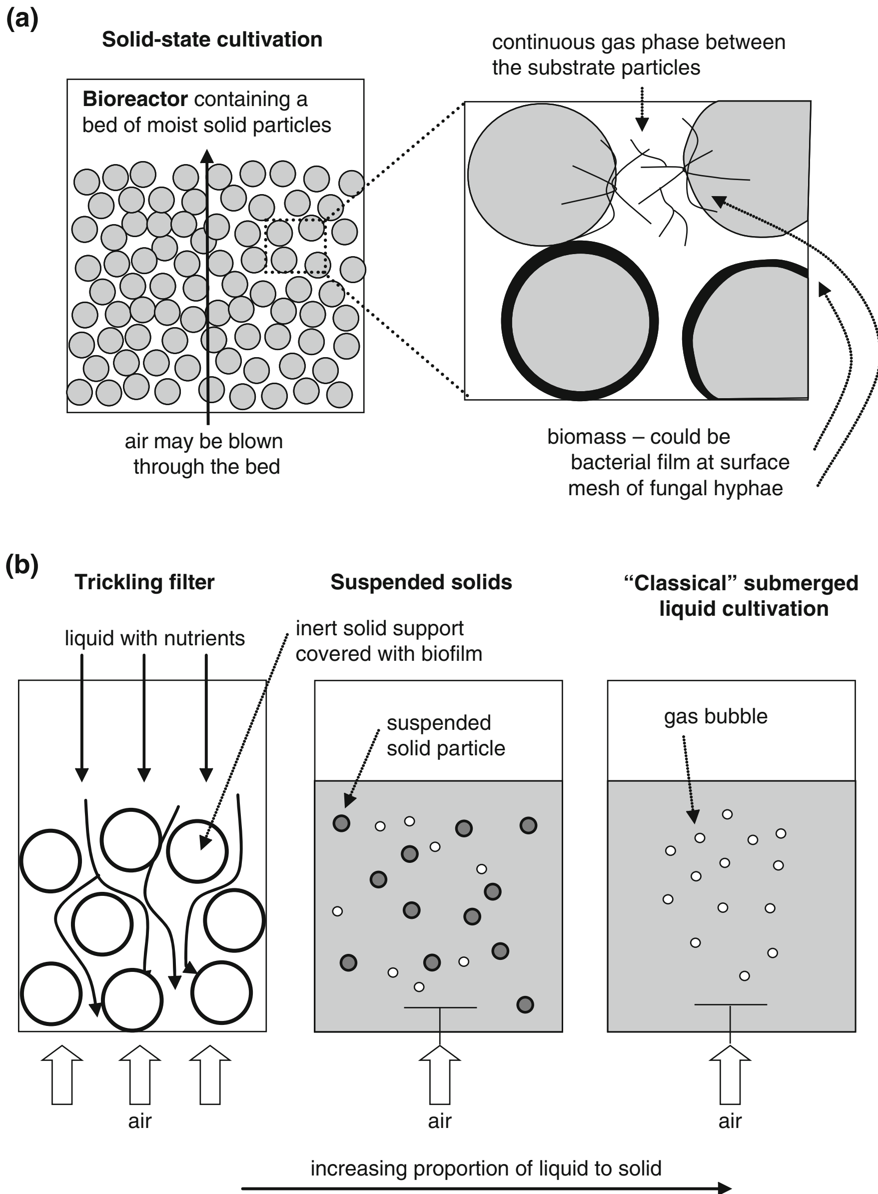

Fig. 7.1. Schematic representation of solid-state cultivation processes, highlighting the main features that define this system and distinguish it from several other cultivation systems (1). (**a**) Solid-state cultivation systems; (**b**) Other cultivation systems, from *left* to *right*: Trickling filter, Solids suspension, and "Classical" submerged liquid cultivation with soluble nutrients.

- *"Classical" submerged liquid cultivation*: the biomass is suspended within a continuous liquid phase, from which it absorbs soluble nutrients, and the gas phase is discontinuous (i.e., present in the form of bubbles).

Solid-state cultivation has a long history of applications, having been used for many hundreds of years in the first stage of soy sauce production, which involves the growth of the fungus *Aspergillus oryzae* on cooked soybeans. During this first stage, the fungus produces a cocktail of enzymes that diffuse into the soybeans. In the second stage, in which the soybeans are steeped in brine, these enzymes work slowly to digest the soybeans. Despite this long history, various technical difficulties with large-scale solid-state cultivation processes mean that submerged liquid cultivation, for which the technology developed significantly over the last half of the twentieth century, is the dominant cultivation method for biotechnological applications. However, in certain situations, solid-state cultivation has advantages over submerged liquid cultivation, especially in various environmentally related applications.

Section 2 classifies various types of environmentally related applications of solid-state cultivation, providing short descriptions of selected examples. Special emphasis is placed on processes that use bioreactors, for example, in situ bioremediation is not considered here. Since the various applications have some significant differences, Sect. 3 draws out some basic features that can be used to classify the nature of the process, and which affect bioreactor design and operation. Section 4 presents the general functions of a bioreactor. Various different bioreactors, which differ in aeration and agitation strategies, can be used for solid-state cultivation processes; Sect. 5 classifies these bioreactors into several groups based on the strategies used. Section 6 then shows the general considerations involved in the design of solid-state cultivation bioreactors and goes on to discuss these more specifically in the context of each different bioreactor type. Section 7 addresses some associated issues that must be considered during the bioreactor design process, namely decisions about the air preparation system and the system for monitoring and control of the bioreactor. This chapter ends with an evaluation of the scope for further improvement of bioreactors and bioreactor design methods.

2. CLASSIFICATION OF ENVIRONMENTAL APPLICATIONS OF SOLID-STATE CULTIVATION PROCESSES

Much of our current interest in solid-state cultivation processes is motivated by environmental concerns. It fits well with the vision proposed by Gunter Pauli in the Zero Emissions Research Initiative (ZERI proposal), in which all wastes of industrial processes are utilized as inputs to other processes, of the same or another industry. This vision requires the integration of various industries with different activities, in which the original wastes undergo a chain of transformations, with the final wastes only being returned to the environment if this can be done without any negative environmental effect (2). Solid-state cultivation has the potential to provide destinations for solid organic wastes from agricultural and forestry activities and from the food-processing industry, often producing products that have environmentally related applications. The following subsections explore several of these applications.

2.1. General Scheme for Classifying Solid-State Processes Used in Environmental Biotechnology

Solid-state cultivation finds several different environmentally related applications that can be divided into several classes based on the nature of the environmental relevance:

1. Class 1: Processes that use as substrates solid residues that would otherwise be discarded as wastes, although the products of these processes do not have environmentally related applications. Utilization of residues in these processes reduces the environmental impact that the wastes would have if they were to be discarded directly.
2. Class 2: Processes that use higher-value solid materials as substrates, for example, grains that could otherwise be used as food for humans or feed for animals, but the products of the process are either applied directly in environmental technology or, upon application, have less negative environmental impact than alternative products.
3. Class 3: Processes that both use solid residues as substrates and produce products that have environmentally related applications.
4. Class 4: Processes that either partially or totally replace other processes for biotransformation of solids, the solid-state cultivation process having a lesser negative impact on the environment than the process that it replaces.
5. Class 5: Pollutant removal processes for treating waste streams or contaminated soils.

Table 7.1 shows a selection of processes that are currently under study, classified according to this general scheme (3–44). In Sect. 2.2, examples of each type of application are briefly explored in order to highlight the environmental importance of these processes.

2.2. *Examples of Environmentally-Related Processes that Use Solid Residues*

2.2.1. *Two Examples of Class 1 Processes*

There are numerous examples of solid-state cultivation processes that use various solid organic residues as substrates that would otherwise simply be discarded, although the products from these processes do not have direct environmental applications (45). Two brief examples are given here (Fig. 7.2). First, wastes from the vegetable and fruit processing industry can be used for the production of a range of flavor and aroma compounds by solid-state cultivation (46). Note that in this case, a solid waste does remain after the flavor and aroma products are recovered. Second, wheat straw can be treated in a solid-state cultivation by either of two processes:

- Treatment with an actinomycete that preferentially utilizes lignin, having little cellulolytic activity, such that this process serves as a biopulping step, enabling the product to be used for paper manufacture (47).
- Treatment with a white-rot fungus, *Phanerochaete chrysosporium*, producing a product with improved digestibility to be used as an animal feed for ruminants (48).

2.2.2. *An Example of a Class 2 Process*

In some solid-state cultivation processes, the substrate is not actually a solid waste, but the product does have environmentally relevant applications. One example for this type of process is the production of spore-based fungal biopesticides, using grains such as wheat or rice. The environmental relevance comes from the fact that, in general, the application of a biopesticide in the environment will have a lower environmental impact than the application of a chemical pesticide. Biopesticides tend to have a much greater specificity for the pest than chemical pesticides. For example, in the case of bioinsecticides, it may be possible to target the pest insect without killing other insects, such as predatory insects; the latter are then able

Table 7.1
A selection of recent studies into environmentally related solid-state cultivation processes

Class 1: Processes that use as substrates solid residues that would otherwise be discarded as wastes, although the products of these processes do not have environmentally related applications

(a) Production of enzymes, including lignocellulolytic enzymes from *Trametes gallica* grown on wheat straw (3), pectinase from *Aspergillus niger* grown on sugar beet pulp (4), alkaline protease from *Bacillus* sp. grown on wheat bran and lentil husk (5), α-galactosidase from *Penicillium* sp. grown on soybean meal and beet pulp (6), α-amylase from *Aspergillus oryzae* grown on coconut oil cake (7), cellulase from *Trichoderma* sp. grown on wheat straw (8), lipase from *Penicillium simplicissimum* grown on soy cake (9), laccase by *Coriolus versicolor* grown on rice bran (10), and chitinase by *Trichoderma harzianum* and *Trichoderma longibrachiatum* grown on wheat bran supplemented with colloidal chitin (11)
(b) Production of secondary metabolites, including griseofulvin from *Penicillium griseofulvum* grown on rice bran (12) and tetracycline from *Streptomyces* spp. grown on groundnut shells, corncob, corn pomace and cassava peels (13)
(c) Production of p-coumaric acid (p-CA) and ferulic acid (FA) from *Sporotrichum thermophile* grown on corn cobs (14)
(d) Production of the aroma compound decalactone by various fungi grown on olive and castor oil press cakes (15)
(e) Production of single-cell protein for use as animal feed, growing various microorganisms on citrus residues and cotton stalk (16)
(f) Growth of *Aspergillus niger* on palm kernel cake to produce an animal feed with improved digestibility (17)
(g) Improving potential commercial value of guava wastes by increasing the phenolic antioxidant content using *Rhizopus oligosporus* (18)

Class 2: Processes that use higher-value solid materials as substrates but the products of the process are either applied directly in environmental technology or, upon application, have less negative environmental impact than alternative products

(a) Production of biological control agents, including *Epicoccum nigrum* grown on a vermiculite-based substrate (19) and *Coniothyrium minitans* grown on wheat grains (20)
(b) Production of phytase, which, when added to animal feeds, improves phosphorus absorption and therefore reduces phosphorus excretion in the feces, by *Aspergillus niger* grown on 1upin flour (21)

Class 3: Processes that both use solid residues as substrates and produce products that have environmentally related applications

(a) Production of xylanase, for use in biobleaching of wood pulp, by *Thermoascus aurantiacus* grown on sugar cane bagasse (22) and by *Bacillus coagulans* grown on soybean residue (23)
(b) Production of xylanase, for the enzymatic hydrolysis of corncob and sugarcane bagasse in the production of biofuels, by *Thermomyces lanuginosus* (24)
(c) Production of a biofertilizer by growing *Azotobacter vinelandii* on technical lignin, derived from the Kraft pulping process (25)
(d) Production of inocula for composting processes using various ligno-cellulolytic fungi grown on pepper plant wastes and almond shell residues (26)

(Continued)

Table 7.1
(Continued)

(e) Production of biocontrol agents, including *Beauveria bassiana* grown on rice straw and wheat bran (27) and *Trichoderma harzianum* grown on dried banana leaf (28)
(f) Production of enzymes, for use in decolorization of dyes, by *Pleurotus ostreatus* grown on wheat straw (29), by *Funalia trogii* grown on wheat bran and soybean residue (30), by *Lentinula edodes* grown on corn cob (31) and by *Trametes versicolor* and *Trametes hirsuta* grown on barley bran, a waste from the brewing industry (32)

Class 4: Processes that either partially or totally replace other processes for biotransformation of solids, the solid-state cultivation process having a lesser negative impact on the environment than the process that it replaces

(a) Use of wood-inhabiting basidiomycetes for biokraft pulping of softwood chips (33) and of wood-rotting polypores and corticioid fungi for the biopulping of Norway spruce wood (34)
(b) Delignification of wheat straw by *Streptomyces cyaneus* as a pretreatment to produce handsheets for the pulp and paper industry (35)
(c) Biobleaching of hardwood kraft pulp by the white-rot fungi *Phaneroehaete sordida* and *P. chrysosporium* (36)

Class 5: Pollutant removal processes for treating waste streams or contaminated soils

(a) Bioremediation of solids contaminated with diethylhexyl phthalate (37) and hydrocarbon (38) in reactors
(b) Benzopyrene removal from soil by *Phanerochaete chrysosporium* by mixing sugarcane bagasse and pine sawdust into the soil (39)
(c) Decolorisation of industrial dyes by *Pleurotus pulmonarius* grown on corn cobs (40)
(d) Fungal biofilters for removal of hexane vapor (41) and other solvents (42) from waste gas streams
(e) Solid-state bioconversion process for domestic wastewater sludge as an environmental-friendly disposal technique, using two mixed fungal cultures, *Trichoderma harzianum* with *Phanerochaete chrysosporium* and *T. harzianum* with *Mucor hiemalis* and two bulking materials, sawdust and rice straw (43)
(f) Biodegradation of chromium shavings in tannery waste by *Aspergillus carbonarius* grown under SSF conditions, permitting recovery, and reuse of chromium (44)

to continue their role in keeping the pest population in check. In addition, bioinsecticides tend not to have the same levels of general toxicity to higher animals as chemical insecticides.

Several biopesticides, especially those based on fungal spores, are better produced in solid-state cultivation than in submerged liquid cultivation. For example, some fungi only sporulate when they grow in the environment provided by solid-state cultivation. Or, if they sporulate in both solid-state cultivation and submerged liquid cultivation, the spore yields are much higher in solid-state cultivation. Another important consideration is the robustness of the spores, since this affects their survival upon application in the field. A greater robustness increases the durability of the spores and, therefore, they remain infective for longer periods. Some fungi produce various different types of spores, with different degrees of resistance. As a general rule, less robust spore types are produced in submerged liquid cultivation, while

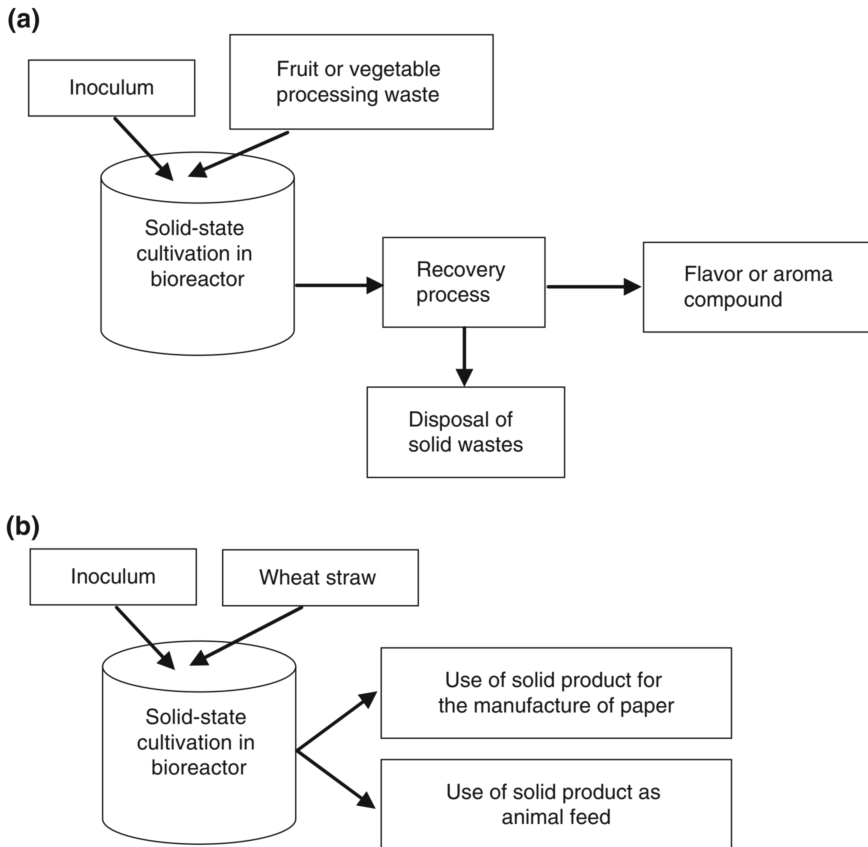

Fig. 7.2. Two examples of solid-state cultivation processes that use solid wastes, although the products do not have environmentally related applications (46–48). (**a**) Production of flavor and aroma compounds from solid fruit and vegetable wastes. In this case, the solid-state cultivation process generates a solid waste. (**b**) Utilization of wheat straw. The solid product from the process is not discarded but rather used either for the manufacture of paper or as an animal feed.

more robust types are produced in solid-state cultivation. A general scheme for the production of fungal spores as biopesticides is shown in Fig. 7.3. As a specific example, spores of the fungus *Metarhizium anisopliae* are produced on rice, as a bioinsecticide against the greyback canegrub, a pest of sugar cane that causes damages worth over $5 million in Australia (49).

2.2.3. *Two Examples of Class 3 Processes*

One example of a process that uses solid residues as raw material and also generates products that have environmentally related applications is the use of babassu oil cake, a solid residue generated during the industrial production of babassu oil, for the cultivation of the fungus *Penicillium restrictum* and the production of an enzymatic pool rich in lipases, proteases, and amylases. This enzymatic pool can be used to hydrolyze oily wastewaters prior to aerobic or anaerobic treatment (50).

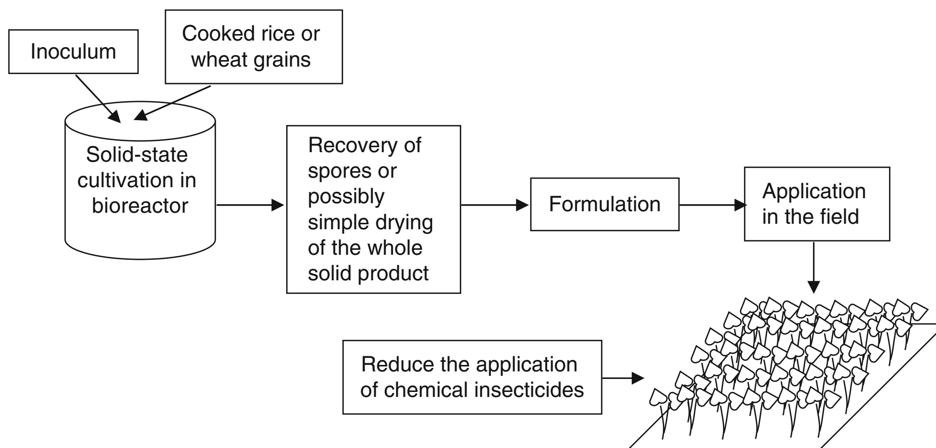

Fig. 7.3. An example of a solid-state cultivation process that uses a higher value solid substrate, but produces a product with environmental benefits (49). In this example, spores of a fungus are produced for application as a bioinsecticide.

Wastewaters with high oil and grease contents, such as effluents of dairy and slaughter plants, present problems to biological treatment systems due to the low biodegradation rate of fats. In anaerobic reactors, fats may cause clogging of the reactor, develop unpleasant odors, cause sludge flotation, and limit the transport of soluble substrates to the biomass, thus reducing the efficiency of removal of COD and BOD. In aerobic systems, the presence of fats leads to the development of filamentous microorganisms of the genera *Sphaerotilus*, *Thiothrix*, *Beggiatoa*, *Nocardia,* and *Microthrix*, which cause an undesirable phenomenon known as "bulking" (51). Furthermore, the presence of oil and grease in aerobic reactors causes the formation of stable foams on the surface of the aeration tanks and generates pellets inside the sludge flocs, hindering biomass flocculation and sedimentation.

Enzymatic hydrolysis of oily wastewaters prior to the biological treatment stages can solve these problems (50). The necessary enzymes can be produced by cultivating fungi of the genus *Penicillium* on babassu oil cake. One to two days after inoculation with fungal spores, fermented solids with high titers of hydrolytic enzymes such as lipases, proteases, and amylases are obtained (52). As shown in Figure 7.4a, the enzymes can be extracted to a liquid buffer and then separated from the solids, resulting in a "liquid enzyme product," which can be added to an oily effluent prior to biological treatment. Alternatively, the fermented solids can be simply dried under mild temperatures and the "solid enzymatic product" that is obtained can be directly added to oily wastewaters, at concentrations of 1 g/L or higher, prior to biological treatment (50).

The benefits of the enzymatic pretreatment step have been demonstrated for both the anaerobic and aerobic treatment of dairy wastewaters, containing elevated fat and grease levels (800 mg/L). Pretreatment of this wastewater with the solid enzyme product increased the efficiency of the subsequent treatment in either an upflow anaerobic sludge blanket reactor (53) or an aerobic batch activated-sludge bioreactor (54).

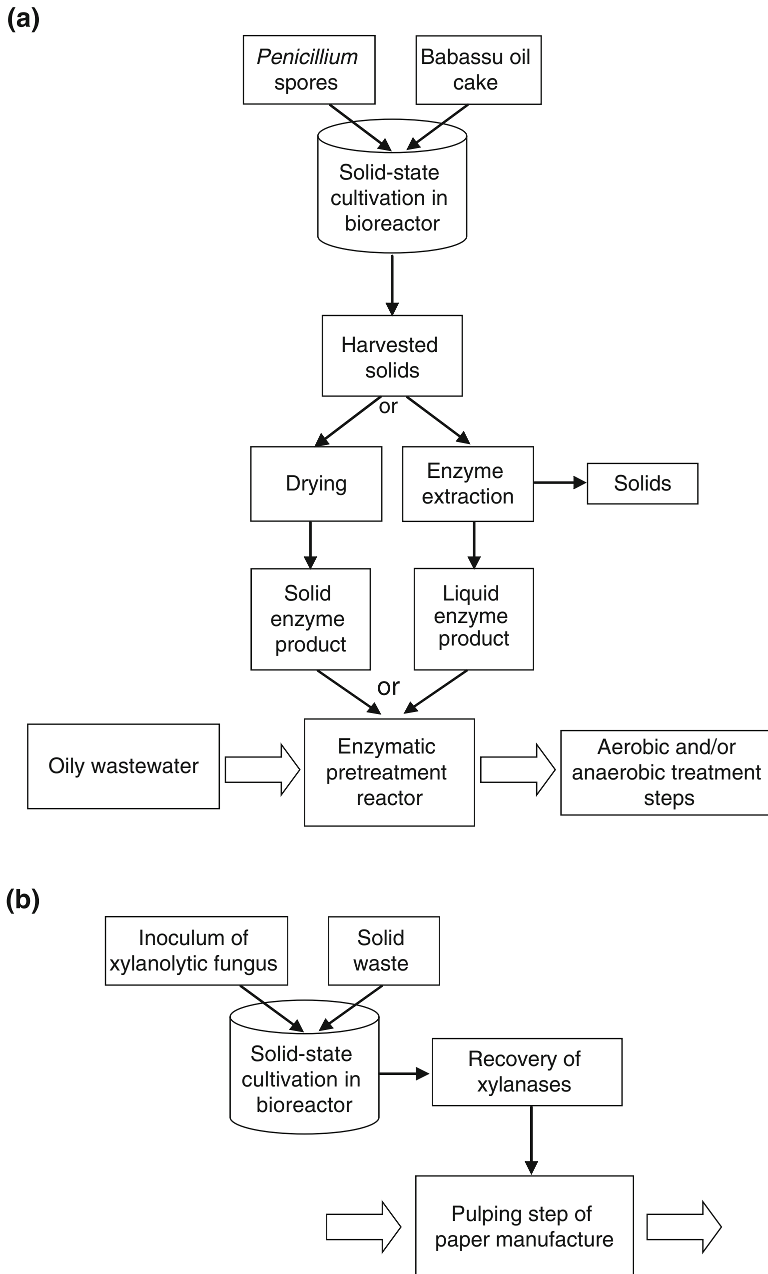

Fig. 7.4. Two examples of solid-state cultivation processes that both use a solid residue and produce a product with environmental applications (50, 56). (**a**) Simplified scheme of the solid-state cultivation of *Penicillium restrictum* on babassu oil cake to produce an enzyme cocktail in either liquid or solid form, and the application of this cocktail in the hydrolysis of oily wastewaters prior to either aerobic or anaerobic treatment. (**b**) Production of xylanases for use in biobleaching.

Comparing the effluent from the anaerobic digester with the enzymatic pretreatment system to one without pretreatment (53), the pretreatment step gave oil and grease removal efficiencies as high as 90% and:

- COD removal was increased from below 50% to as much as 90%;
- Turbidity was reduced by 75% (from 760 nephelometric turbidity units to below 200);
- Volatile suspended solids were reduced by 90% (from 940 mg/L to below 100 mg/L).

In the case of an aerobic batch activated-sludge system (54):

- As the oil and grease concentration in the dairy wastewater increased (400, 600, and 800 mg/L), the COD removal efficiency in the activated-sludge system without pretreatment decreased (86%, 75%, and 0%, respectively) while with the same effluent but with the enzymatic pretreatment step, COD removal efficiency in the activated-sludge bioreactor was maintained at high levels (93%, 92%, and 82%, respectively).
- At an oil and grease concentration of 800 mg/L, the effluent from the activated-sludge system without pretreatment had final volatile suspended solids values ten times higher (2,225 mg/L) than the effluent from the system fed with pre-hydrolyzed effluent (200 mg/L).

Beyond the technical aspects, economic issues must also be taken into account when considering the use of enzymes in wastewater treatment. In this context, the use of solid-state cultivation to produce hydrolytic enzymes is more economical than the use of submerged cultivation techniques. Using *Penicillium restrictum* and babassu oil cake for the production by solid-state cultivation of a lipolytic "liquid enzyme product" and considering a production scale of 100 m^3 per year, the total capital investment needed is 78% lower than that needed for a submerged liquid cultivation process and the unitary production cost for solid-state cultivation is 47% lower than the market price of an existing liquid lipolytic product. The solid-state cultivation process is very attractive from an economic point of view, with a payback time of 1.5 years, a return on investment of 68% and an internal return rate of 62% for a 5-year-project life (55). These economic advantages of solid-state cultivation for the production of hydrolytic enzymes are mainly due to the low capital investment and to the very cheap raw material used. Considering that products for environmental applications should be extremely cheap, the direct addition of the dried fermented solids to the effluents is a further economic advantage of the process, since no operations concerning enzyme extraction and solid-liquid separation are needed.

A second process that both uses waste residues and produces an environmentally relevant product is the production of cellulase-free xylanase preparations for use in biobleaching of kraft pulp in paper manufacture. The action of the xylanases facilitates the extractability of lignins by conventional bleaching chemicals, resulting in a reduction of the consumption of bleaching chemicals by 20–30%. The use of these chemicals generates toxic byproducts that are mutagenic and persist in the environment, and this reduced consumption leads to a reduction of organic halogen loads in the effluents by 15–20% (56).

2.2.4. An Example of a Class 4 Process

An example of a process for the biotransformation of solids that partially replaces processes that have a more negative impact on the environment is biopulping. Biopulping involves the

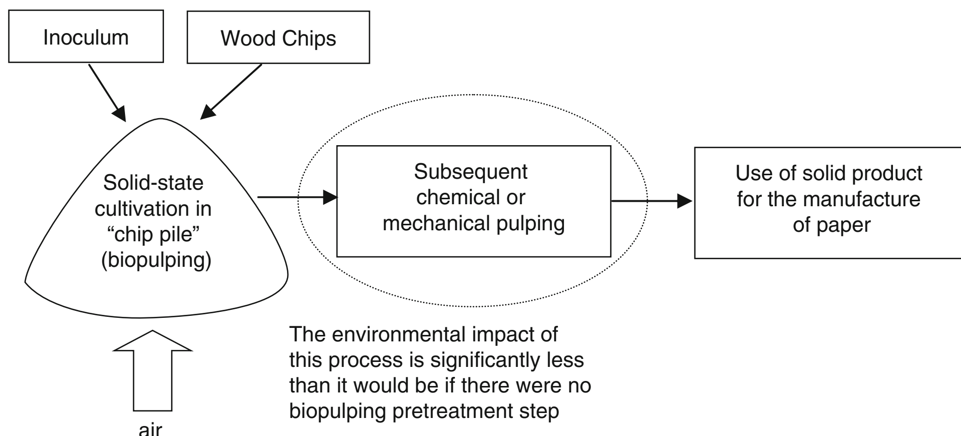

Fig. 7.5. Biopulping of wood chips, an example of a solid-state cultivation process that partially replaces the less environmentally-friendly chemical alternative (57).

pretreatment of wood chips, prior to either mechanical or chemical pulping, with lignin-degrading fungi such as *Ceriporiopsis subvermispora*. This biological pretreatment can lead to energy savings of 40–50% (57). Due to the large volumes that need to be processed, in-vessel treatment would be too expensive; rather the process is carried out in aerated "chip piles" (Fig. 7.5).

2.2.5. Two Examples of Class 5 Processes

Two important examples of solid-state cultivation processes for pollutant removal are biofilters and ex situ bioremediation.

Many industries produce waste gases that contain toxic or odorous substances. Various processes are available to reduce or even eliminate these substances. Biofiltration, in which the gas is passed through a bed of solids on which a mixed microbial population grows and consumes volatile organic compounds from the gas phase (Figure 7.6a), has some advantages over other purification techniques (58). The pollutants are not simply adsorbed and held in another phase, as occurs in adsorption processes, but rather are converted into harmless oxidation products. Moreover, the investments and operational costs of biofilters are cheap when compared to chemical or catalytic oxidation. Recently, the applications of biofiltration have been widely extended due to improvements in filter technology and more severe regulations on the emission of gases.

As an example of ex-situ bioremediation, soil contaminated with petroleum can be removed from the contaminated site, mixed with nutrients and structuring agents, and heaped in piles, up to 3 m high, 5 m wide and 30 m long. These piles are made on the top of a network of air pipes that, in turn, rest on top of an impermeable tarpaulin, in order to prevent the leaching of pollutants into the ground at the treatment site. Treatment times may be as long as 3–12 months, with periodic addition of nutrients. The degree of removal of pollutants depends on

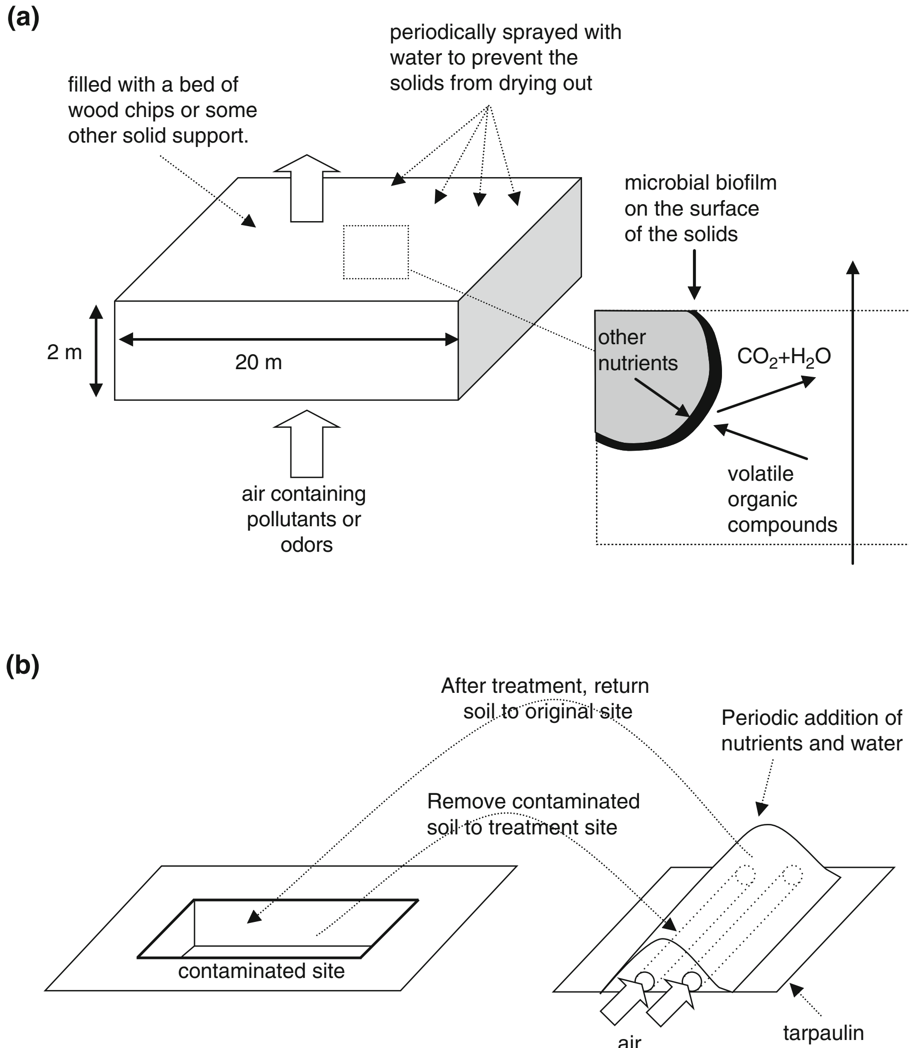

Fig. 7.6. Two examples of solid-state cultivation processes that remove pollutants (58, 59). (**a**) A biofilter used to remove volatile organic compounds that are either pollutants or odors from a waste gas stream. (**b**) Bioremediation of contaminated soil by the "biopile" method.

the efficiency of oxygenation of the soil, and is also affected by temperature, which is not controlled (59).

3. CLASSIFICATION OF PROCESS TYPES

It is possible to analyze the above processes, and other environmentally related applications of solid-state cultivation, according to several different criteria. Each of these criteria has implications for how the bioreactor for the process will be designed and operated. Processes may:

- *Be aerobic or anaerobic*. The majority of solid-state cultivation processes involve the aerobic growth of organisms. However, some environmentally related processes do involve anaerobic growth, such as solid-phase anaerobic digestion and ethanol production from solid residues. The need to supply O_2 to the bioreactor is obviously an important design consideration.
- *Be operated in batch or continuous mode*. The solid nature of the substrate means that the well-mixed continuous culture method that is used in submerged liquid culture is not appropriate, since newly-added substrate particles are not immediately colonized. However, continuous culture of the plug-flow type is feasible, and has been used for an initial composting step in the stabilization of municipal solid waste. Figure 7.7a highlights the differences between batch and continuous operation.
- *Involve the use of pure cultures, defined mixed cultures, or "selected microflora."* For example, the production of the lipolytic enzyme mixture by *Penicillium restrictum* (described in Sect. 2.2.3) involves a pure culture whereas composting processes involve an undefined microflora selected from the original microflora of the material being composted. The use of

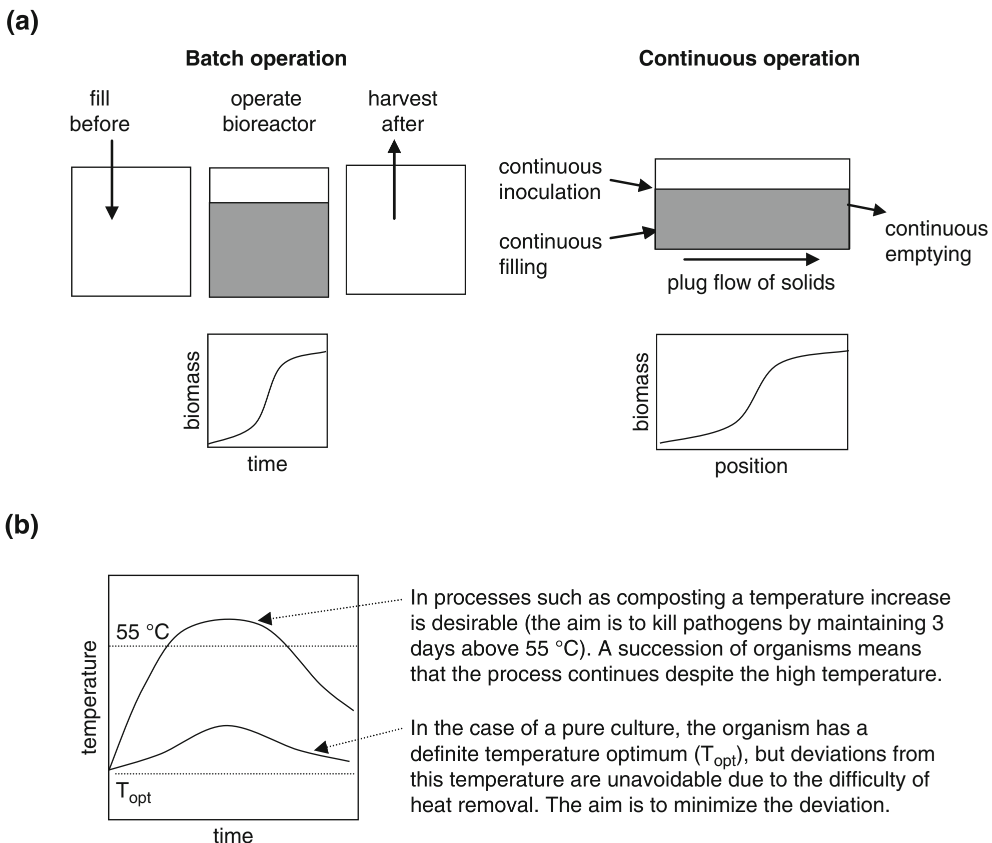

Fig. 7.7. Two of the aspects of bioreactor operation that have implications for process design (60). (**a**) The difference between batch cultivation and continuous operation in the plug-flow mode. (**b**) In some processes, it is highly desirable to control the temperature in the substrate bed, while in others, it is desirable for the temperature to reach high values.

pure cultures is often correlated with a need to design the bioreactor so as to minimize the entry of contaminants during the cultivation. On the other hand, if the culture conditions select for a particular microflora, there may be no particular need to prevent the entry of contaminants.

- *Require good temperature control or either tolerate or need significant variations in the bed temperature during the process*. For many processes, especially those that use pure cultures or defined mixed cultures, there is an optimum temperature for the process (Figure 7.7b) and the bed temperature should be maintained at this optimum value or as close to it as possible. On the other hand, in composting, a desired succession of microbial types accompanies temporal variations in the temperature. In fact, in order to kill pathogens, it is desirable to maintain temperatures above 55°C for several days.
- *Require a high degree of asepsis or not be particularly demanding in terms of asepsis*. Depending on the process conditions and the microorganisms used, solid-state cultivation processes may or may not be resistant to contamination. For example, processes operated at water activities below 0.98 with fungi such as *Penicillium* or *Aspergillus* are typically resistant to contamination by bacteria. If started with a vigorous inoculum, it is often not crucial to go to great lengths to prevent contamination during the process. In some cases, the process organism might be an opportunistic pathogen, produce spores that can trigger allergy in process workers, or present an environmental risk. In these cases, it is desirable to prevent release of the process organism from the bioreactor. These considerations will affect the necessity to include aseptic seals and filters in the bioreactor design.
- *Require to be operated by relatively unskilled workers.* Some processes will be operated at a large-scale central facility wherein relatively skilled labor will be available, allowing the implementation of more complex bioreactor types and more sophisticated technology. However, some processes will be applied in domestic or local industries, requiring more simple technologies, such as cultivation in trays, pots, or bags.

4. THE FUNCTIONS THAT THE SOLID-STATE CULTIVATION BIOREACTOR MUST FULFILL

This section addresses the design and operation of bioreactors for the various processes discussed in Sect. 2. Clearly, the various process classifications presented in Sect. 3 will affect the specific design and operational features of the bioreactor and, as a result, several quite different bioreactor types are used in solid-state cultivation processes. The following sections will outline the various bioreactor types available and the important considerations necessary in order to design and operate them efficiently. The current section will outline in general terms the functions that a bioreactor may have to fulfill. Bioreactors for specific processes may not have all of the features mentioned here, but these specific differences will be made clearer when the various different bioreactors are presented in the later sections.

When cultivating microorganisms in a bioreactor, the aim is to maintain optimal conditions for growth and product formation or for the execution of a desired metabolic activity. The ability to control the conditions in the bed depends on the operating variables that can be manipulated, such as air flow rates and mixing regimes, and on the effectiveness of heat and mass transport phenomena within the bed. Both the available operating variables and the effectiveness of heat and mass transfer depend on the particular bioreactor type used. It is often either impossible or prohibitively expensive to maintain optimal conditions across the

whole bed during the whole cultivation. In this case, the aim becomes to maintain near-optimal conditions over as much of the bed and during as much of the cultivation as possible.

In the majority of solid-state cultivation processes, two key process variables that need to be controlled are the bed temperature and the moisture content. For aerobic processes, it is also necessary to ensure high O_2 concentrations within the void spaces in order to provide an adequate O_2 supply to the particle surface. These variables are important because it is possible to influence them through manipulation of the operating variables. Of course, there are many other variables that affect the growth process. However, "intra-particle variables", such as the dissolved O_2 concentration, the nutrient concentration, and the pH, are typically only affected very indirectly by manipulations of operating variables such as the air flow rate. In fact, our inability to undertake control actions to maintain intra-particle variables at optimum values is a limitation intrinsic to the solid-state cultivation method.

For some processes, there is a single set of conditions that should be maintained throughout the cultivation. For other processes, the optimal conditions change during the process, in which case the aim is to provide the optimal temporal profile. As an example, in producing fungal spores for use as biopesticides, it may be desirable to have higher water activities within the bed during the early stages in order to favor growth, but to have lower water activities in the latter stages in order to favor spore formation. As another example, in composting, it is desirable that the bed temperature should initially increase rapidly, reach and then maintain values of over 55°C for several days before declining again.

In order to allow control of the key process variables, and to fulfill various other functions that a bioreactor has, the bioreactor may need to be designed to (Fig. 7.8):

- *Hold the substrate.* The bioreactor size will be determined on the basis of calculations of the required throughput and the expected average productivity per unit volume of the bioreactor. The size will affect the selection of the material for construction of the bioreactor, based on the required mechanical strength.
- *Enable sterilization*. Sterilization will be most important for those processes in which pure cultures or defined mixed cultures are used. If sterilization is necessary, it must be decided whether the bioreactor and substrate are to be sterilized separately, or the substrate is to be sterilized within the bioreactor. If the substrate is to be sterilized within the bioreactor, the bioreactor must be designed in such a way as to enable as uniform a sterilization of the solid bed as possible and may need to resist high internal pressures. In cases in which the process conditions are highly selective for the process organism, it may be possible simply to cook the substrate rather than to sterilize it.
- *Provide a barrier against contamination or contain the process organism.* These considerations will depend on whether pure or defined-mixed cultures are used, the selectivity of the process conditions for the process organism and the health or environmental risks presented by the process organism.
- *Enable the removal of metabolic heat into cooling water.* Adequate removal of the metabolic heat generated by the process organism is typically the central challenge in the design of solid-state cultivation bioreactors. It may be desirable for the bioreactor to have water jackets or internal heat transfer surfaces to remove heat, although the effectiveness of this method of heat removal is limited by the relatively poor conductivity of solids.
- *Enable air to be supplied to the substrate bed*. Many solid-state cultivation processes are aerobic, and in such processes O_2 must reach the organism growing at the surface of the substrate

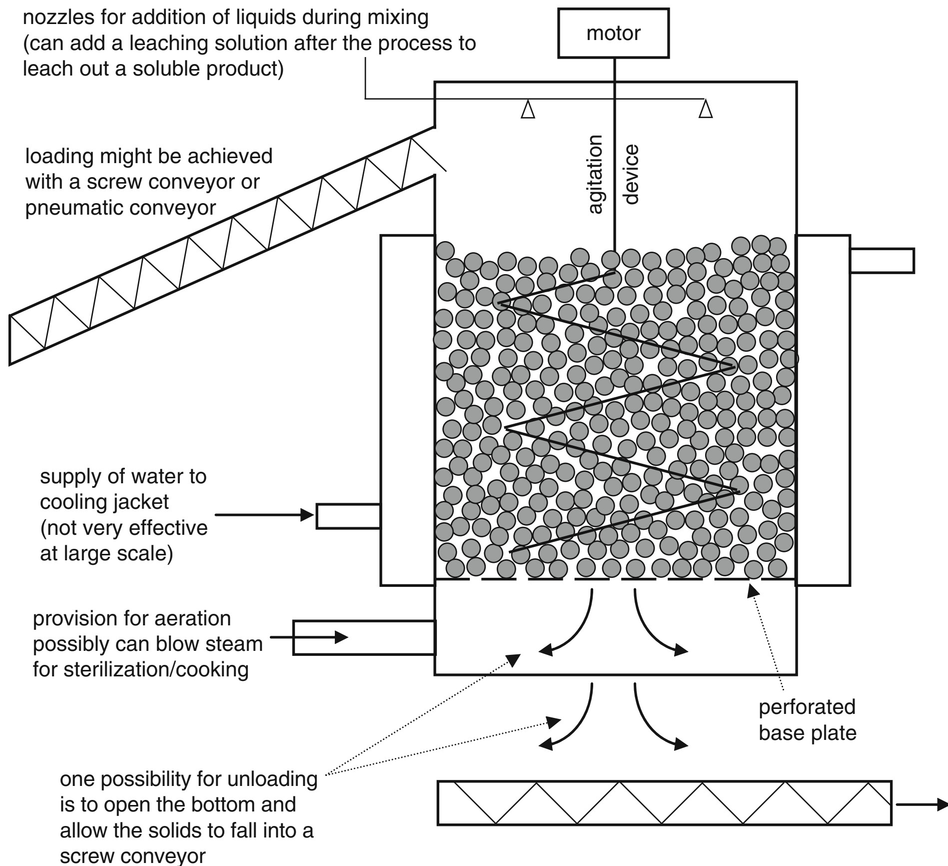

Fig. 7.8. A schematic diagram of a bioreactor for solid-state cultivation, showing various features that it may need (60). Note that some bioreactors appear quite different and not all bioreactors will incorporate all the features shown here.

particles. Beyond this, gas supplied to the substrate bed may play an important role in removal of heat from the bed by convective and evaporative cooling. Two aeration strategies are possible. Air may be blown across the bed surface, without forcing it to pass through the bed, or, alternatively, air may be forcefully blown through the bed. As a general principle, aeration will be more effective as contact increases between the supplied air and the bed. In other words, it is most effective to blow air through the bed; this should be done in such a way as to ensure an even distribution of the air, avoiding preferential flow through cracks in the bed or between the bed and the bioreactor wall.

- *Enable the contents of the bed to be mixed.* As a general principle, mixing promotes uniformity of conditions within the bed, this being desirable in order to obtain uniformity of the microbial processes that occur. However, it may also cause undesirable damage to the process organism, especially in processes in which fungi are used. Three mixing regimes are possible. The substrate bed may be completely static, it may be left static part of the time but suffer intermittent mixing, or it may be continuously mixed. The mixing of a bed of solid particles is a challenging task

that can be greatly affected by the properties of the substrate such as the stickiness, mechanical rigidity, and size and shape of the particles.

- *Facilitate the solids loading and unloading steps*. The design of the bioreactor must take into account the loading and unloading operations. Note that it is not as easy to move solids around as it is to pump liquids into a vessel and drain them out. The difficulty of these solids handling processes increases as the scale of the process increases, that is, as the volume of the solids to be processed increases. The manner in which the bioreactor design provides for the loading and unloading steps will also depend on whether the process is to be operated in batch or continuous mode.
- *Enable the addition of liquids during the cultivation.* In some cases, it might be desirable to add water to the solids during the process to avoid them drying out. Alternatively, it might be necessary to add nutrient solutions or pH-correcting solutions. The uniform distribution of such additions will be important, and, as a general principle, such additions will best be made by misting the solution onto the substrate bed as it is being mixed.
- *Take part in downstream processing operations*. For example, product drying or extraction by leaching of a soluble product could be done in situ within a bioreactor.

5. CLASSIFICATION OF BIOREACTORS USED IN ENVIRONMENTALLY-RELATED SOLID-STATE CULTIVATION PROCESSES

Two key design considerations for bioreactor operations are the aeration and agitation schemes, since these have the greatest effect on the key process variables, namely the bed temperature and water content and the void space O_2 concentration. It is useful to classify bioreactors in groups in terms of how they are aerated and agitated, because of the many similarities in operating variables that can be manipulated to optimize bioreactor performance as well as the design strategies used (60). Four basic groups can be identified:

- Group I – Bioreactors that are neither agitated nor forcefully aerated
- Group II – Bioreactors that are not agitated but are forcefully aerated
- Group III – Bioreactors that are agitated but are not forcefully aerated
- Group IV – Bioreactors that are both agitated and forcefully aerated

The basic features of the bioreactors in these various groups are outlined in the following subsections. Note that the distinction in terms of the agitation regime is not clear-cut. Intermittently mixed bioreactors might either be grouped with unmixed bioreactors or with continuously mixed bioreactors, depending on the frequency of the mixing events. In this context, "frequently mixed" means that the mixing events are sufficiently frequent for the substrate not to become anaerobic for long periods.

5.1. *Group I Bioreactors: Not Aerated Forcefully and Not-Mixed*

In Group I bioreactors, air is not blown forcefully through the bed but rather is circulated around the bed surfaces. The substrate bed either remains static during the whole process or is mixed only very infrequently, of the order of once or twice per day. Figure 7.9 shows several types of bioreactors that are classified within this group. The classical process is carried out in

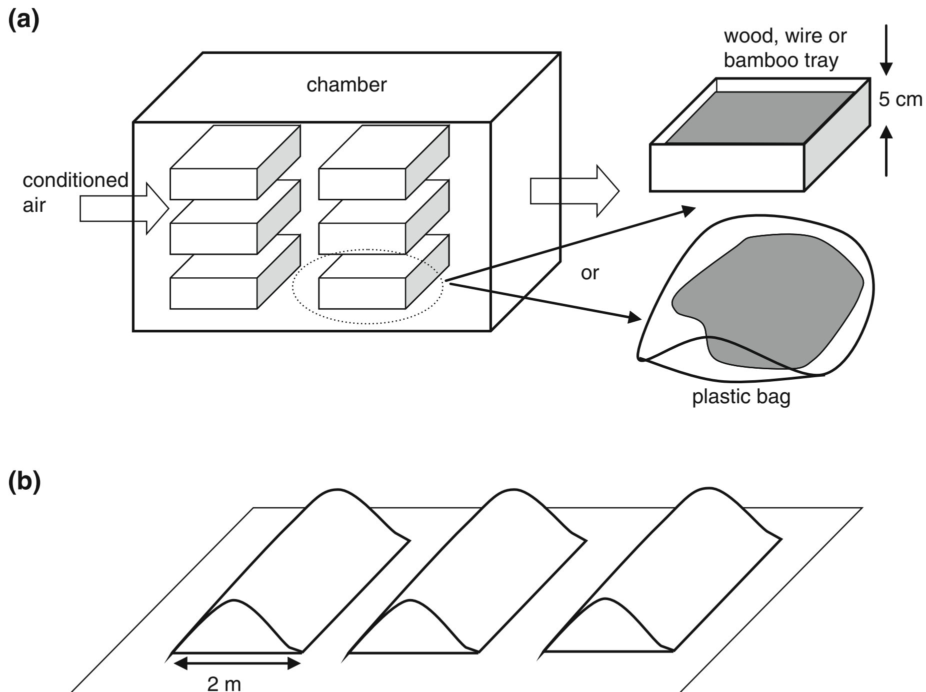

Fig. 7.9. Various bioreactors classified in Group I, that is, bioreactors that are neither forcefully aerated nor mixed (60). (**a**) Tray-type bioreactors. (**b**) Unaerated windrows for composting.

trays, which may be made of wood, bamboo, plastic, or metal. Some processes are carried out in plastic bags. The plastic allows for the exchange of O_2 and CO_2 but minimizes the exchange of water and does not allow the entry of contaminants. These trays or bags are incubated within a chamber, which, depending on the scale of the process, might actually be a room.

Tray bioreactors are appropriate for situations in which relatively small volumes are to be produced with relatively simple technology. For example, tray-type cultivations can be used for the production of biopesticides by local producers. At a very large scale, those composting processes that are based on the use of unaerated, infrequently turned windrows would be classified within this group of bioreactors.

5.2. Group II Bioreactors: Aerated Forcefully but Not-Mixed

The general feature of Group II bioreactors is that the substrate bed is forcefully aerated but remains static; in some processes, it remains static for the whole cultivation, while in others, it may be mixed infrequently, on the order of once or twice per day. The consequence is that the performance of this bioreactor is highly dependent on convective flow phenomena, which, as described later, means that there is a tendency to establish axial gradients (that is, gradients along the direction of air flow).

Typically, in this type of bioreactor, the substrate bed sits on a perforated base and air is forced through the bed. It is more common for the air to enter at the bottom, although it is also

possible to have the air entering at the top (Figure 7.10). A very simple large-scale application of this type of bioreactor is that of forcefully aerated windrows used in composting. The so-called Zymotis bioreactor is an interesting variant of this bioreactor type, in which heat transfer plates are inserted into the bed (61). This bioreactor has been used for the production

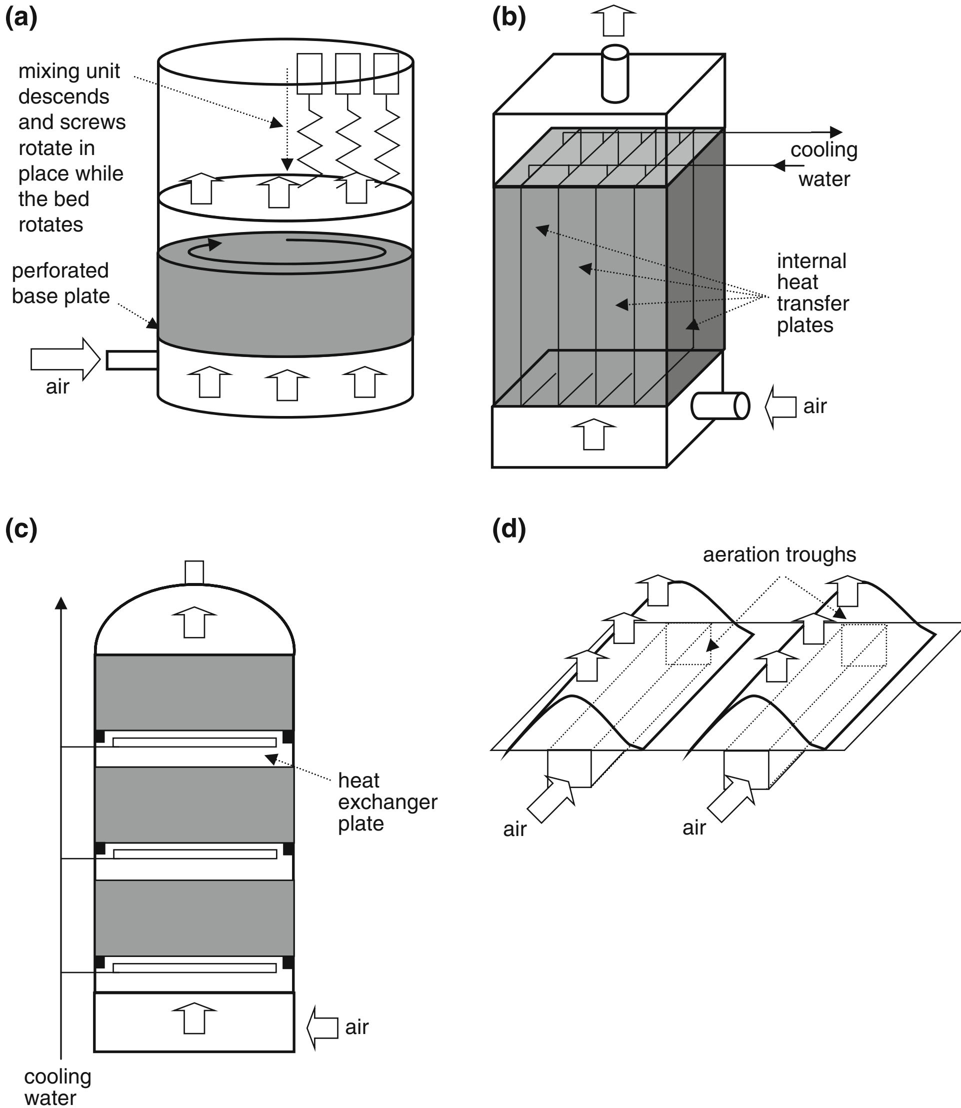

Fig. 7.10. Various bioreactors classified in Group II, that is, bioreactors that are forcefully aerated but not-mixed, or, if mixed, mixed only very infrequently (60–62). (**a**) Traditional design, but with provision for infrequent mixing events. (**b**) The Zymotis bioreactor, which has heat transfer plates within the substrate bed. (**c**) The bioreactor patented by Prophyta, which has multiple beds with a heat transfer plate under each bed. (**d**) Aerated windrows used in composting.

of cellulases using a mixture of two residues (sugar cane bagasse and wheat bran in 80:20 ratio by weight) (61). Another design, recently patented, the so-called Prophyta design, consists of a stack of packed beds with heat exchanger plates at the bottom of each bed and has been used for the production of biopesticides (62). Various designs used for the production of soy sauce *koji*, a non-environmentally related application, could be adapted to environmentally related processes (63).

5.3. Group III Bioreactors: Not Aerated Forcefully but Mixed

The general feature of Group III bioreactors is that the substrate bed is continuously mixed, but air is not blown forcefully through the bed; rather it is blown past the bed surface. Note that, in some cases, the mixing may be intermittent, but occurring at frequent intervals. Most frequently, such bioreactors consist of drums with their central axis being either horizontal or slightly inclined to the horizontal, are partially filled with substrate and have air blown through the headspace. For such bioreactors, there are two mixing options (Figure 7.11). In the "rotating drum" option, the bioreactor body is rotated to provide the mixing action, this possibly being facilitated by internal lifters. In the "stirred drum" option, the bioreactor body remains stationary and the substrate bed is mixed by paddles or scrapers that rotate around a central shaft.

In some cases, attempts have been made to inject air into the substrate, for example, through small holes in the end of each paddle in a stirred bed (64). However, unless provisions are made to distribute this air over a wide area, the effect of this aeration will be limited to a relatively small proportion of the substrate in the bed and such a bioreactor will operate more like a Group III bioreactor than the well-mixed, forcefully aerated bioreactors of Group IV.

Group III bioreactors have been used in various environmentally related applications of solid-state cultivation. Large composters, up to 3.5 m diameter and 45 m in length, have been used for composting of municipal solid-wastes (65). In some cases, compost is produced to be sold as a soil conditioner while in other cases composting is used simply to stabilize the wastes before landfilling, in order to reduce the environmental impact of the landfill.

5.4. Group IV Bioreactors: Aerated Forcefully and Mixed

Group IV bioreactors are both forcefully aerated and continuously mixed, or, if intermittently mixed, they are mixed every hour or two, or more frequently still. Various bioreactors that fall into this group are shown in Figure 7.12. In air-solid fluidized beds, the mixing action is not mechanical (Figure 7.12a). Such a bioreactor of 8,000 L working volume has been used to produce amylases and proteases from wheat bran powder (66). Air-solid fluidized beds have not yet been used for environmentally related applications, but could be expected to have reasonably high operating costs due to the high aeration rates needed to fluidize the bed. In addition, uniform fluidization might be difficult to achieve if heterogeneous waste materials are used as the substrate. In other designs, the bed is mixed mechanically (Figs. 7.12b–d). In some designs, the mixer itself operates continuously, but only mixes a relatively small portion of the bed at any one time and must travel along the bed in order to mix the whole bed, such that any particular region of the bed is mixed only intermittently (Figs. 7.12c, d).

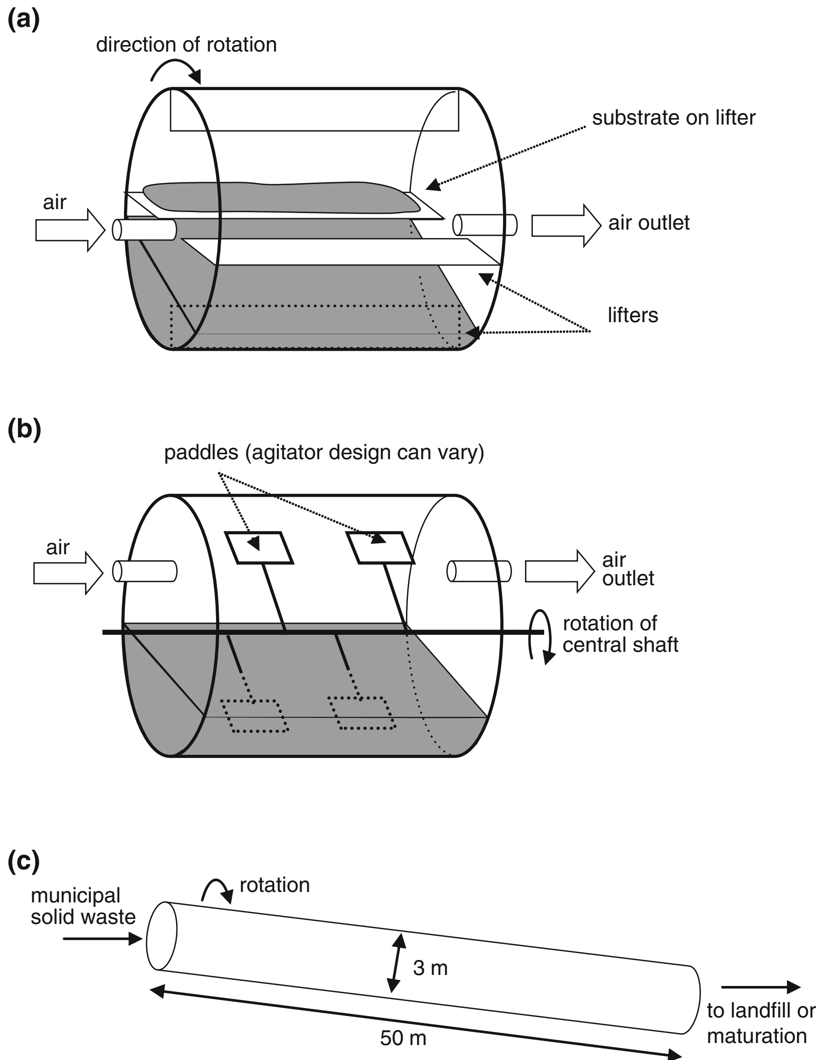

Fig. 7.11. Various bioreactors classified in Group III, that is, bioreactors in which the bed is not forcefully aerated, but are mixed either continuously or frequently (60). (**a**) Rotating drum bioreactor. (**b**) Stirred-drum bioreactor. (**c**) Eweson or Dano-type composters for stabilizing municipal solid waste.

Mechanically mixed Group IV bioreactors have been used at relatively large scales, including various applications of environmental relevance. Various designs have been used in composting processes (65). A bioreactor developed originally at INRA (Platform for Development in Biotechnology), in Dijon, France (67), has been used for the protein enrichment of agro-industrial byproducts for use as animal feed and for the production of enzymes and biopesticides (68). This bioreactor has a bed capacity of 1.6 m^3, enabling it to hold one ton of moist sugar beet pulp (at 75% water content). A larger version 17.6 m in length, 3.6 m in width,

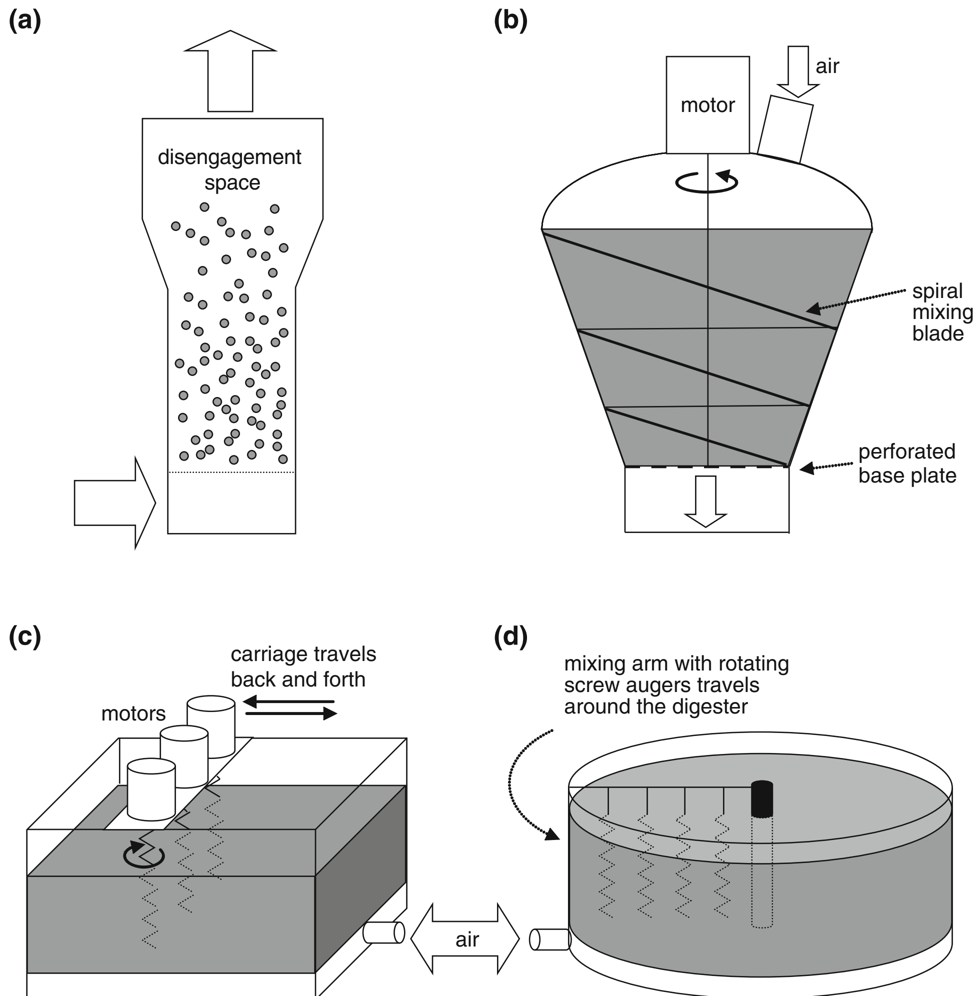

Fig. 7.12. Various bioreactors classified in Group IV, that is, bioreactors in which the bed is both forcefully aerated and mixed either continuously or frequently (60, 65–70). (**a**) An air-solid fluidized bed bioreactor, in which the bed is mixed by the air flow. (**b**) A mechanically-stirred bioreactor without a traveling agitator, an adapted conical solids mixer (70). Note that the bioreactor shown in Fig. 7.8 is another version of this type of stirred-bed bioreactor, and that, in this type of bioreactor, the agitator may be designed differently, for example, planetary mixers may be used. (**c**) A larger scale version of a mechanically-stirred bioreactor with a traveling agitator, as developed Durand and Chereau (67). (**d**) The Fairfield-Hardy digester, which has a traveling agitator and has been used for composting processes (65).

and with a 2.0 m bed height has been used for protein enrichment of 25 ton batches of sugar beet pulp (at 80% water content) (69). Conical commercial solids mixers with helical-blades have also been suggested as bioreactors for solid-state cultivation (Figure 7.12b) (70).

6. DESIGN OF BIOREACTORS FOR ENVIRONMENTALLY-RELATED SOLID-STATE CULTIVATION PROCESSES

Given the various different bioreactor designs (Sect. 5) that can be used and the various different process considerations (Sect. 3), bioreactor design for solid-state cultivation processes is not a simple matter. This section will concentrate on bioreactor design for those environmentally related processes in which it is desirable for the bioreactor to control the temperature at, or as near to as possible, a fixed optimum value throughout the cultivation. Special considerations are required for the design and operation of bioreactors for in-vessel composting, in which temperature variations are desirable and for in-vessel bioremediation and biofilters, in which the main consideration is the removal of pollutants. These will not be discussed here.

6.1. General Considerations for the Selection and Design of Bioreactors

Before addressing methods for the design of specific bioreactor types, it is worthwhile to give a general outline for the bioreactor selection and design process. Various bioreactors that have been developed for environmentally related applications of solid-state cultivation technology in the past have been highly inefficient because they were designed based on a "best-guess" strategy. Our knowledge is currently sufficient to enable the use of quantitative calculations to guide bioreactor design. The process of selecting and designing a solid-state cultivation bioreactor should, therefore, be based on these calculations and will have three steps, each of which can be characterized by a basic question:

- *Understanding the process organism*: What are the key phenomena that, first, affect the process significantly and, second, can be affected in the way that the bioreactor is designed and operated? It is necessary to understand how the organism grows and produces its product and how this growth and production are affected by key process variables. This chapter concentrates on those processes for which the key process variable is the bed temperature. Note that the water content of the bed becomes another key variable in those cases in which evaporation plays a role in temperature control. In other environmentally related processes, such as bioremediation and biofiltering, in which metabolic activities are not so high and, therefore, the generation of waste metabolic heat is lower, high temperatures may not be such a crucial problem; rather, the lowering of the pollutant to acceptable levels may be the crucial design consideration. The tolerance of the process organism to agitation also needs to be understood at this stage.
- *Bioreactor selection*: What is the best bioreactor, taking into account any external constraints? For example, there may be limits on operating costs or on the level of technology to be used. Note that bioreactor selection will be significantly affected firstly by the rate of growth of the organism and the consequences this has for efficient heat removal and secondly by the degree to which the process organism tolerates agitation of the bed.
- *Bioreactor design and optimization*: Having selected the best bioreactor for a particular process, what is the best way to design and operate the selected bioreactor in order to maintain the key process variables at, or as near as possible to, the optimum values for the process? This requires an understanding of the heat and mass transfer phenomena that affect the values of the key process variables, and how the various operating variables influence the efficiency of this heat and mass transfer. As will become clear in the sections addressing the design of the various different bioreactor types, mathematical models that incorporate both the kinetic behavior of the organism and the key heat and mass transfer processes are the powerful tools for guiding the design and optimization of operation of bioreactors.

The design process can be attacked at various levels of sophistication (Figure 7.13). Typically, in the design of an environmentally related process, the interest is in avoiding undue complexity, while making reasonable decisions. The desire to avoid undue complexity means that intra-particle phenomena will typically not be of interest. As described in the following section, this means that relatively simple empirical kinetic equations tend to be used.

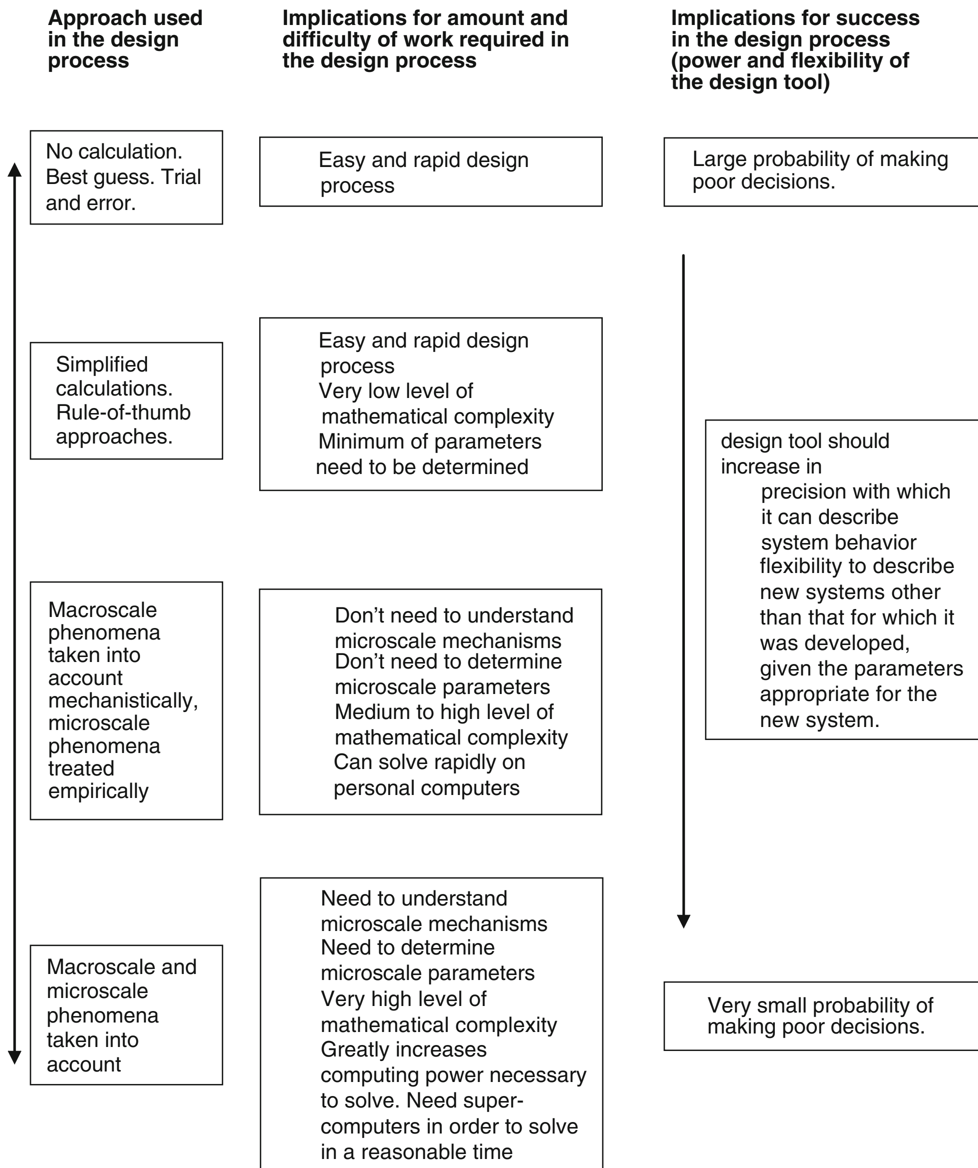

Fig. 7.13. A continuum of approaches to the design task, from the most simple to the most complex (60).

As mentioned earlier, this chapter focuses on those processes in which growth is sufficiently fast for temperature control to be the major challenge in bioreactor design. For these "fast-growth/temperature-problematic" processes, several key questions will guide the design process:

- What is the optimum temperature of the substrate bed?
- What is the degree of aeration that the process requires?
- What is the optimum water activity of the substrate? Note that water becomes an important variable since metabolic heat production promotes evaporation and, furthermore, the bioreactor might be operated in such a manner as to maximize evaporation in order to take advantage of the high heat removal potential of evaporative cooling, but this can potentially decrease the water activity of the substrate to values too low for good growth.
- What are the important limitations on bioreactor operation that might derive from properties of the substrate or organism, such as limitations on the type and frequency of mixing that can be used? Specifically, in processes that involve fungi, the fungal hyphae may be damaged significantly by the mixing action. In addition, certain mixing actions may tend to compact the substrate bed, which would impede O_2 supply to the organism at the particle surface.

With the answers to these questions, the design problem can be further characterized by a more specific set of questions:

- What is the aeration type that should be used, and at what flow rate, temperature, and relative humidity this air should be supplied? Note that it might be advantageous to vary the inlet air properties during the course of the cultivation.
- What is the mixing type that should be used, and what mixing regime should be used, in terms of frequency of mixing, the duration of mixing events if mixing is not continuous, and the intensity of mixing?
- Will there be other significant contributions to heat removal, such as removal through cooling surfaces, and in this case, what will the cooling fluid be (typically air or water) and what should its flow rate and temperature be? Note that it might be advantageous to vary the cooling fluid properties during the course of the cultivation.
- Is it worthwhile to implement monitoring and control schemes to try to maintain the specified key process variables at their desired values by manipulating the regimes for aeration, mixing and cooling through cooling surfaces?

Note that decisions about aeration rates will be most strongly influenced by heat removal considerations, and aeration rates required for adequate heat removal will be adequate to maintain relatively high O_2 concentrations in the void spaces, so considerations of O_2 levels typically do not enter as primary design considerations.

These questions will appear, collocated in a more specific manner, in the individual bioreactor sections, where it will be argued that these questions are best answered on the basis of models of the process in which mass and energy balances over the bioreactor are formulated (Figure 7.14). These balance equations can be formulated in manners that are more complex or simpler to use. These cultivation processes are dynamic processes, that is, the process variables vary significantly over time. In bioreactors in which the bed is not well mixed, there are also often significant spatial variations, which the model should also describe (Figure 7.15). Models that describe both the spatial and temporal variations will then require the solution of partial differential equations, which is more challenging than solving the ordinary differential

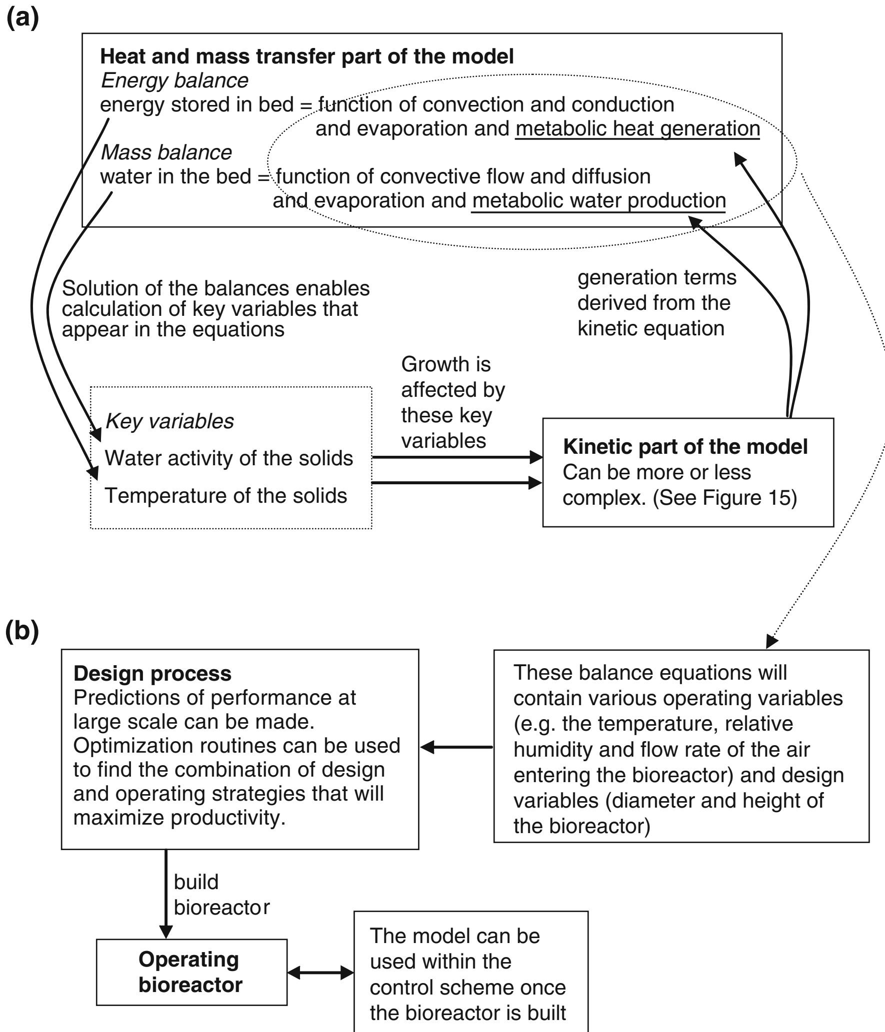

Fig. 7.14. Models as tools for bioreactor design (60) (**a**) The basic structure of models used as tools for guiding the bioreactor design process. (**b**) An indication of how models can be used in the design process.

equations used to describe the temporal profile for a well-mixed system. However, as will become apparent, it is also typically possible to make pseudo steady-state approximations, since the growth processes tend to be slower than the transport processes. This has the effect of transforming a model with partial differential equations into a model with ordinary differential equations in which the independent variable is the spatial position, and of transforming a model with ordinary differential equations into a model with simple algebraic equations.

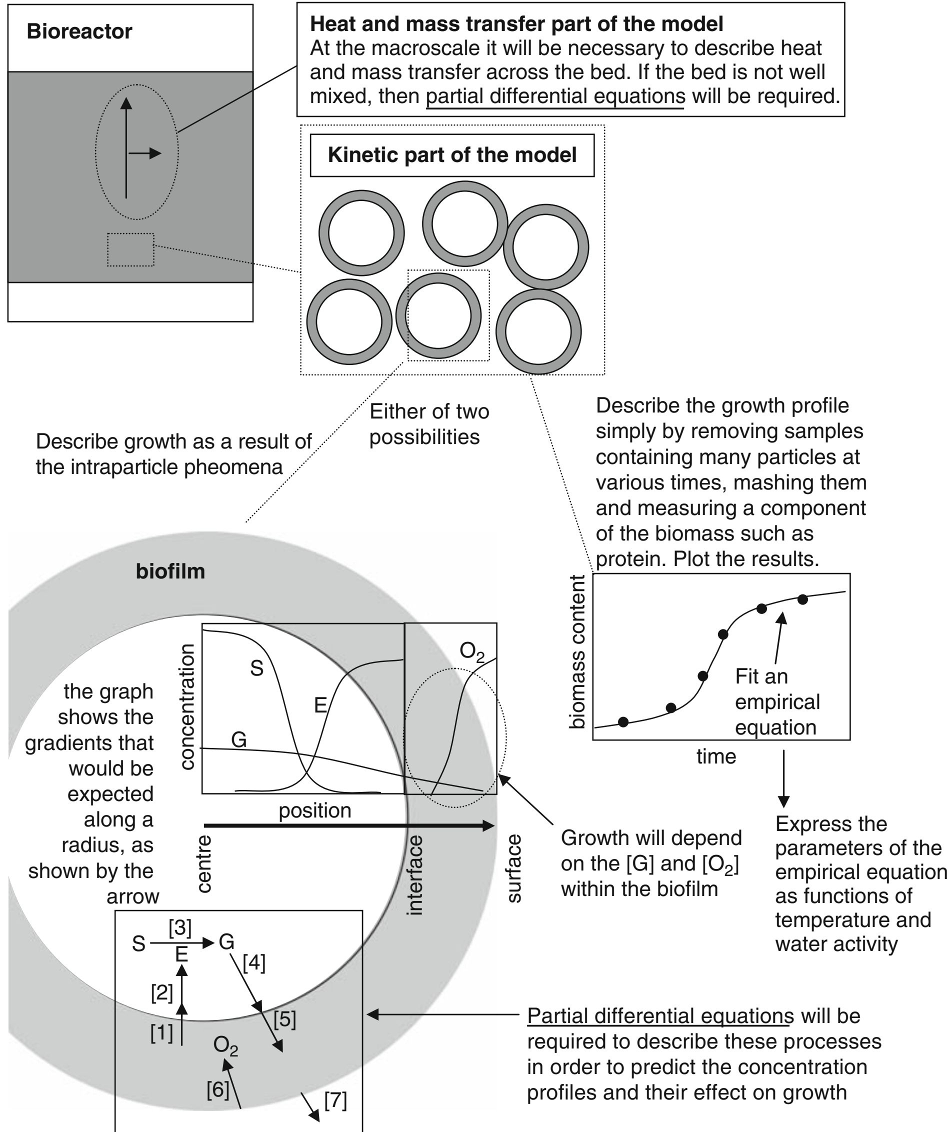

Fig. 7.15. The appropriate level of complexity for the kinetic part of the model (60). As shown by the figure, which represents a process in which a biofilm of a glucoamylase-producing bacterium grows on the surface of a starchy substrate particle, intra-particle variables such as starch, enzyme, and glucose concentrations do affect growth but lead to complex mathematical equations. The empirical approach will lead to much simpler equations. Key: [1] Release of enzyme (E) by the biomass; [2] Diffusion of enzyme into the substrate particle; [3] Action of the enzyme to hydrolyze starch (S) to glucose (G); [4] Diffusion of glucose within the substrate particle; [5] Diffusion and uptake of glucose within the biofilm; [6] Diffusion and uptake of oxygen within the biofilm; [7] Expansion of the biofilm as more biomass is produced.

6.2. The Importance of Characterizing the Growth Kinetics of the Microorganism

As pointed out at the beginning of the previous subsection, in all cases, it is necessary to understand the basic kinetics of growth and product formation by the process organism before making any design decisions. Actually, this is potentially quite a complex task, since ideally the kinetic equation should take into account the key factors that influence the growth of microorganisms in solid-state cultivation systems. Note that several "intra-particle variables" can be important in affecting the growth kinetics, such as local nutrient and dissolved O_2 concentrations and local pH values within the substrate particle (Figure 7.15). However, there are typically significant gradients within the particle, and it is a highly complex matter to characterize these gradients in order to know exactly what "intra-particle conditions" are experienced by the biomass. It is desirable to avoid such complexity in the design process. As a result, a simple empirical approach to characterizing growth kinetics in solid-state cultivation processes is used (Figure 7.15). It involves two steps:

- Empirical characterization of the basic form of the growth profile, describing it with an equation that does not include intra-particle nutrient or O_2 concentrations or the pH;
- Expression of the parameters of the growth equation as functions of the key process variables, these being the temperature and possibly also the water activity of the substrate.

An extensive characterization of growth kinetic profiles in solid-state cultivation systems showed that the logistic equation gives a reasonable approximation of around 75% of growth profiles (71) and, as a result, this equation has been used to describe the growth kinetics in mathematical models for various solid-state cultivation bioreactors. The differential form of the logistic equation, which expresses the growth rate of the organism, can be presented in terms of the volumetric concentration of the biomass (X, kg of dry biomass per m^3 of bed volume):

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_{max}}\right) \tag{1}$$

where μ is the specific growth rate constant (per hour) and X_{max} is the maximum volumetric biomass concentration reached. Integration of this equation with constant values for the parameters gives a temporal profile of the form shown on the right in Fig. (7.15). For use within a bioreactor model, the specific growth rate constant is expressed as a function of the bed temperature and water activity.

Given that the development of mathematical models of bioreactors requires a large amount of modeling and programming work to write and solve the equations and a large amount of experimental work to determine the parameters, the following bioreactor design sections will show only the equations that are used in simplified design methods. Typically, these rules require only an estimate of the peak heat production rate under optimal growth conditions, avoiding the necessity for extensive characterization of the effect of temperature and water activity on growth. The peak heat production rate occurs at the time of maximum growth rate. Differentiating Eq. (1) with respect to biomass after bringing the term μX inside the

parentheses and then setting the differential to zero gives:

$$\frac{\mathrm{d}\left(\mu X - \frac{\mu X^2}{X_{\max}}\right)}{\mathrm{d}X} = \mu - \frac{2\mu X}{X_{\max}} = 0 \tag{2}$$

which can be solved to show that the maximum growth rate occurs when $X = 0.5X_{\max}$. Substituting this into Eq. (1), the maximum growth rate can be calculated:

$$\left.\frac{\mathrm{d}X}{\mathrm{d}t}\right|_{\max} = \mu 0.5 X_{\max}\left(1 - \frac{0.5X_{\max}}{X_{\max}}\right) = 0.25\mu X_{\max} \tag{3}$$

The maximum volumetric heat production rate (R_{Qmax}, W/m^3) can then be calculated if the stoichiometric relationship between growth and heat production (Y_{QX}, J/kg) is known:

$$R_{\mathrm{Q\,max}} = Y_{\mathrm{QX}} \left.\frac{\mathrm{d}X}{\mathrm{d}t}\right|_{\max} \tag{4}$$

Note that it is not essential to know the growth kinetics in order to use these equations. In fact, the measurement of biomass concentrations in solid-state cultivation systems is often highly problematic, due to the difficulty in separating the microorganism from the solids. An experimental measurement of the O_2 consumption rate will suffice, since the maximum heat generation rate should be directly proportional to the maximum O_2 consumption rate ($R_{\mathrm{O2\,max}}$). The stoichiometric coefficient (Y_{QO}) is 16,562 kJ/kg (72). In this case, the maximum heat production rate would be estimated as:

$$R_{\mathrm{Q\,max}} = Y_{\mathrm{QO}} R_{\mathrm{O2\,max}} \tag{5}$$

The use of this estimate of $R_{\mathrm{Q\,max}}$ will be described in the following subsections.

6.3. Design of Group I Bioreactors

The design and operation decisions that need to be made for Group I (tray-type) bioreactors are:

- If the chamber in which the cultivation to take place is to have conditioned air, how should the inlet and outlet of air in the chamber be designed? What should be the arrangement of trays or bags in the chamber to promote a uniform circulation of air within the chamber itself?
- At what values should the temperature and relative humidity of the air circulated past the trays or bags be controlled?
- How large should each tray or bag be and especially, what depth of the substrate layer should be used in each tray or bag?
- Where infrequent mixing is to be undertaken, what frequency, duration, and intensity of the mixing should be used?

These questions can be answered on the basis of considerations of heat and mass transfer in static, unaerated beds. Note that heat transfer is essentially limited to conduction and mass transfer of O_2, CO_2, and water vapor is essentially limited to diffusion within the inter-particle spaces. There is a tendency to reach high temperatures and low O_2 concentrations within the interior of the bed, with the shape of the profiles depending on whether the bottom of the bed is perforated or not (Figure 7.16).

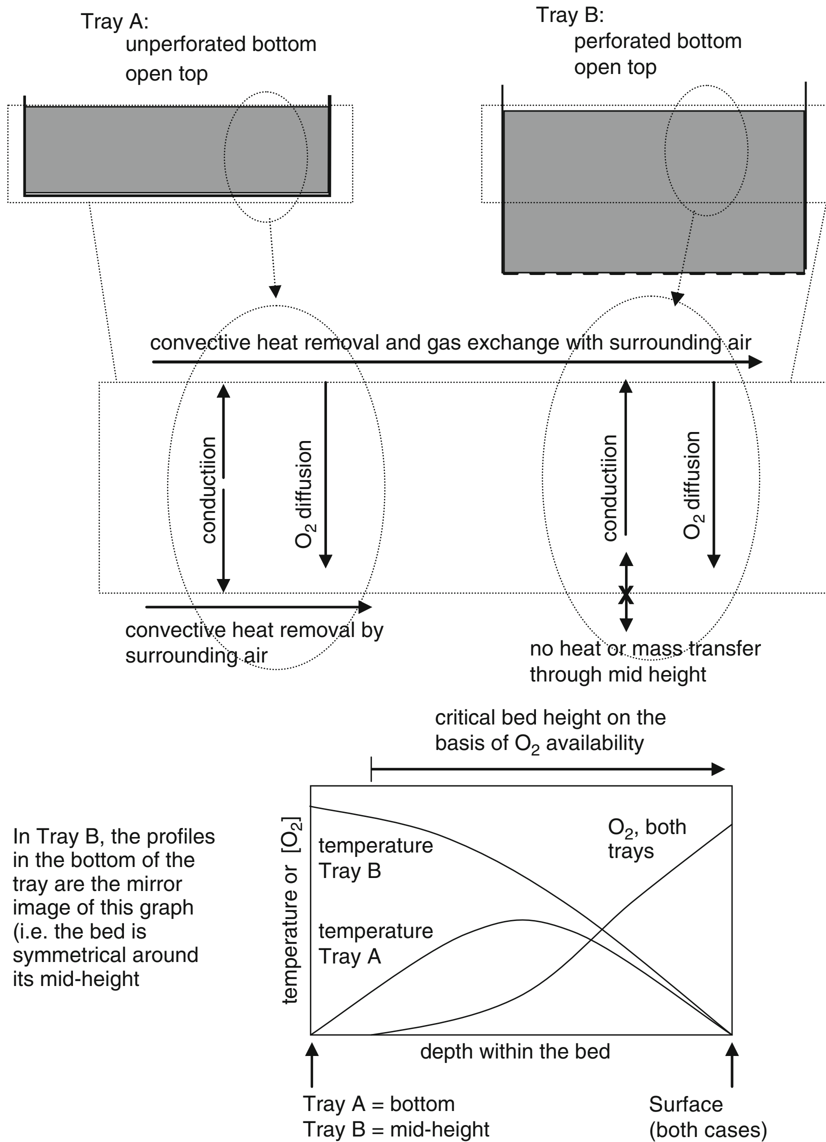

Fig. 7.16. Key heat and mass transfer mechanisms in trays and the resulting effect on temperature and inter-particle O_2 concentration profiles within the bed (73–75). Note that whether or not the bottom of the tray is perforated affects the relative shapes of the O_2 and temperature profiles. Also shown is the critical bed height, defined by Ragheva Rao et al. (73) as being the height at which the O_2 concentration falls to zero at some time during the culture.

6.3.1. Simple Approaches to Making Design Decisions About Group I Bioreactors

A pseudo steady-state approximation for O_2 diffusion and consumption within the substrate bed within a tray gives an equation for the critical bed height (H_c), namely the depth at which O_2 reaches zero concentration at some time during the cultivation (73):

$$H_c = \sqrt{\frac{2D_e C_g Y_{xo}}{R_{O2\,max}}} \tag{6}$$

where D_e is the effective diffusivity of O_2 within the bed, C_g is the O_2 concentration in the gases surrounding the tray, Y_{xo} is the yield coefficient of biomass from O_2, and $R_{O2\,max}$ is the maximum value of the O_2 consumption rate.

Estimating the values of the parameters of this equation is not necessarily an easy task. The effective diffusivity of O_2 within the bed is likely to change during the culture as the microorganism grows into the inter-particle spaces. Ragheva Rao et al. (73) suggested a value of 0.03 cm^2 h. They used a value of 1.07 kg/kg for Y_{xo}. The value of $R_{O2\,max}$ will depend on the particular growth and O_2 consumption kinetics. Ragheva Rao et al. (73) used a value of $R_{O2\,max}$ of 1.62 g/(cm^3 h). With these values, and assuming that the air surrounding the tray was 21% O_2 (v/v), they determined the critical bed height as 4.8 cm. Of course, these calculations will need to be repeated for each new process; however, they do give an order of magnitude estimate of maximum bed heights for Group I bioreactors on the basis of considerations of O_2 consumption.

In terms of heat removal considerations, a pseudo steady-state assumption gives an equation for the temperature profile within the bed as a function of depth below the surface (74):

$$T = -\xi^2\Theta + \frac{N_{Bi}}{N_{Bi}+1}(T_s - T_a + \Theta)\xi + \frac{T_s + \Theta + N_{Bi}T_a}{N_{Bi}+1} \tag{7}$$

where $\Theta = R_Q\delta^2/(2k)$, $N_{Bi} = \alpha\delta/k$ and $\xi = z/\delta$. In these equations, T is the temperature at a particular height z, T_s is the surface temperature of the bed, α is the bed-to-air heat transfer coefficient, R_Q is the volumetric rate of heat generation by the organism, δ is the overall height of the bed, k is the thermal conductivity of the bed, Θ is the temperature difference between the bottom of the solid medium and the upper surface in the case where heat is not transferred across the bottom surface to the air, N_{Bi} is the Biot number and T_a is the temperature of the surrounding air.

To use Eq. (7), it is necessary to have an estimate of the bed-to-air heat transfer coefficient (α), or at the very least, to use a reasonable estimate of the Biot number. A reasonable value may be ten (75). Szewczyk (74) did not actually determine values for the surface to air heat transfer coefficient, but rather used a more complete model to explore the relationship between α and the predicted bed surface temperature, repeating this for various values of the metabolic heat generation rate (R_Q). Such a relationship must actually be provided in order to solve Eq. (7), since it includes both T_s and α and T_s depends on α. In the absence of an explicit equation relating T_s to α, Eq. (7) is not in a form that is easy to apply, however, the analysis undertaken by Szewczyk (74) does give general guidance. For a bed height of only 3 cm, with an air temperature of 30°C and with a low bed-to-air heat transfer coefficient (10 W/(m^2 °C)),

which might be expected if the air is not circulated past the bed at a high rate, bed temperatures can potentially reach values as high as 37°C (if there is reasonable heat transfer through the bottom of the tray) or even 46°C (if there is no heat transfer through the bottom of the tray).

6.3.2. *Model-Based Approaches to Making Design Decisions About Group I Bioreactors*

Rajagopalan and Modak (75) developed a mathematical model to describe both heat and mass transfer within a tray. The model incorporated the heat and mass transfer phenomena shown for "Tray B" in Figure 7.16. It is therefore capable of predicting both the bed temperature and the O_2 concentration in the inter-particle gas phase within the bed as a function of both bed height and time. Both temperature and O_2 concentration were predicted to be limiting factors, although at different times. During the early stages of growth, the temperature was the dominant factor. At intermediate stages, O_2 limitation was important at the bottom of the bed, while temperature limitation was important in the center of the bed.

The model was used to explore the effects of several design and operating variables. The velocity of the air flow past the surface of the bed had relatively little effect on the temperatures within the bed. The surrounding air temperature and the bed height were more important. Simulations were undertaken to identify the optimum combination of these two variables for an organism with an optimum growth temperature of 38°C. Optimum growth was predicted to occur for a 3 cm bed with surrounding air temperatures of 30–35°C. With larger bed heights, growth was poorer, regardless of what surrounding air temperature was used.

6.3.3. *Synthesis of Our Knowledge About How Best to Operate and Design Group I Bioreactors*

For a majority of processes, bed heights should probably not exceed 3–5 cm. Given that these bed heights will already have been used in laboratory studies, this means that processes must be scaled up by increasing the total tray area, while maintaining the height of the substrate layer within the tray constant. On a large scale, processes involving Group I bioreactors are likely to be highly labor intensive; however, in processes with batches of the order of 10–100 kg of substrate, such as might occur in small-scale or domestic industries, this type of bioreactor could be appropriate.

6.4. *Design of Group II Bioreactors*

The design and operation decisions that need to be made for Group II (packed-bed type) bioreactors are:

- What should the height of the bed be?
- What is the required overall capacity? Note that this, combined with the bed height, will determine the necessary horizontal dimensions.
- What should the temperature and flow rate of the process air be?
- Should internal heat transfer surfaces be provided and, if so, what should be the temperature and flow rate of the cooling water?
- Is the bed to be infrequently mixed? In the case that infrequent mixing is to be used, what should be the humidity of the inlet air? Note that unsaturated air can be used in the case of an infrequently mixed bed since water can be added to the bed during the mixing event. For a bed that is to remain static throughout the process, then the inlet air should be saturated in order to minimize

the evaporation rate, otherwise growth may be limited simply because the bed dries out to water activities low enough to inhibit microbial growth.
- If the bed is to be infrequently mixed, what should be the trigger for mixing, what should be the duration and intensity of the mixing events and what additions, if any, should be made to the bed during these events?
- If the bed is to be infrequently mixed, what should the design of the agitator be, and should it be removed from the bed during the periods of static operation?

The decisions that will be made in response to these questions will be affected by the following considerations:

- There is a tendency for bed temperature to increase with bed height for forcefully aerated static beds due to convective cooling, and, therefore, the decision on the bed height to be used will be influenced by the effects of high temperature on the process organism.
- The bed height may also be decided on the basis of expected pressure drops through the bed. Pressure drop can potentially be a problem in static packed beds, especially in processes involving fungi, since growth of fungal hyphae into the void spaces impedes the flow of air through the bed, necessitating a higher pressure at the inlet in order to maintain the flow rate. Note that considerations of pressure drop mean that beds should be of uniform thickness. If a bed is not of uniform thickness, then the air will tend to flow preferentially through those regions of the bed that are less thick (Figure 7.17a).
- Decisions about when to mix might be made on the basis of the need to make intermittent additions, the need to prevent the pressure drop through the bed from becoming too large or the need to destroy any channels that might occur due to the bed either cracking or pulling away from the bioreactor walls. Such channeling is undesirable since it means that the air will flow preferentially through the cracks and not through the bed itself.

6.4.1. Simple Approaches to Making Design Decisions About Group II Bioreactors

The following equation can be used to estimate the maximum outlet temperature as a function of the maximum heat production rate and the air flow rate (76):

$$\mathrm{Da_M} = \frac{R_\mathrm{Q}}{\rho_\mathrm{a}(C_\mathrm{pa} + f\lambda)V_\mathrm{z}(T_\mathrm{out} - T_\mathrm{in})/H} \tag{8}$$

This so-called "modified Damkohler number" ($\mathrm{Da_M}$) is a dimensionless number that represents the ratio of the volumetric heat production rate (R_Q, $\mathrm{J/(m^3\,h)}$) to the volumetric heat removal rate. The volumetric heat production rate is estimated by assuming that the air enters the bioreactor saturated at the air inlet temperature and leaves saturated at the outlet air temperature, and takes into account the contributions of the sensible energy of the air and the evaporation of water into the gas phase. The factor f represents a linear approximation to the saturation humidity curve, this approximation being reasonable over short temperature intervals.

Equation (8) is most useful if the modified Damkolher number is set equal to 1, and the equation is rearranged to be explicit in the bed height (H). If a maximum allowable temperature can be identified (T_max), this being a temperature that should not be exceeded in any part of the bed, at any time during the cultivation, then it is possible to calculate the maximum height that the bed can have (H_max). In order to do this, the volumetric metabolic heat production

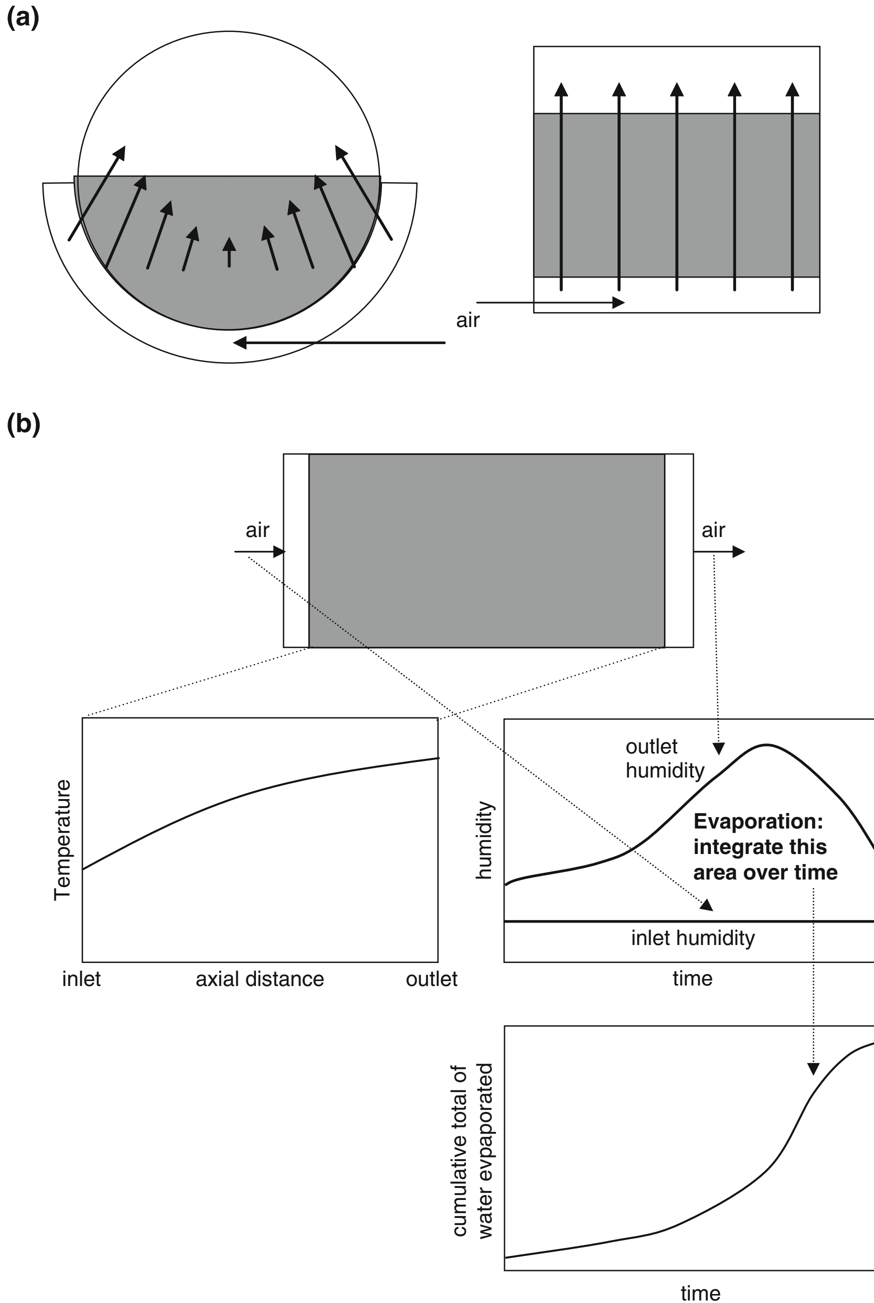

Fig. 7.17. Considerations in the design of packed beds (76). (**a**) The necessity of having a bed of constant thickness in order to obtain uniform aeration. If the bed is not uniformly thick, then the air will preferentially flow through those regions that are less thick because the pressure drop is lower. (**b**) The tendency of packed beds to have axial temperature profiles and the consequence that this has for evaporation. Note that in this case, the packed bed is shown lying on its side.

rate is set to its maximum value ($R_{Q\,max}$) and T_{out} is set to T_{max}, since the highest temperature occurs at the air outlet end of the bed. These manipulations and substitutions give the following equation (76):

$$H_{max} = \frac{\rho_a (C_{pa} + f\lambda) V_z (T_{max} - T_{in})}{R_{Q\,max}} \tag{9}$$

It is then possible to explore the effect of the superficial velocity of the air (V_Z, obtained by dividing the volumetric flow rate of the air by the total cross-sectional area of the bed) and the inlet air temperature (T_{in}) on the maximum allowable height of the bed. In order to do this, the density and heat capacity of air (ρ_a and C_{pa}) can be taken from a reference book, while over the range of 25–45°C, the factor f can be estimated as 0.00246 kg-water/(kg-air °C) (76).

This type of approach can be extended to include considerations of the water balance (77). The temperature profile tends to be steeper at the inlet end of a packed bed, becoming ever less steep with height. This, combined with the exponential nature of the saturation vapor pressure curve, means that the vapor pressure increases approximately linearly with height, suggesting that the bed will dry out relatively uniformly along its length. The drying rate can then be estimated if the expected temporal profile for the outlet air temperature is known, simply by using the outlet air temperature to estimate the difference between the inlet and outlet humidities (y_{out}–y_{in}) and then integrating this profile against time (Figure 7.17b). However, this method can only be applied if the profile for outlet temperature is known. Therefore, it will be most useful in a system in which the inlet and outlet temperatures of a large-scale bioreactor are monitored and used to estimate evaporation.

6.4.2. Model-Based Approaches to Making Design Decisions About Group II Bioreactors

Mathematical models have been proposed for several packed-bed bioreactors. The Zymotis bioreactor, with its internal heat transfer plates, might be used in processes in which absolutely no mixing is desired during the cultivation. A model of this bioreactor (78) that takes into account the energy balance, but not the water balance (Figure 7.18a), can be used to explore the effect of various design and operating variables on bioreactor performance:

- Design variables: bed height, spacing between the heat transfer plates.
- Operating variables: air flow rate and temperature, temperature of the cooling water passed through the heat transfer plates.

Simulations with the model show that use of internal heat transfer plates has the potential to decrease, although not remove, the radial and axial temperature gradients (Figs. 7.18b, c). However, careful consideration needs to be undertaken before using this type of bioreactor. Optimal performance requires relatively small spacing between the heat transfer plates, as little as 6 cm for processes involving fast-growing organisms (79). The presence of the heat transfer plates will likely complicate loading operations, it being essential to obtain an even packing of substrate in the compartments in order to prevent channeling. Any changes in bed volume due to bed shrinkage away from the walls during the process will also prevent proper aeration of the bed.

In some solid-state cultivation processes, mixing does have deleterious effects, but infrequent mixing events do not have an unduly negative effect. In such cases, mixing will be used

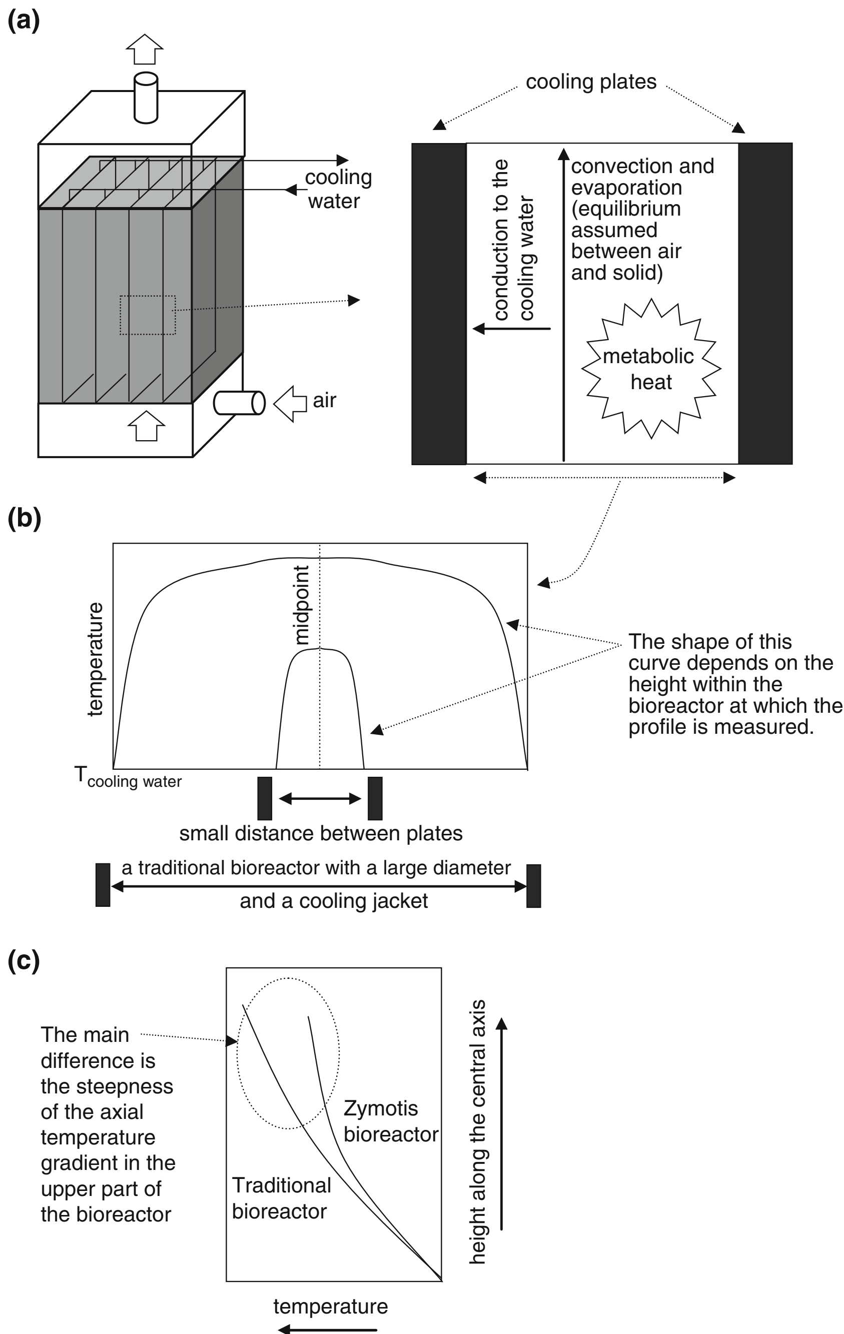

Fig. 7.18. Modeling of the Zymotis bioreactor (78). (**a**) Representation of how the model of Mitchell and von Meien (78) treats the processes occurring. (**b**) and (**c**) Predictions of the model about how the small spacing between the cooling plates reduces (**b**) the radial temperature gradients in the bed and (**c**) the axial temperature gradients in the bed, compared to a traditional packed-bed bioreactor of large diameter with a cooling jacket.

not in an attempt to control temperature, which would require frequent mixing events, but rather in order to allow the replenishment of water to the bed, which opens up the possibility of using unsaturated air at the air inlet in order to promote evaporative cooling. Note that the replenishment of water is only feasible during mixing events, due to the difficulties of uniformly distributing water in a static bed. For example, any attempt to percolate water downwards from the top of the bed would simply flood the top of the bed. von Meien and Mitchell (80) developed a model to describe such a system. It treats the air and solid phases as separate subsystems and, instead of assuming equilibrium between the air and solid phases, describes heat and mass transfer as a result of driving forces (Figure 7.19a).

The predictions of this model allow various insights into how to design and operate infrequently mixed packed beds. An appropriate strategy for initiating mixing events would be to monitor the relative humidity of the outlet air and to initiate a mixing event when this falls below a certain threshold value (Figure 7.19b). During this mixing event, water would be added to the substrate to bring its water activity back to values that are favorable for growth. Such bioreactors should be able to operate with two or three mixing events over a period of 30 h, in the case of a fast-growing process organism. The ability to add water means that the limitation of growth by low water activities in the substrate bed can be avoided (Fig. 7.19b), which leaves temperature control as the most important problem: even though evaporative cooling can be promoted with the use of dry air at the air inlet, axial temperature gradients will still limit the bed height that can be used. Of course, the higher the superficial velocity of the air, the less steep is the axial temperature gradient and the higher the bed can be. The model of von Meien and Mitchell (80) can be used to explore this relationship. There will be limits on acceptable air flow velocities, either due to the operating costs of the aeration system, or due to the fact that at very high velocities the bed will fluidize, although the model is not yet sufficiently sophisticated to take these into account.

6.4.3. Synthesis of Our Knowledge About How Best to Operate and Design Group II Bioreactors

Static packed beds and intermittently stirred packed beds are likely to be suitable for many environmentally related solid-state cultivation processes, especially for less shear-tolerant organisms, as they minimize shear damage to the organism and also minimize operating costs associated with the mixing of the substrate bed. On the basis of the results of the quantitative design approaches described earlier, plus the experience of the soy sauce *koji* industry (63), it is likely that maximum bed heights will be of the order of 0.5–1.0 m, necessitating large areas if large volumes are to be processed.

Order of magnitude design decisions can be made using the simplified strategies outlined in Sect. 6.4.1. However, these methods do make some assumptions that are questionable. For example, Weber et al. (81) showed experimentally that the outlet gas from packed-beds is not necessarily saturated, falling to below 90% relative humidity during periods of peak heat production. Models that allow for this situation, such as that developed by von Meien and Mitchell (80), will be more accurate design tools. However, use of such models does require estimates of the solid-to-gas heat and mass transfer coefficients, which can potentially vary significantly between processes and are not simple to measure experimentally.

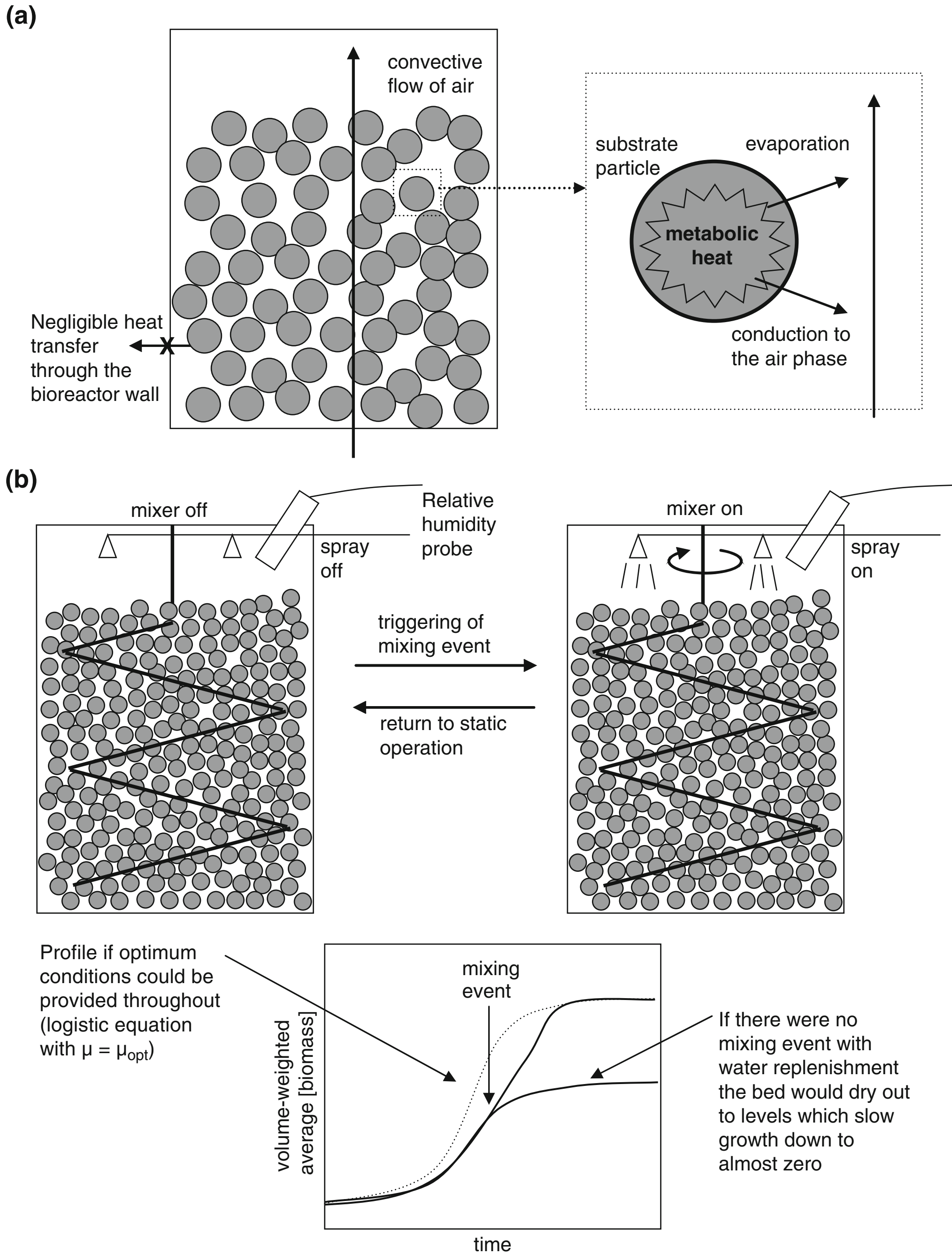

Fig. 7.19. Modeling of intermittently-agitated packed-bed bioreactors (70). (**a**) How the model of von Meien and Mitchell (80) treats the processes occurring. (**b**) Details of the operating strategy and the predicted performance of the bioreactor with this mode of operation.

6.5. Design of Group III Bioreactors

The design and operation decisions that need to be made for Group III (rotating-drum and stirred-drum type) bioreactors are:

- How large should the bioreactor be, and what should be the length to diameter ratio?
- How much of the total bioreactor volume should the substrate bed occupy?
- At what values should the headspace air temperature and relative humidity and flow rate be controlled?
- In rotating drums, should lifters be incorporated and, if so, how many and of what size and shape? In stirred drums, what is the best design for the agitation system?
- Should the bioreactor axis be horizontal or inclined?
- What rotation rate should be used, for the drum body for rotating drum bioreactors and for the agitator for stirred-drum bioreactors?
- Should the outer surface be cooled and, if so, in what manner? Note that it is possible to blow air past the bioreactor, to spray cooling water onto the outer surface or to incorporate a cooling jacket into the design.

The decisions that will be made in response to these questions will be affected by how the various design and operating variables influence the heat and mass transfer phenomena within the bioreactor. As shown by Fig. 7.20, these include:

- *Flow patterns within the solid substrate bed.* In rotating drums in the absence of lifters, axial and radial mixing of the bed can be quite inefficient, especially at the relatively low rotational rates that are typically used. In rotating drums with lifters, the lifters will promote radial mixing, and can be designed in such a manner as to promote axial mixing by pushing substrate back and forth within the drum. In any case, mixing in horizontal drums tends to be inefficient when the bed occupies more than 30% of the total drum volume, so this can be taken as an upper limit as to how full the drum should be.
- *Flow patterns of gas within the headspace*. These flow patterns affect the efficiency of bed-to-headspace heat and mass transfer.
- *Bed-to-headspace heat and mass transfer*. This transfer will be improved by those factors that increase the overall area of contact.

These considerations mean that lifters should typically be used in rotating drums, since lifters prevent the slumping flow regime, even at very low rotation rates. This improves radial mixing patterns within the bed, exposing the substrate more uniformly at the surface. In addition, as the particles are lifted and then fall back as a curtain of particles, there is intimate contact between these particles and the headspace, promoting bed-to-headspace heat and mass transfer. In order to achieve good radial mixing and bed-headspace contact in rotating drums without lifters, it would be necessary to use high rotation rates to obtain the cataracting flow regime. Note that lifters can also be used to promote axial mixing, in which case it is probably best to have the bioreactor at an angle such that the movement imparted by the lifters to one end of the drum is counterbalanced by return of the substrate due to gravity (82).

6.5.1. Simple Approaches to Making Design Decisions About Group III Bioreactors

Hardin et al. (83) developed what they called a "dimensionless design factor" (DDF) for rotating drum bioreactors. It assumes that both the headspace and substrate are well mixed, and represents the ratio of the estimated maximum heat production rate (R_Q) to the heat

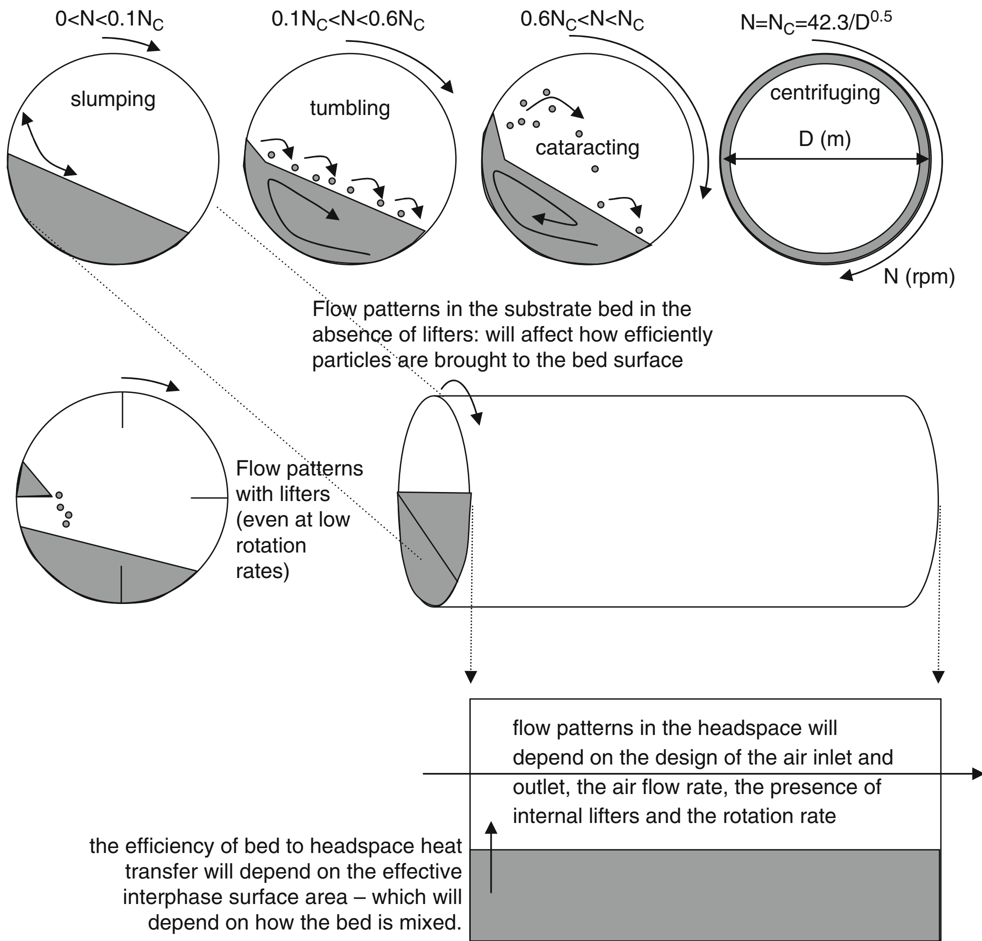

Fig. 7.20. Various design and operating variables that influence the heat and mass transfer phenomena within rotating drum bioreactors (60). Note that in the slumping regime, there is minimal mixing within the bed; it simply rises and falls as a single mass. N_C is the critical drum speed at which the solids are centrifuged against the sides of the drum.

removal rate.

$$\mathrm{DDF} = \frac{R_Q V_{bed}}{F_a C_{pa}(T_{out} - T_{in}) + F_a(y_{out} - y_{in})\lambda + h_{con}\pi D(D/2 + L)(T_{out} - T_a)} \quad (10)$$

where V_{bed} is the volume of the bed, F_a is the air mass flow rate, T_{in}, T_{out}, and T_a are the inlet air, outlet air, and surrounding air temperatures, respectively, y_{in} and y_{out} are the inlet and outlet humidities, respectively, D and L are the drum diameter and length, respectively, C_{pa} is the heat capacity of the air, λ is the heat of evaporation of water and h_{con} is the overall coefficient for heat transfer from the bed to the surrounding air by conduction through the drum wall.

Equation (10) can be used to provide an estimate of the air flow rates required by setting the DDF to 1 (that is, putting the rate of heat removal equal to the rate of heat production), R_Q to its maximum expected value ($R_{Q\,max}$), and the outlet air temperature to its maximum allowable value (T_{max}):

$$F_a = \frac{R_Q V_{bed} - h_{con}\pi D(D/2 + L)(T_{max} - T_a)}{C_{pa}(T_{max} - T_{in}) + (y_{out} - y_{in})\lambda} \quad (11)$$

Various parameter values need to be known in order to use this equation. As with Eq. (9), C_{pa} and λ can be obtained from reference books. The humidities y_{in} and y_{out} can be calculated from the Antoine equation, assuming that the air enters the bioreactor saturated at T_{in} and leaves saturated at T_{out}. The challenge is then to find a suitable estimate for the overall heat transfer coefficient of the bioreactor (h_{con}), before actually building the bioreactor, since the equation is being used for design purposes. Stuart et al. (84) used an equation developed by Kays and Bjorklund (85), which takes into account the effect of the drum diameter and rotational speed on convective heat removal from the drum surface. However, these have relatively little effect, so Stuart et al. (84) recommended the use of a value of h_{con} of 18,000 J/(h m^2 °C). Once these values are substituted, Eq. (11) gives an estimate of the necessary air flow rate for a given drum size and geometry, that is, for given values for the drum length and diameter. This air flow rate will depend on the values used for the operating variables T_{in} and T_a.

Hardin et al. (83) rearranged Eq. (11) to be explicit in T_{max} and used this rearranged equation to predict maximum temperatures expected for various experimental systems from the literature in which the operating conditions of the rotating drums were reported. This simplified approach overestimated the maximum temperature achieved in the bed by 2–5°C, meaning that it could be quite useful as a conservative design tool.

6.5.2. Model-based Approaches to Making Design Decisions about Group III Bioreactors

The DDF approach of Hardin et al. (83) assumes that the bed and headspace are in thermal and moisture equilibrium, with the headspace gases being saturated at the temperature of the solids, which is probably not a good assumption in practice. Two mathematical models have been proposed for rotating drum bioreactors that do not assume equilibrium but rather take into account bed-to-headspace heat and mass transfer. One model assumes that the substrate bed and headspace gas phases are each well-mixed in both in the axial and radial directions (84). This could be a reasonable approximation if the length to diameter ratio of the bioreactor is small, or even if the bioreactor is longer, but only if provisions are made for end-to-end movement of the solids, through adequate design of the lifters, and if multiple air inlets and outlets are provided. The other model assumes that both the substrate bed and the headspace gases are well mixed in the radial direction but that there is no mixing of either phase in the axial direction (86).

The model of the well-mixed rotating-drum bioreactor not only recognizes the substrate and headspace phases, but also formally treats the bioreactor body as a separate phase (Fig. 7.21a). Heat transfer between the various phases is described, as well as evaporation from the substrate bed to the headspace. The model agreed well with experimental data obtained at a laboratory scale (84). Using correlations that enable the estimation of heat

transfer coefficients for different scales (87), the model can be used to explore the design of large-scale bioreactors. If geometric similarity is maintained (that is, a constant L:D ratio), then heat removal through the bioreactor wall to the surrounding air makes an ever decreasing contribution to overall heat removal as the scale increases. This suggests that, if laboratory-scale studies are undertaken, the outer surface of the bioreactor should be insulated, in order to simulate the poor contribution of this heat removal mechanism at large scale. This strategy will enable better estimation of the air flow rate and relative humidity that should be used at a large scale.

The model's predictions give insights regarding the order of magnitude of air flow rates that would be necessary in order to enable adequate temperature control at small and large scale. For adequate temperature control, this being defined as bed temperatures never exceeding 38°C when a fast-growing organism with an optimum temperature of 30°C is cultivated, aeration rates as high as 0.3 vvm (volumes of 15% relative humidity air per total volume of bioreactor per minute) are necessary for a process involving 2 kg of cooked wheat bran in a 20 L bioreactor. With 250 kg of substrate, the aeration rate must be of the order of 3 vvm whereas with 2,000 kg of substrate, the aeration rate must be as high as 8 vvm.

Exploration of the predictions of the model that describes a rotating drum bioreactor in which there is no axial mixing suggests that for this type of operation, depending on the combination of operating conditions, significant axial temperature profiles can occur or the whole bed may reach high temperatures (Fig. 7.21b) (86). For example, in a simulation of the growth of *Aspergillus oryzae* in a 20 L rotating drum bioreactor with 2 kg of cooked wheat bran, the axial temperature profile in the bed was as large as 5°C. The model was used to predict the order of magnitude of the aeration rates that will be necessary to achieve adequate temperature control in larger-scale bioreactors. If the inlet air were maintained at 90% relative humidity throughout the cultivation, then normalized superficial velocities (that is values of V_Z/L, where V_Z is the volumetric flow rate divided by the cross sectional area of the headspace) of around 0.2/s would be required for a 204 L bioreactor and of around 1.2/s would be required for a 2,400 L bioreactor. However, if a control system were incorporated that switched the inlet air from 90% to 15% relative humidity whenever the outlet air temperature exceeded a set-point, then adequate temperature control could be achieved in the 2,400 L bioreactor with a normalized superficial velocity of only 0.5/s. Note that the use of dry air increases the evaporation rate and, therefore, necessitates the addition of water to the substrate during the cultivation. However, this is not a problem for rotating drums, as water can be distributed evenly simply spraying it onto the surface of the revolving bed.

6.5.3. Recent Directions in Characterizing the Phenomena in Group III Bioreactors

Recent studies have shed some light on the appropriateness of the idealized mixing regimes assumed by Stuart et al. (84) and Mitchell et al. (86). Hardin et al. (88) used CO as a tracer gas and analyzed the residence time distribution patterns in order to infer the flow pattern within the headspace. The data were consistent with the flow pattern shown in Fig. 7.21c. However, it is difficult to apply these results generally to rotating drum bioreactors, since headspace flow patterns are significantly affected by the design of the air inlet and outlet, the air flow rate, and the presence of lifters. Once such flow patterns are determined, it is possible to use them

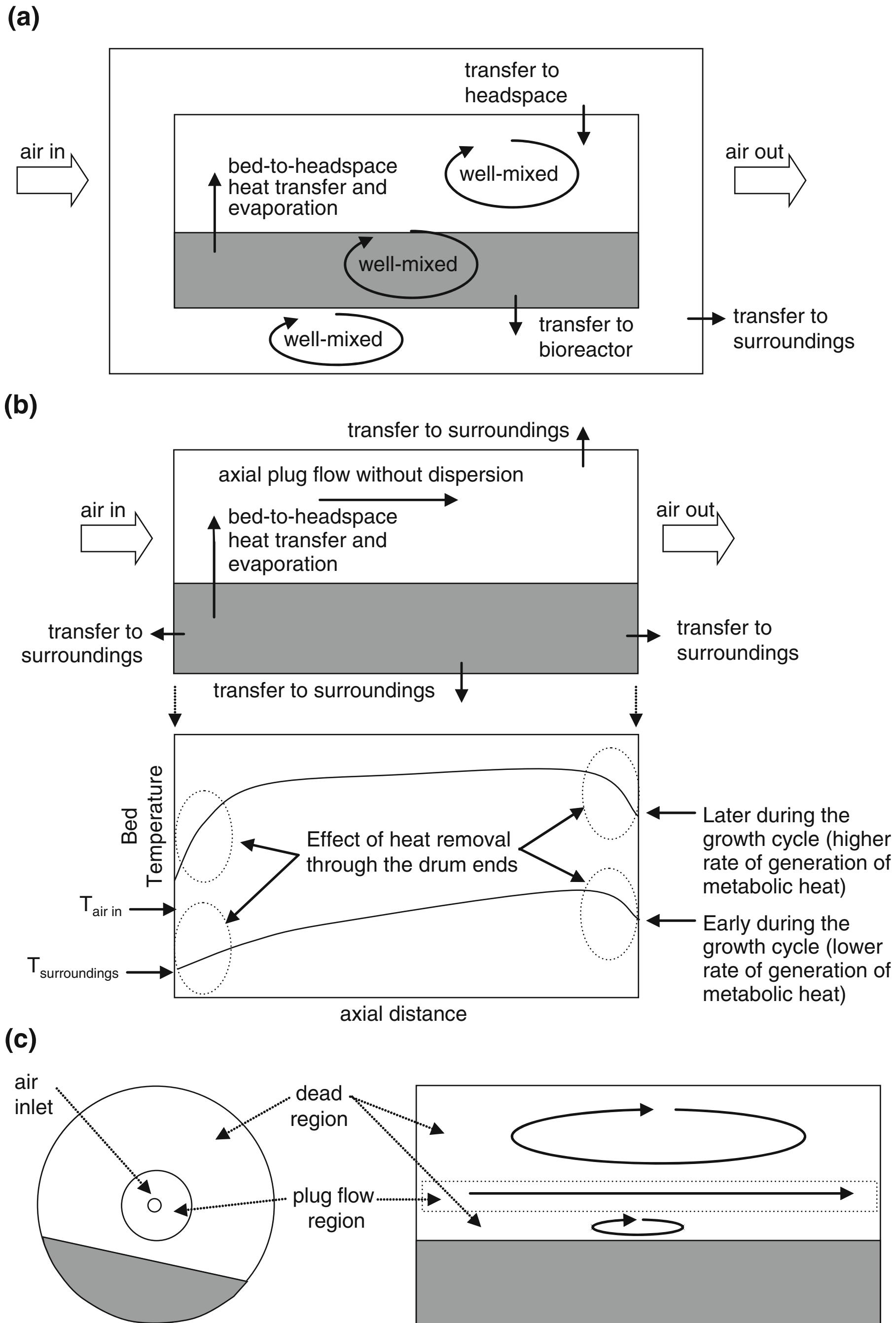

Fig. 7.21. Modeling of rotating drum bioreactors (84, 86, 88). (**a**) How the model of Stuart et al. (84) describes a well-mixed rotating drum bioreactor. (**b**) How the model of Mitchell et al. (86) treats a rotating drum bioreactor in which axial mixing is insignificant, and typical predictions that this model makes about axial temperature profiles in the substrate bed. (**c**) More realistic headspace flow patterns as determined by Hardin et al. (88).

to obtain estimates for the overall coefficients that characterize the bed-to-headspace heat and mass transfer and to express them as functions of the air flow rate and the rotation rate (89). However, as yet there is insufficient knowledge for the development of general relationships; it will be necessary to undertake experimental studies with a wider range of geometries.

Studies have also been undertaken regarding the degree of axial mixing within the substrate bed in rotating drums, using colored substrate particles (90, 91) and nuclear magnetic resonance (92). More recently, Schutyser et al. (82) introduced the powerful tool of discrete particle modeling into the analysis and prediction of mixing in solid-state cultivation bioreactors. They applied this technique to investigate baffles designs in rotating drums and showed that the use of curved baffles in an inclined drum would lead to the most effective radial and axial mixing.

6.5.4. *Synthesis of Our Knowledge about How Best to Operate and Design Group III Bioreactors*

It is possible to draw some general conclusions about the design and operation of rotating drum bioreactors in batch mode:

- Lifters are highly desirable in order to promote bed-to-headspace heat and mass transfer and radial mixing, and can also be designed so as to promote axial mixing.
- In order to avoid significant axial temperature gradients, it is best not to have a very high length to diameter ratio, in which case, curved baffles can give adequate axial mixing of the substrate bed. If high length to diameter ratios are used, then multiple air inlets and outlets should be provided.
- At large scale, air flow rates of as much as 10 volumes of air per bioreactor volume per minute will be required for adequate temperature control.
- Although discontinuous rotation has been suggested as a possible operating strategy (93), it is unlikely to enable adequate temperature control at large scale.

More work is required to characterize flow patterns with the headspace and beds and, based on these studies, to arrive at correlations that allow bed-to-headspace heat and mass transfer coefficients to be estimated as functions of key design and operating variables.

Although rotating drums are well suited to continuous processes, little attention has been given to developing quantitative design rules for this mode of operation. Further, relatively little attention has been given to the operation of stirred drums. They will have many similarities with rotating drums, however, the design of the agitator and the resultant mixing patterns in the bed have received little attention.

6.6. *Design of Group IV Bioreactors*

The design and operation decisions that need to be made for Group IV bioreactors are:

- How large should the bed be, and what should be its geometric proportions?
- What should the flow rate, temperature, and humidity of the process air be? Note that since water can easily be added uniformly to a mixed bed, unsaturated air can be used at the air inlet in order to promote evaporative cooling.
- When should additions (e.g., replenishment of water) be made to the bed?
- Should any cooling surfaces be provided, and, if so, what should be the cooling water flow rate and temperature?

- What type of mixing action should be provided and, for mechanically-mixed beds, how should the agitator be designed?
- What should be the intensity of mixing? Should mixing be intermittent but frequent? If so, what should be the frequency and duration of mixing events? Note that, for frequently-mixed beds, mixing plays a role in temperature control, unlike infrequently-mixed beds wherein its role is to allow water replenishment or prevent high pressure drops (Sect. 6.4).

The decisions that will be made in response to these questions will be affected by the following considerations:

- The rate of heat generation by the process organism.
- The degree to which evaporative cooling is used as a cooling mechanism.
- The effect of the various options for mixing method and mixer design on the organism and substrate.

6.6.1. Simple Approaches to Making Design Decisions about Group IV Bioreactors

The modified Damkohler number of Mitchell et al. (76) (Eq. (8)) can be modified to be applied to well-mixed beds, assuming that heat removal through the bioreactor wall makes a contribution that is sufficiently small to be ignored:

$$\mathrm{Da_M} = \frac{R_Q V_{\mathrm{bed}}}{F_a (C_{\mathrm{pa}} + f\lambda)(T_{\mathrm{out}} - T_{\mathrm{in}})} \tag{12}$$

where the symbols are as previously defined. By setting this modified Damkolher number to 1, Eq. (12) can be used for to estimate the aeration rate (F_a) that will be necessary in order to maintain the bed temperature below a certain upper limit ($T_{\max}$):

$$F_a = \frac{R_{Q\,\max} V_{\mathrm{bed}}}{(C_{\mathrm{pa}} + f\lambda)(T_{\max} - T_{\mathrm{in}})} \tag{13}$$

6.6.2. Model-Based Approaches to Making Design Decisions about Group IV Bioreactors

If it is reasonable to assume that the substrate bed is well-mixed, then the water and energy balance equations will be ordinary differential equations, which are reasonably easy to solve with standard numerical integration programs. Consequently, researchers who have developed models for Group IV bioreactors have typically paid more attention to the kinetics of growth and product formation. Such models can then be used to explore the effect of operating variables on predicted bioreactor performance. This has been done in several studies:

- Nagel et al. (64) proposed a model that divides the water in the substrate particle into intracellular and extracellular water, and used the model to explore on-line control of moisture content and temperature.
- dos Santos et al. (94) used a model to describe the effect of operating conditions on the bed temperatures reached within a simple well-mixed bioreactor, and the effect of these temperatures on the denaturation of enzymes produced by the process organism.

More recently, Schutyser et al. (70) applied discrete particle modeling to investigate mixing patterns in the conical helical-blade mixer, which they proposed as a potentially useful design for well-mixed solid-state cultivation bioreactors.

Models for fluidized bed bioreactors have not considered the energy balance because the high aeration rates provide sufficient cooling. The main design challenges are related to adequate fluidization of the substrate particles, a subject that will not be addressed here.

To refine these models, it is probably interesting to incorporate the effects of mixing on growth into the model, especially for the case in which the process organism is a fungus. Although the deleterious effect of agitation on fungal hyphae is well known, as yet there is insufficient quantitative information to describe this effect mathematically.

7. ASSOCIATED ISSUES THAT MUST BE CONSIDERED IN BIOREACTOR DESIGN

7.1. A Challenge in all Bioreactor Types: Design of the Air Preparation System

For almost all bioreactors, it is necessary to design an air preparation system. The only exceptions are those tray-type bioreactors that are incubated in a chamber without conditioned air. The basic task is to provide air at the required flow rate, temperature, and humidity. The required conditions for the inlet air are deduced from quantitative bioreactor design strategies, such as those outlined in Sect. 6. Note that it is not necessarily the case that the design and optimization of operation of the bioreactor is decided and only then the air preparation system is designed. Limitations on what it is feasible or economical to do with the air preparation system can affect decisions about how to operate the bioreactor.

Typically, it will be advantageous to change the conditions of the inlet air during the process, this being because the aeration system has a key role to play in temperature control and the need for heat removal typically varies significantly through the growth cycle of the organism. Note that the simple design approaches represented by Eqs. (9), (11), and (13) can be used to calculate the aeration rates necessary at the time of peak heat production. These rates should be sufficient to cool the bioreactor during the entire growth cycle. However, use of an aeration rate based on the peak heat production rate means that the aeration rate will be unnecessarily high during the great majority of the growth cycle. Therefore the model-based approach, which incorporates growth kinetic equations, has the advantage of being able to be used to determine how the need for heat removal changes during the process and consequently, how the required aeration conditions change during the process.

In order to vary the cooling effect provided by the aeration system, any of various variables can be manipulated, namely the inlet air temperature, humidity, and flow rate. It is possible to design an air preparation system to control humidity and temperature simultaneously, however, it would be economically unfeasible due to the sophistication that would be necessary since air humidity has a non-linear dependence on the temperature. In general, solid-state cultivation processes operate at low profit margins, especially those in environmentally related applications. As a result, the air preparation system must have low operating and maintenance costs. A good compromise can be obtained using a humidification column that provides saturated air at a particular temperature with a by-pass if evaporative cooling with dry air is required. In the system suggested in Fig. 7.22, which is similar to that of Agosin et al. (95), the humidifying column assures air saturation and the water temperature can be used to control the temperature of the outlet air.

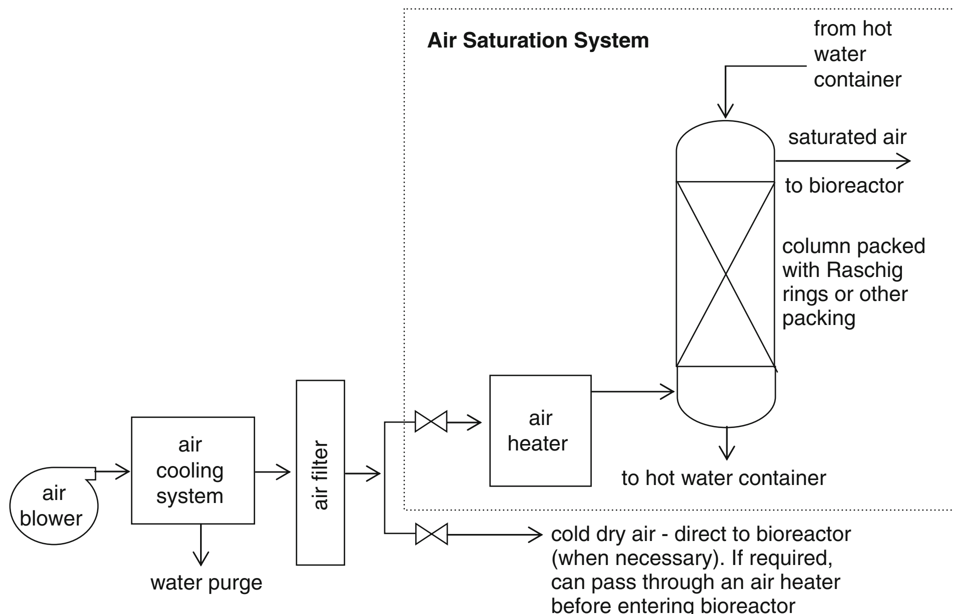

Fig. 7.22. Basic features of an air preparation system that can be used to provide saturated or dry air to a bioreactor, as required (95). A control system will be needed to direct the air flow in the desired direction (i.e., to provide saturated or dry air to the bioreactor).

7.2. *Monitoring and Control Systems for Bioreactors*

Given that the required operating conditions change over time with changes in the microbial activity, it is essential to have a monitoring (data acquisition) system and a control system for the bioreactor that makes on-line measurements of key process variables and makes changes in selected operating variables (manipulated variables), in the most effective manner possible, in response to these measured values.

7.2.1. Equipment for On-Line Monitoring

Key process variables for which it would be desirable to have on-line measurements include:

- *The bed temperature, possibly at different locations within the bed.* Bed temperature is important because it has significant effects on microbial growth and when it deviates from the optimum value it will be necessary to undertake control actions.
- *The pressure drop over the bed.* Pressure drop is important in those bioreactors that are forcefully aerated, since it represents a resistance to air flow and, if the pressure drop gets too high, it may be desirable to undertake a control action, such as agitating the bed to increase the bed porosity.
- *The rate of growth of the organism.* This is a key variable that can be used to evaluate process performance. Note that direct measurement of growth rates is highly problematic in solid-state cultivation systems. However, an indirect estimate can be obtained from respiration rates.
- *The water activity of the bed.* The water activity of the bed is important because it has significant effects on microbial growth. If it is likely to deviate significantly during the process, which

depends on the sorption isotherm of the substrate and how the bioreactor is operated, then it will be necessary to undertake control actions. Note that if the sorption isotherm of the substrate is known, it may be sufficient to determine the water content, although the presence of the microorganism and changes in the substrate due to growth-related processes may mean that the sorption isotherm changes during the process, a phenomenon that has received little attention.
- *The flow rate, temperature and humidity of the air supplied to and leaving the process.* As discussed in Sect. 1, it is important to control the inlet air conditions at the values required by the process. However, it is not a simple matter to do this, and it is necessary to monitor the inlet air variables to ensure that they are in fact at their desired values and, if they are not, to activate the system responsible for controlling the inlet air. The outlet air conditions can give valuable information about water losses from the bed.

Note that other key variables, such as average biomass and average nutrient concentrations, can only be measured off-line and do not give results quickly enough to be used in control systems. This section focuses on equipment and methods for on-line measurements. However, the necessity to remove samples for off-line measurements does raise the issue of the design and operation of the sampling systems. Note that it will typically be desirable to remove samples from the substrate bed during the process. If the bed remains static, it will probably be necessary to remove samples from several locations since the conditions in the bed and the resulting growth and product formation will be heterogeneous. This may need to be done without disturbing bioreactor operation, but this will be difficult. For example, the removal of samples from the interior of a packed-bed will typically leave holes that will allow preferential flow of the air, diminishing the effectiveness of the aeration of the solids. Note also that for processes that are not resistant to contamination, it will be necessary to design the sampling system so as to operate aseptically.

In designing the bioreactor, it is necessary to consider what on-line monitoring equipment should be incorporated into the design. Various possibilities, some of which are illustrated in Fig. 7.23, are listed below.

- *Temperature measurement.* Bed temperatures can be measured with thermocouples or thermistors inserted into the substrate bed. Although thermistors are more expensive and more fragile than thermocouples, they are more stable and precise. The temperature probes may need to be withdrawn from the bed before the bed is mixed. The measurement of temperature at different positions gives information about the efficiency of heat removal and will allow the implementation of a more adequate and robust control strategy.
- *Measurement of the pressure drop across the bed.* The pressure drop across the bed can be measured using differential pressure meters that work on the piezoelectric principle. These meters have short response times and are not affected by the gas temperature.
- *Measurement of bed water content.* Although various probes could potentially be used to measure bed water contents on-line, such as capacitance or conductivity-based devices, these will be affected by the probe-solid contact, which can vary significantly during the process. Possibly the most promising devices are those based on the emission of microwaves and analysis of the reflected signal by Time Domain Reflectometry (96). Potentially, this method can be used to map moisture contents in three dimensions, although the methods for doing this are still under development (96).
- *Measurement of gas phase temperature and relative humidity.* Gas phase relative humidity in the inlet and outlet gas can be measured with various probes in which the capacitance or resistance

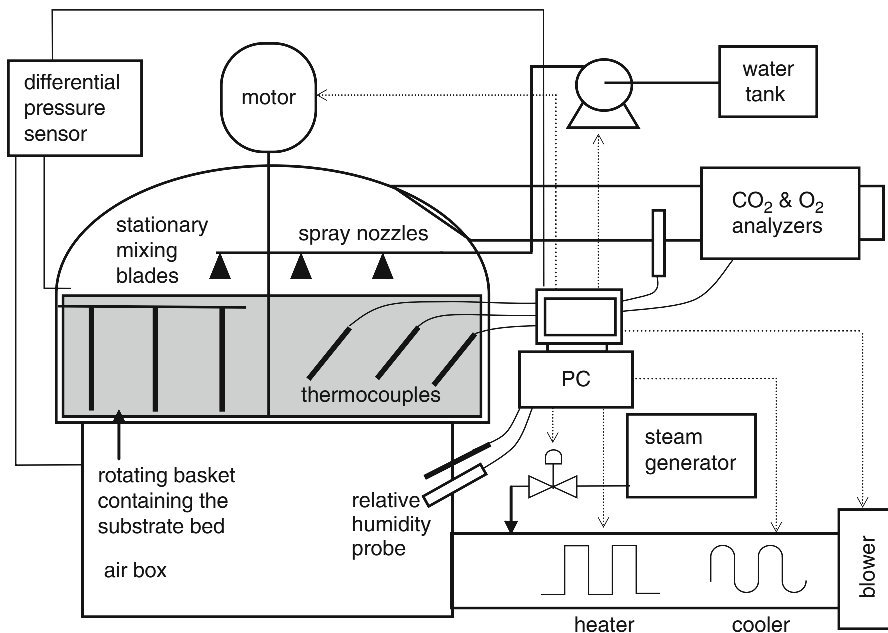

Fig. 7.23. A possible system for monitoring and control of a solid-state cultivation bioreactor, based on the system used by Agosin et al. (95).

of a sensor varies with the relative humidity of the gas phase with which it is in equilibrium. However, these tend to have relatively long response times. Relative humidity meters that operate on the dew point principle may be preferable as they have rapid response times (96). The temperature measurement can be done using thermocouples or thermistors.

- *Measurement of gas phase O_2 and CO_2 concentrations*. The respiration rate of the process organism, calculated through monitoring O_2 and CO_2 concentrations in the off-gases from the bioreactor, can give valuable information about the growth rate and metabolic state. Although on-line gas chromatography can be used to monitor not only these gases but also various volatile metabolites, gas analyzers, such as paramagnetic analyzers for O_2 and infrared analyzers for CO_2, tend to be more precise and have faster response times. Although they are relatively expensive, they are durable, precise, and suffer relatively little from interference with other gases if the gas is dried before analysis.
- *Measurement of gas flow rates*. There are many methods available to measure gas flow rates. The volumetric flow rate of the gas can be measured with Pitot tubes, Venturi tubes, rotameters, anemometers, and turbines, to name a few. The mass flow rate of the gas can be determined with thermal mass meters, which are more expensive.

Note that this survey of available measuring devices is only very brief. In order to choose the appropriate equipment when more than one device is available for a given measurement, it is necessary to understand the abilities and limitations of the various devices and to weigh this against its capital and maintenance costs.

In some cases, equipment for a desired measurement is not available or is prohibitively expensive. In these cases, it may be possible to develop inference models (so-called “soft-sensors”), which use other measurable variables to estimate the desired variable. For example,

Penha y Lillo et al. (97) used a mass balance, based on the relative humidity of the inlet and outlet gases and the consumption and production of water in growth-related processes, to estimate the water content of the substrate bed. This brings up the need for data filtering. All measuring devices suffer from random or systematic measurement errors, and data filtering will be necessary to smooth out the readings in order to identify the underlying "real" trends.

7.2.2. Control Strategies for Solid-State Cultivation Bioreactors

When measured process variables such as those discussed in the previous section deviate from values that are optimal for the process, it is necessary to manipulate the operating variables in a manner such as to counteract the deviation and, as far as is possible, to bring the process variables back to their optimal values. For example, when the bed temperature rises above the optimum, the flow rate of the inlet air might be increased or its temperature decreased, or both. The design of solid-state cultivation bioreactors involves not only decisions about the physical appearance of the bioreactor, but also decisions about the operating strategy, in other words part of the design task is to devise the control strategy.

A discussion of process control strategies is beyond the scope of this chapter. However, experience in solid-state cultivation systems has shown that simplistic control strategies, in which a single operating variable is manipulated in response to a single measured variable, are inadequate for controlling solid-state cultivation bioreactors (98). Given that evaporative cooling is often involved in temperature control, simultaneous control of the temperature and moisture levels can only be achieved with a multiple-input, multiple-output scheme.

Data filtering, mentioned in the previous section, is an essential part of any control scheme, as it obviously makes no sense to undertake control actions in response to random noise or even systematic deviations of the data acquisition equipment. Therefore, before being used in the control algorithm, the data is treated with mathematical filtering procedures such as Kalman filtering or Butterworth filtering to eliminate this measurement noise.

Given the complex nature of solid-state cultivation systems, and that much of the behavior of the system is driven by the growth kinetics of the process organism, which follow defined rules, it is appropriate to use predictive control algorithms, such as Dynamic Matrix Control, that take into account the predicted future behavior of the system based on a model of the process, rather than simpler algorithms, such as PID, which is simply reactive in nature.

Control of solid-state cultivation systems in large-scale bioreactors has proved to be a very difficult task, and is still far from a satisfactory solution (99, 100). Indeed, there are only few examples of rational use of modern control strategies in the optimization of large-scale bioreactors. Figure 7.23 shows an example of the monitoring and control system for a pilot-scale bioreactor built in Chile (95, 101).

8. FUTURE PERSPECTIVES

As discussed in this chapter, quantitative design rules for bioreactors for solid-state cultivation processes are already available, including simplified methods that can give answers of the right order of magnitude to design questions. Mathematical models are potentially more powerful design tools. Various of these models are discussed in greater detail in Mitchell et al. (102). However, the models that have been developed to date have not yet been converted

into fully flexible and user-friendly tools. In other words, for an engineer to adapt one of the current models to their own system, which might simply have a different isotherm, a different kinetic equation for microbial growth or a different geometry, it would be necessary to change the original source code, which requires the engineer to have modeling and programming skills. An ideal design tool would allow such equations to be entered directly into a specific dialog box on the computer screen. Of course, incorporating such flexibility into a program is a challenging task. Furthermore, there is much scope for improvement of the models themselves, including the following:

- Development of general relationships to express key model parameters (e.g., heat transfer coefficients) as functions of design and operating variables;
- Improvement in the modeling of growth kinetics, especially in the face of conditions that vary during the growth cycle;
- Extension to describe systems more realistically. For example, in various models, it will be appropriate to replace assumptions of equilibrium between the solid and gas with equations that describe heat and mass transfer.

The discrete particle modeling approach that has been used to simulate mixing patterns in rotating drum bioreactors can be extended to other bioreactors and, with the incorporation of water transfer and heat transfer into the model, it is potentially a powerful tool for use in the bioreactor design process (103). However, despite the promise of this approach, it will probably be some time before discrete particle modeling is used widely in the design of solid-state cultivation bioreactors, since the method requires advanced mathematical skills and the models make very heavy demands on computing power. Such models may take days or even weeks to run, during which time several cultivations could be undertaken experimentally.

ACKNOWLEDGMENTS

This chapter represents the combined input of a team working to develop bioreactors for solid-state cultivations under a grant given by the Brazilian-Argentinean Biotechnology Center (CBAB, Centro Brasileiro-Argentino de Biotechnologia), using funds provided by the Brazilian Ministry of Science and Technology (MCT, Ministério de Ciência e Tecnologia) and administered by the Brazilian National Council for Scientific and Technological Development (CNPq, Conselho Nacional de Desenvolvimento Científico e Tecnológico). David Mitchell, Nadia Krieger, José Domingos Fontana, Geraldo Lippel, Leda Castilho, and Denise Freire thank CNPq for research scholarships.

NOMENCLATURE

α = bed to air heat transfer coefficient, $J/(h\,m^2\,^\circ C)$
ξ = dimensionless height within the bed, dimensionless
δ = overall bed height within a tray, m
Θ = temperature difference between the bottom of the solid medium and the upper surface in a tray in the case where heat is not transferred across the bottom surface to the air, °C
λ = enthalpy of evaporation of water, J/kg
μ_{opt} = specific growth rate constant under optimal conditions, per hour

μ = specific growth rate constant, per hour
ρ_a = density of air, kg/m^3
BOD = Biological Oxygen Demand, mg/L
COD = Chemical Oxygen Demand, mg/L
C_g = O_2 concentration in the gas phase surrounding the tray, kg/m^3
C_{pa} = heat capacity of air, J/(kg °C)
D = diameter, m
Da_M = modified Damkohler number, dimensionless
D_e = effective O_2 diffusivity within the substrate bed within a tray, m^2/h
f = rate at which the saturation humidity of air increases with temperature, kg/(kg °C)
F_a = air flow rate, kg/h
h_{con} = coefficient for heat transfer through the bioreactor wall, $J/(h\,m^2\,°C)$
H_c = critical bed height for a tray, m
k = thermal conductivity of the bed, J/(h m °C)
L = length, m
N_{Bi} = Biot number, dimensionless
N_C = Critical drum rotational speed, rpm
$R_{O2\,max}$ = maximum volumetric rate of O_2 consumption $kg/(h\,m^3)$
R_Q = volumetric rate of heat generation, $J/(h\,m^3)$
$R_{Q\,max,}$ = maximum volumetric heat production rate, $J/(h\,m^3)$
t = time, h
T = bed temperature, °C
T_a = surrounding air temperature, °C
T_{in} = Inlet air temperature, °C
T_{out} = Outlet air temperature, °C
T_s = temperature at the surface of the bed, °C
V = volume of the substrate bed, m^3
V_Z = superficial velocity of the air, m/h
Y_{QO} = stoichiometric coefficient relating heat yield with oxygen consumption, J/kg
X = biomass content, kg/m^3
X_{max} = maximum possible biomass content, kg/m^3
y_{out} = humidity of the outlet air, kg/kg
y_{in} = inlet air humidity, kg/kg
Y_{QX} = stoichiometric heat yield from growth, J/kg
Y_{xo} = Yield of biomass from O_2, kg/kg
z = height within the bed, m

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