11 Continuous Solid-State Fermentation Bioreactors

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11.1 Introduction

The previous chapters have presented solid-state fermentation (SSF) bioreactors that operate in batch mode. Although batch operation is the most common type of operation in SSF processes to date, it is also possible to design and operate continuous SSF bioreactors. However, there are challenges faced in the operation of continuous SSF bioreactors that are not faced in classical continuous submerged liquid fermentation (SLF) processes and, consequently, true continuous-flow SSF bioreactors (CSSFBs) are currently scarce in industry. Improved design procedures and sensors promise a better future for these bioreactors.

This chapter deals with the design and operation of continuous SSF bioreactors and discusses the potential advantages that continuous operation can bring to SSF processes and also the various considerations that need to be addressed in order to arrive at a well-performing continuous process.

11.2 Basic Features of Continuous SSF Bioreactors

11.2.1 Equipment

In general, continuous chemical reactors can be classified into one of three groups: stirred tank reactors, tubular flow reactors, and designs that are between these two types (i.e., which combine some characteristics of both stirred and tubular flow reactors). This is also true for CSSFBs. Readers interested in exploring possible designs further are encouraged to consult references that deal with equipment used for mixing of solids (Sastry et al. 1999) and for feeding of solids (Bell et al. 2003), many of which could be adapted to act as CSSFBs.

In this chapter we will discuss three possible CSSFB designs: the Continuous Stirred Tank Bioreactor (CSTB), the Continuous Rotating Drum Bioreactor (CRDB), and the Continuous Tubular Flow Bioreactor (CTFB). The principles of operation of screw conveyors and belt conveyors are the same as those of CTFBs,

so in this chapter these conveyor bioreactors will be used as examples of this group.

11.2.1.1 Continuous Stirred Tank Bioreactors (CSTBs) for SSF

Continuous Stirred Tank Bioreactors are designed to mix the whole content of the bioreactor thoroughly. In the ideal case, mixing is said to be *perfect* which means all the properties are identical everywhere inside the vessel at a given time.

In the case of SSF processes, it is impractical to *mix perfectly* due to two limitations imposed by the solid nature of the system. Firstly, wet solids have limited capacity for flowing and this makes mixing difficult. Secondly, wet solids tend to show a significant degree of flow segregation. The term *flow segregation* refers to the tendency of particles that have been in the vessel for different periods of time to remain segregated in different groups (Fogler 1999). As a result, any CSTB that is used for a continuous SSF process will behave to some degree as an intermittently mixed bioreactor. Despite these problems, perfect mixing behavior remains as an ideal model that serves as a paradigm for the analysis and design of these systems as we will see later in this chapter.

Note that there is a further limitation on *perfect mixing*. In *perfectly mixed* CSTBs for SLF, mixing is perfect even at the molecular level. However, in SSF, the bed of solids cannot be mixed at the molecular level unless the solid substrate particles are completely destroyed. If the solid particles are to remain intact, then *perfect mixing* can only occur at the "supra-particle" scale, with no mixing at the intra-particle scale. Further, transfer of liquid or biomass between particles will typically be quite limited. As a result, in SSF, even for a *perfectly-mixed* CSTB, each particle essentially acts as a "batch micro-bioreactor".

The main design variables for CSTBs are:

- The geometry of the vessel. Figure 11.1 shows a conical geometry that could favor both the mixing of the solids within the bioreactor and their flow through the bioreactor. The height to diameter ratio of the vessel and the way it is positioned (i.e., vertical, inclined or horizontal) will influence the agitation devices that should be used and also the portions of the flow that will be moved as plug-flow and as perfectly mixed flow.
- The availability of heat transfer devices. Temperature control is easier in this bioreactor because of mixing, so different approaches could be explored, such as the use of water jackets or water-cooled impellers.
- The design of the aeration system. The air can be circulated through the headspace or blown forcefully through the bed. If blown through the bed, the air flow can be in the same direction, in the opposite direction or normal to the solids flow. Of course the aeration system can be designed to allow changes in the direction of air flow during the process.
- The type and number of impellers. The solids mixing efficiency depends strongly upon the type of impeller used (Sastry et al. 1999). A careful study should be conducted to select the appropriate design and positioning.

- The features of the solids addition and removal devices. They may need to be designed to prevent the entry of contaminants into the bioreactor. The solids inlet and outlet should be designed and positioned in order to minimize the possibility of short-circuiting. That is, added solids should be mixed into the bed and should not simply flow directly from the solids inlet to the solids outlet. In the case of external recycling of part of the solids that exit the vessel, the design of the recycling system must prevent contamination and mix the recycled solids well into the fresh solids stream.
- The features of equipment for addition of water and nutrients. A large part of the metabolic heat may be removed via evaporation, in such cases continuous or semi-continuous water replenishment will be required. The equipment for makeup water distribution should be designed to allow an even distribution. Minerals and soluble carbon sources can be added by the same system.

The operating variables include:

- The dilution rate. This is defined as the ratio of the mass flow rate into the bioreactor to the total mass of solids within the bioreactor. It is a key factor in optimizing the productivity of the process and maximizing the concentration of products. Theoretically *washout* flow could occur, in a similar manner to that which occurs in continuous CSTB processes in SLF.
- Impeller velocity and frequency of stirring. These factors will influence the quality of mixing and will be very important in determining the distribution of temperatures and concentrations within the vessel.

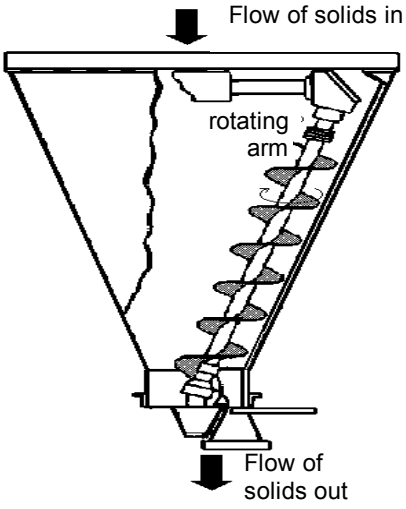

Fig. 11.1. Schematic representation of a Continuous Stirred Tank Bioreactor (CSTB) that could be used for SSF processes. Note that air could be blown into this bioreactor either at the top or at the bottom. Alternatively, it may even be possible to blow air into a hollow mixing device, with appropriately positioned holes allowing the air to pass into the bed

11.2.1.2 Continuous Rotating Drum Bioreactor (CRDB)

This bioreactor is similar to those of the stirred tank group but differs in the manner in which mixing is achieved: the CRDB consists of a cylinder that rotates horizontally around its axis. Bioreactors of this kind fall between perfectly mixed bioreactors and plug-flow bioreactors and hence might be referred to as mixed-flow bioreactors. Indeed, as in solid-drying equipment of this shape (see Moyers et al. 1999), they can have internal devices that promote forward and backward mixing. These devices could be static mixers, like the baffles in Fig. 11.2, or dynamic mixers, which stir and transport the solid internally within the vessel.

The design and operating variables of CRDBs are similar to those of CSTBs. Nevertheless the fact that the drum rotates without the motion of an internal agitator produces particular features in the stirring mechanisms. The number, shape, and position of the baffles are important factors that affect the flow through the drum and consequently the performance of this bioreactor.

In addition to heat removal by convection to the air flowing through the headspace, different strategies can be tried for removal of waste metabolic heat from the bed of fermenting solids. For example, the lower part of the external wall of the vessel could be immersed in a water bath.

The speed of rotation of the drum and the angle of inclination of the body of the bioreactor to the horizontal are very important factors affecting solids mixing and transportation. Rotational speeds as low as 2 to 3 rpm are commonly used in batch systems (Hesseltine 1977; Pandey 1991), although higher speeds have also been reported. The substrate normally occupies 10% to 40% of the volume of the bioreactor (Stuart 1996).

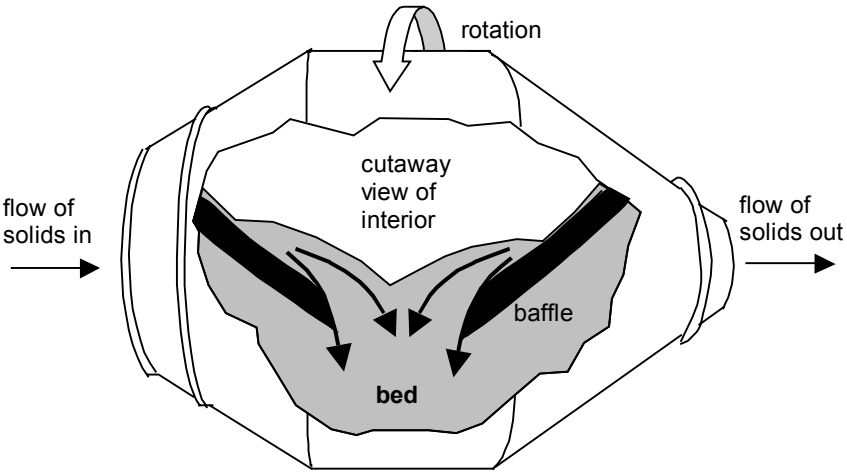

Fig. 11.2. Continuous Rotating Drum Bioreactor, which, in terms of solids-flow regimes, is placed between perfectly-mixed bioreactors and plug-flow bioreactors

Van de Lagemaat and Pyle (2001) used a 1-m-long CRDB with a diameter of 8 cm. By adjusting the baffle arrangement and the inclination of the central axis of the bioreactor to the horizontal, they achieved near-perfect mixing of un-inoculated solid substrate particles. The main goal of this design was to achieve sufficient back mixing so that the sterile feed could be inoculated by the fermented particles within the bioreactor, in such a manner as to remove the need for an external inoculation system. However, the efficiency of such "back-inoculation" has not yet been directly investigated. Further, as will be explained later, there may be problems with product uniformity in back-mixed CSSFBs.

A special kind of CRDB was tested for fermentation of a mixture of feedlot waste and coarsely cracked corn (Hrubant et al. 1989). The 91.5-cm-long bioreactor had a diameter of 22.8 cm and consisted of three chambers aligned axially and separated by bulkheads. Each bulkhead had a centrally located hole to permit unidirectional passage of fermenting substrate sequentially through the chambers. Each chamber had several baffles to ensure perfect mixing of the fermenting solids within the chamber and therefore this bioreactor acted like three perfectly-mixed continuous bioreactors in series. Fermentation runs as long as two months were conducted with this bioreactor. A pilot-scale bioreactor of this kind having three 468-liter chambers was also used at Illinois University.

11.2.1.3 Screw and Belt Conveyor Bioreactors

Screw conveyors and belt conveyors, which are examples of continuous tubular flow bioreactors (CTFBs), can move solids with almost zero mixing in the direction of flow (Fig. 11.3). When mixing is desired, which is often the case, static or dynamic mixers can mix the bed in the radial direction and, if desired, also in the axial direction. The current subsection focuses on the situation without axial mixing. Due to lack of back mixing, internal back-inoculation is not possible, however, external inoculation might be done by recycling a part of the fermented product, avoiding the need for a separate process for inoculum production.

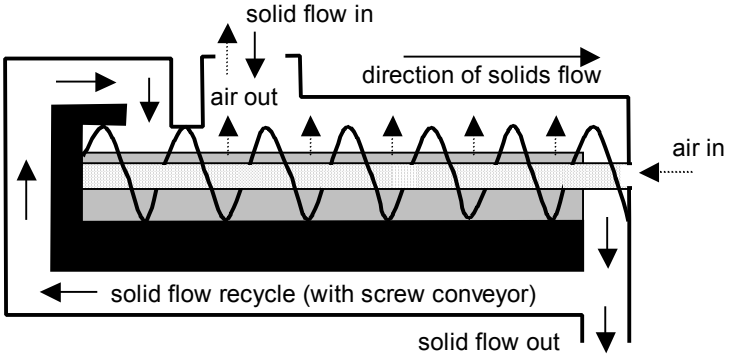

Fig 11.3. Screw bioreactor with recycling. The central axis is hollow and perforated, to allow the flow of air into the bed. The screw blade is mounted on this axis, which rotates

Gibbons et al. (1984, 1986) investigated a continuous screw-type bioreactor for farm-scale fuel ethanol production from various solid substrates such as fodder beets and sweet sorghum. Their system was not aerated and only anaerobic or microaerophilic fermentations could be carried out. Moreover, the void spaces between the solid particles contained significant quantities of liquid, meaning that the process actually represented a borderline case between SSF and a "slurry fermentation". In any case, their bioreactor could be adapted for true SSF processes, although an aeration system would need to be incorporated for the cultivation of aerobic organisms.

Some of the large-scale *koji* production bioreactors can work in this mode. For example, the rotary disk bioreactor shown in Fig. 10.2 can be operated in a manner in which the rotating disk acts as a circular conveyor belt. As the disks slowly rotate, particles are transferred from the upper disk to the lower disk. The empty space on the upper disk is then filled with freshly inoculated particles. Each particle entering the upper disk spends the same time before being transferred to the lower disk. Each particle entering the lower disk then spends the same time before being harvested. Production rates as high as 4150 kg h^{-1} have been reported (Yokotsuka 1985; Chisti 1999). Tower-type CSSFBs used in certain composting processes operate in a similar manner, with a semi-continuous flow of substrate from one chamber to the next.

11.2.2 Flow Patterns: Real-Flow Models

As for any other continuous chemical reactor, the flow of materials from the inlet to the outlet of a CSSFB could potentially fall anywhere between the ideal plug-flow and perfect-mixing regimes. In plug flow, all of the particles have the same residence time within the bioreactor as they move along parallel paths at the same speed. On the other hand, if the particles are mixed parallel to the direction of flow, they may spend different lengths of time in the reactor. In other words, different particles may have different residence times. In a completely mixed reactor, the residence time distribution for the population of particles is wide, some particles may exit almost immediately after they enter, while some other may remain within the reactor for longer times.

Theoretically, in order for all fermented substrate particles exiting a CSSFB to have the maximum possible growth and product formation, each particle should spend the same amount of time in the bioreactor between when it is inoculated at the solids inlet and when it is harvested at the solids outlet. The importance of this can be seen in a simple example. Let us assume that it requires 24 h, measured from the time of inoculation, for the microorganism on a particular substrate particle to produce the maximum activity of a desired enzyme, and that the enzyme activity falls off after 24 h due to denaturation or degradation by proteases. In this case, any particles exiting a CSSFB with residence times lesser than or greater than 24 h will have an enzyme activity less than the maximum possible value. In such a case it would be desirable for the residence time distribution to be as narrow as possible, with a mean of 24 h.

This is the consideration of "uniformity amongst harvested substrate particles", which is desirable, but may be difficult or impossible to achieve in practice. Note that "true" continuous operation does guarantee a uniform product regardless of whether all substrate particles have the same residence time or not, but in this case the concept of uniformity is applied differently: if a CSSFB does manage to establish a steady state, then the exiting product will have a uniform composition, averaged over the population of exiting substrate particles. In other words, the proportions of "young" substrate particles and "old" substrate particles in the harvested product will remain constant over time for true continuous operation, regardless of the flow regime and residence time distribution of the particles. However, in terms of bioreactor productivity, the exiting of a mixture of younger and older particles is disadvantageous when compared to the exiting of a uniform population of "fully-fermented" particles. There is a further consideration: heterogeneity of the inlet raw material and the presence of non-ideal flow patterns, dead volumes, air channeling, and solids short-circuiting may all contribute to fluctuations in the quality of the product exiting a CSSFB. These issues have received very little attention in SSF.

The wideness of the residence time distribution depends on the direction and extent of mixing (Fig. 11.4). Mixing of fermenting solid particles perpendicular to their flow direction in a bioreactor would typically be desirable, especially if the bed were forcefully-aerated with the air flow being perpendicular to the solids flow direction. In the absence of vertical mixing in the bioreactor shown in Fig. 11.4, undesirable temperature and moisture gradients, similar to those noted for packed-bed bioreactors (Chap. 7), would arise along the direction of the air flow. If it were possible to mix the solids in this bioreactor vertically (i.e., perpendicular to the solids flow direction) without any horizontal movement of the solids (i.e., parallel to the solids flow direction), then such mixing would have no influence on the residence time of the solid particles. However, this is an ideal that is impossible to achieve in practice: Mixing perpendicularly to the solids flow direction will also cause some mixing parallel to the solids flow direction. Mixing parallel to the solids flow direction, often called flow dispersion, leads to a broadening of the residence time distribution pattern.

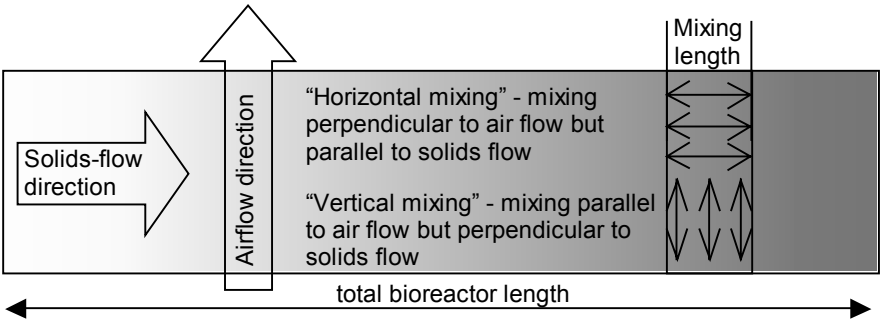

Fig. 11.4. Two main mixing directions in continuous SSF bioreactors

The amount of flow dispersion will depend on the design and operation of the bioreactor and the mixer and on the number of mixers used. It may be assumed to be roughly proportional to the sum of mixing lengths divided by total bioreactor length (see Fig. 11.4). For example, in a CSSFB composed of a single long belt carrying fermenting solids from inlet to outlet, the bed might not be mixed at all or it may be mixed occasionally so that sum of mixing lengths is negligible compared with total belt length. On the other hand, in a CSSFB composed of a rotating drum with curved baffles, the mixing length may be equal to total bioreactor length, leading to a wide residence time distribution.

11.3 Continuous Versus Batch Mode of Operation

11.3.1 Reduction of Upstream and Downstream Investment

In the batch mode of operation, a quantity of feed equal to the bioreactor capacity must be ready for loading at the start of each cycle. Chemical changes cannot be prevented when moist solid substrates are stored for long times, and there is always the danger of the growth of contaminants, so it is not feasible to prepare and cook the substrate gradually. Neither is it feasible to inoculate the substrate gradually if the bioreactor is to be operated in batch mode. Hence, the upstream equipment for substrate preparation and inoculation must be large enough to be able to prepare the whole batch of required substrate within a few hours before the start of each cycle. In contrast, in the continuous operation mode, smaller equipment can be used to process, on an hourly basis, the smaller substrate quantities that are fed into the bioreactor. In this manner, continuous operation can reduce the investment in upstream processing equipment. The degree of reduction becomes greater as the cycle time is increased. For example, suppose that a bioreactor with a capacity of 1000 kg is used in batch mode in a SSF process that has a 50-hour fermentation time. If the substrate is required to be prepared no sooner than 10 h before the start of each fermentation, then the capacity of the upstream equipment must be 100 kg h^{-1}. On the other hand, if the same bioreactor were used in continuous mode, then the required capacity of the upstream processing equipment would be 20 kg h^{-1}. Moreover, if the fermentation time were 100 h, the upstream equipment capacity required for batch mode would not change, while that for continuous mode would be reduced to 10 kg h^{-1}.

In the same manner, continuous operation will reduce downstream equipment costs. Continuous operation of the bioreactor will require continuous downstream processing since the fermented solids are chemically and biologically active and if stored for long times before a large batch is sent for downstream processing, then the fermentation may continue, leading to undesirable changes. For example, labile products may be degraded if they are not recovered from the solid medium soon after the fermentation. Consequently, fermented solids leaving the fermenter must be processed as soon as possible to the final product or stabilized via means such as drying or freezing. In a continuous system in which the fermented solids

exit the bioreactor gradually, relatively small equipment could process them into the final product or a stabilized product. This contrasts with batch operation, in which a large amount of fermented solids is discharged from the fermenter during a very short time period, requiring equipment with a large capacity in order to minimize storage time.

Replacing a single large batch fermenter with several smaller ones having staggered start times would reduce the required capacity and cost of upstream and downstream equipments. However, this would be accompanied by an increase in the investment required in the bioreactor section of the process.

11.3.2 Uniformity of the Product from Batch and Continuous Bioreactors

Continuous operation permits a more uniform product than batch operation, especially in cases in which the solid bed is mixed only intermittently. This can be seen by comparing an intermittently-mixed, forcefully-aerated bioreactor operated in batch mode with the same bioreactor type operated in continuous mode. Note that, as shown in Fig. 11.5, in both bioreactors the air flow is perpendicular to the flow of solids. As described in Chap. 10, this design has been successfully proven in batch bioreactors at pilot scale. Note that the intermittent mixing is necessary in order to break up aggregates of solid particles and also to allow the replenishment of evaporated water.

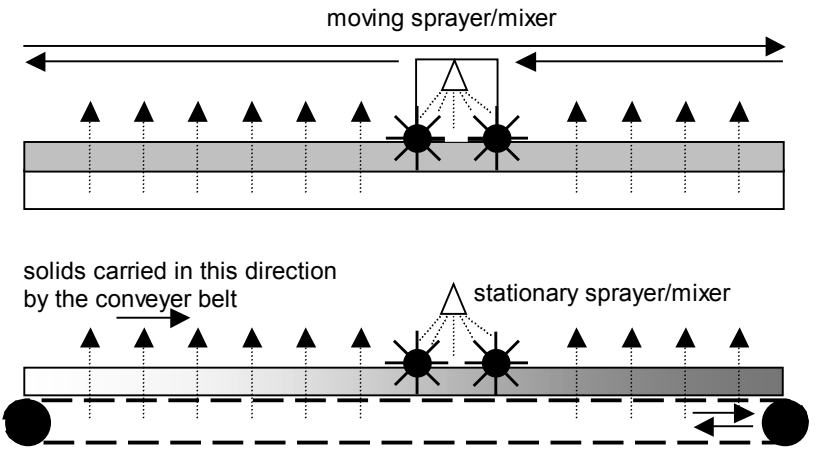

Fig. 11.5. Mixing schemes in intermittently-mixed, forcefully-aerated bioreactors operated in **(a)** batch mode, in which the mixer moves back and forth along the whole length of the bioreactor; **(b)** plug flow continuous mode, in which the mixer stays in place and the bed moves past it

In batch bioreactors of this type, mixing is often performed by a moving mixer, although in some cylindrical *koji* bioreactors the mixing system is stationary and the bed is moved past it via rotation of the base plate. In both cases, simultaneous mixing of the whole bed is difficult to achieve. In a system such as that shown in Fig. 11.5(a), parts of the bed located to the left of the mixer are mixed soon after the start of mixing while parts located to the right of the mixer are mixed only after a lag time that depends on the bed length and the speed with which the mixer travels back and forth along the bed. For large-scale bioreactors the lag time may become considerable, especially in cases in which the mixer travels slowly in order to enable homogeneous distribution of added water. Such lag times could have adverse effects on the product uniformity. Use of several mixers could reduce time lags in the batch mode but will imply a more expensive mixing system. On the other hand, in the continuous system shown in Fig. 11.5(b), the mixer stays in place as the substrate is moved past it and all of the fermenting solids are mixed or wetted at the same time interval after their entrance into the bioreactor, leading to more uniform product.

11.3.3 Enhanced Production Rates

Changing of the mode of bioreactor operation from batch to continuous saves the time required for loading, discharging, and cleaning of the bioreactor, since in continuous operation loading and discharging proceed simultaneously with the fermentation whereas in batch processes the bioreactor is not producing product while these "turnaround" operations are taking place. Assuming the same fermentation time for both batch and continuous operations, the saved time means that the volumetric productivity of the continuous plug-flow bioreactor is higher.

The extent of the increase in productivity depends on ratio of the turnaround time to the fermentation time. For example, assuming that the turnaround and fermentation times are 10 and 40 h, respectively, the volumetric productivity will increase by 25% upon changing from batch to continuous mode. The productivity increase is smaller for higher ratios of fermentation time to turnaround time. For example, with the same turnaround time of 10 h but a fermentation time of 70 h, the increase in productivity gained by changing from batch to continuous operation is only 14%.

As mentioned previously, replacing a single large batch bioreactor with several small ones operating in a staggered manner will reduce the difference in productivities. However, once again, it must be highlighted that this implies higher investment costs.

11.3.4 Contamination

The risk of contamination seems to be the major barrier to be overcome in the development of continuous SSF bioreactors.

In the batch mode of operation, the air flow and added water are the only streams that need to enter the bioreactor during the fermentation. It is typically not difficult to perform these operations aseptically. On the other hand, continuous operation involves a constant flow of a feed stream into the bioreactor and a product stream out of the bioreactor. It is more difficult to ensure that these operations are done aseptically, so the risk of contamination is higher in continuous operation than in batch operation. Moreover, the consequences of occasional contamination are more severe in continuous mode. Typically, the initial concentration of the contaminant is much less than that of the inoculated process organism. Hence, if the growth rate of the contaminant is not significantly higher than that of the process organism, it is not able to reach high concentrations before the fermentation terminates when the bioreactor is operated in batch mode. In the continuous plug-flow mode the situation may or may not be different. If the contaminant is simply carried along with the flow, it poses no greater a problem than it poses for batch operation. However, any back-mixing that occurs allows some particles to remain in the bioreactor for longer times and also it is possible for some contaminated particles to attach to stationary surfaces in a particular part of the bioreactor. This may allow sufficient time for the contaminant to reach high levels on some particles, which would act as seed for inoculation of the contaminant onto other particles. Note that in batch mode the attached contaminant is killed by sterilization operations carried out between successive runs while in continuous mode it can remain within the bioreactor and become a source for continuous contamination.

The situation would be more severe still in a well-mixed continuous fermenter, in which some particles have very long residence times. Of course, the severity of the problem would depend on the efficiency with which the contaminant was passed from particle to particle. However, it is possible that in this mode a contaminant may eventually conquer the whole bioreactor if it competes better than the process organism, even if the initial contamination level is very low.

Problems with contamination are often claimed to be less severe for SSF than for SLF. For example, it is often claimed that, in SSF processes using filamentous fungi, the water activity or pH of the substrate can be adjusted to values low enough to be unfavorable for most bacteria, although of course such conditions may not select against other fungi. With a fast-growing organism it may be sufficient to have a high density of vigorous inoculum and to provide optimum growth conditions early during the process in order to give the process organism a head start. In fact a large number of commercial SSF processes such as *koji* production and beet pulp protein enrichment are usually carried out under non-sterile conditions (Durand 2003). In those SSF processes in which the process organism has a selective advantage over any contaminants, contamination problems may in fact not be a serious barrier do continuous operation. However, for slow-growing microorganisms aseptic operation will be essential, and processes involving these organisms may be difficult to adapt to continuous operation due to contamination problems.

The acceptable degree of sterility depends also on the kind of product and legislative constraints. Pharmaceuticals should be produced under sterile conditions while *koji* can have a contamination of 10^9 bacteria per gram (Yokotsuka 1985).

So, from the point of view of contamination problems, continuous operation seems to be feasible for SSF processes in which fast-growing fungi are cultivated, provided that the product does not have to meet strict sterility standards. The degree of back mixing needs to be reduced to decrease the impact of any contamination. Moreover, internal surfaces should be highly polished. The temperature should be controlled near the optimum growth temperature of the process organism. If possible, water activity and pH should be kept as low as possible while not unacceptably retarding growth of the process organism.

11.4 Comparison by Simulation of the Three CSSFBs

Detailed experimental information on the performance of CSSFBs is not available. With this lack, simulation is a useful tool in understanding the potential of the various bioreactors. Note that almost no attention has been given to the modeling of the continuous operation of SSF bioreactors in the literature. The intention of the present section is to present simple models, while recognizing that many improvements in these models will be necessary in order for them to describe continuous performance reliably. For example, the models presented here for the mixed bioreactors do not take into account the fact that each particle is a batch micro-bioreactor and therefore will be most appropriate for very small particle sizes.

The different systems described above will be simulated using different flow models. The kinetic information has been taken from Ramos-Sánchez (2000), in which the logistic model is used to describe the growth kinetics of the yeast *Candida utilis* for the enrichment of sugarcane byproducts. The logistic model is frequently used to describe the growth kinetics in SSF (see Sect. 14.4) hence it is interesting to simulate the behavior of these systems using this kinetic model. The parameters of this model are the initial biomass content (X_o), the maximum possible biomass content (X_{max}), and the specific growth rate constant (μ). In these simulations X_o is set at 2.5 g kg-dry-matter^{-1}, X_{max} is set at 263 g kg-dry-matter^{-1}, and μ is set at 0.3 h^{-1}.

Constant temperature is assumed; heat and mass transfer phenomena are not modeled. The performance of each system is evaluated on the basis of the productivity of single-cell biomass (g-biomass kg-dry-matter^{-1} h^{-1}).

11.4.1 Continuous Tubular Flow Bioreactors (CTFBs) with Recycling

The operation of tubular flow SSF bioreactors with recycling, such as those shown in Figs. 11.3 and 11.5, can be simulated using a plug-flow bioreactor with a recycle stream (Fig. 11.6). The operating variables are the dilution rate (kg-solids kg-solids^{-1} h^{-1}), defined as in Sect. 11.2.1.1, and the ratio of recycled solid-flow to entrance mass-flow ($\gamma = f_R/F$), the so-called "recycle ratio" (dimensionless).

The results of the simulation are presented in Fig. 11.7 from which it is possible to conclude that:

- There is an optimal dilution rate above which the productivity falls rapidly with increasing dilution rate, as is characteristic of continuous SLF processes.
- Since the feed is inoculated with biomass, there is always biomass in the exit stream, regardless of dilution rate. Note that the graph therefore appears different from the graphs for "classical" continuous SSF processes in which there is no biomass in the feed and therefore above a critical dilution rate the steady state biomass concentration is zero. Of course, if a continuous SLF process were to have a certain level of inoculum in the inlet stream, then at dilution rates greater than the critical rate, the outlet stream would have a concentration of biomass equal to the inlet concentration, in the same manner as occurs for the situation in the CSSFB shown in Fig. 11.7 at high dilution rates.

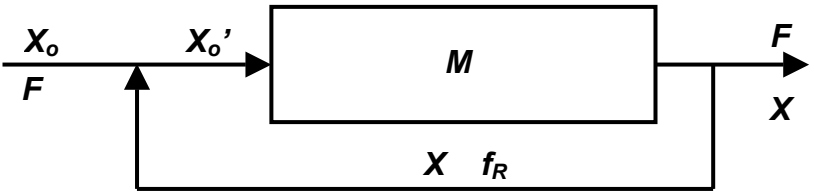

Fig. 11.6. Flow-model for screw conveyor and belt conveyor bioreactors with recycling, these being examples of Continuous Tubular Flow Bioreactors (CTFBs). *M* represents the mass of solids in the bioreactor, *F* represents the inlet flow rate, f_R the recycle flow rate, and *X* represents the biomass concentration

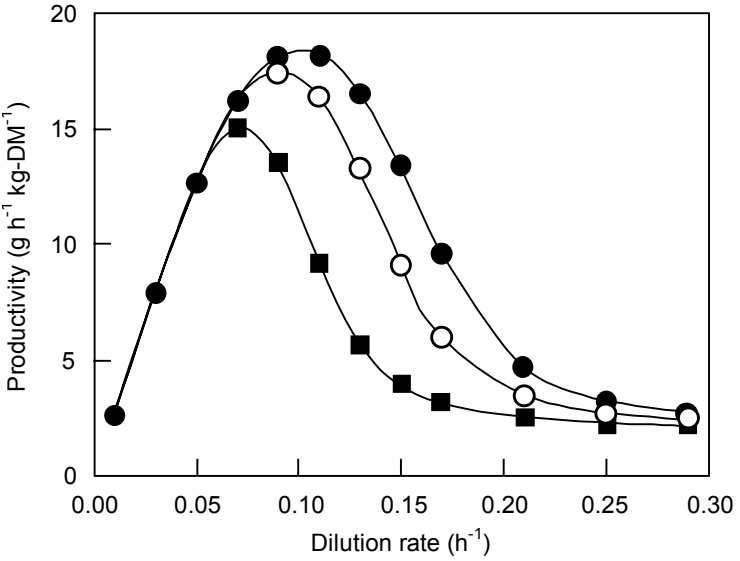

Fig. 11.7. Simulation of a Continuous Tubular Flow Bioreactor (CTFB) with recycling at different dilution rates and recycling ratios. Key: (■) $\gamma = 0.1$; (○) $\gamma = 0.3$; (●) $\gamma = 0.5$

- For dilution rates less than the optimal one, the fraction of mass-flow recycled back to the entrance has no influence on the productivity of the bioreactor.
- If high levels of the product are the main objective, the system can operate at a low dilution rate and with a low recycle ratio. In these cases the task is to find the optimal dilution rate, this being constrained by the minimum acceptable product concentration. The recycle ratio will not be of great importance.
- When high productivities are necessary at high dilution rates, the system will demand greater recycling ratios. The design problem in this case is more complicated and would include finding a combined optimum for both variables, namely the dilution rate and recycle ratio.

11.4.2 Continuous Rotating Drum Bioreactor (CRDB)

One of the many possible flow models for describing the micro-mixing inside a CRDB has been presented by Ramos-Sánchez et al. (2003). The pattern that describes the micro-mixing and, consequently, the behavior of this bioreactor, is a combination of a plug-flow reactor, a perfectly mixed reactor, and a recycle stream (Fig. 11.8). There are three main operating variables for such a system: The fraction of the flow that passes through the plug-flow bioreactor ($\alpha = f_p/f_m$), the fraction of the "in-bioreactor" mass that is contained by the plug-flow bioreactor ($\beta = M_p/M_m$), and the fraction of the flow that it is recycled back to the entrance of the CRDB ($\gamma = f_R/F$).

Figure 11.9 shows the simulations for a given set of α and β at different values of the dilution rate and recycled fraction γ. The behavior is similar to that shown in Fig. 11.7, but some important differences should be pointed out:

- Above the productivity maximum, the decrease in productivity with dilution rate is less pronounced than it was in the case of the former bioreactor (compare the profiles in Figs. 11.7 and 11.9). This means that the operation in this region is more stable, which is more desirable for practical purposes. In fact, for high dilution rates, for example, greater than 0.15 h^{-1}, the CRDB will have higher productivities than the CTFB.

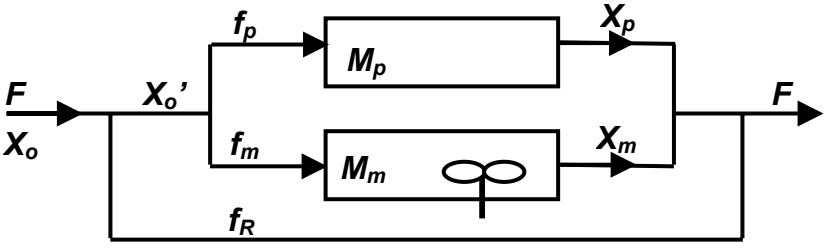

Fig. 11.8. Combined flow-model of a CRDB. M_p is the mass of solids in the plug-flow region while M_m is the mass of solids in the well-mixed region

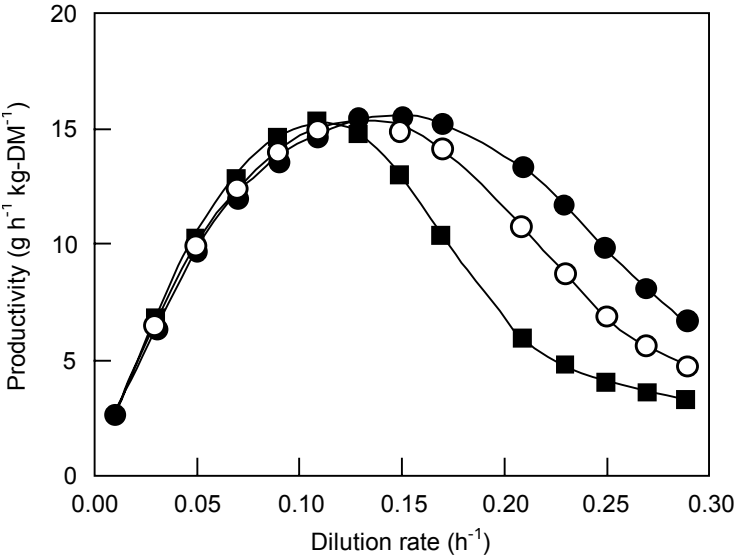

Fig. 11.9. Simulation of a Continuous Rotating Drum Bioreactor (CRDB) where $\alpha = 0.30$ and $\beta = 0.40$. Key: (■) $\gamma = 0.1$; (○) $\gamma = 0.3$; (●) $\gamma = 0.5$

- The maximum productivity is not as sensitive to increments in the recycle ratio (γ) as it was in the previous case, remaining between 15 and 16 g h^{-1} kg-dry-matter^{-1} as the recycle ratio is varied from 0.1 to 0.5.
- In the bioreactor simulated in Fig. 11.9, the maximum of productivity at a value of γ of 0.5 is 16% less than that in Fig. 11.7.

11.4.3 Continuous Stirred Tank Bioreactor (CSTB)

A real CSTB has a complex flow-pattern due to the solid nature of the system and the limitations on stirring imposed by the sensitivity of the microorganisms to shear damage. However, given that the flow patterns in such bioreactors have not been studied, a model assuming perfectly mixed flow is used for the simulations. Note that it is assumed that the particles are inoculated as they enter the bioreactor.

In the case in which there is no recycle stream, the dilution rate is the only operating variable. Figure 11.10 shows the results of the simulations for this bioreactor as a function of dilution rate, together with simulations of the two former bioreactors at a recycle ratio of 10%. The behavior of the CSTB is similar to that of the previous bioreactors but some important differences should be noted:

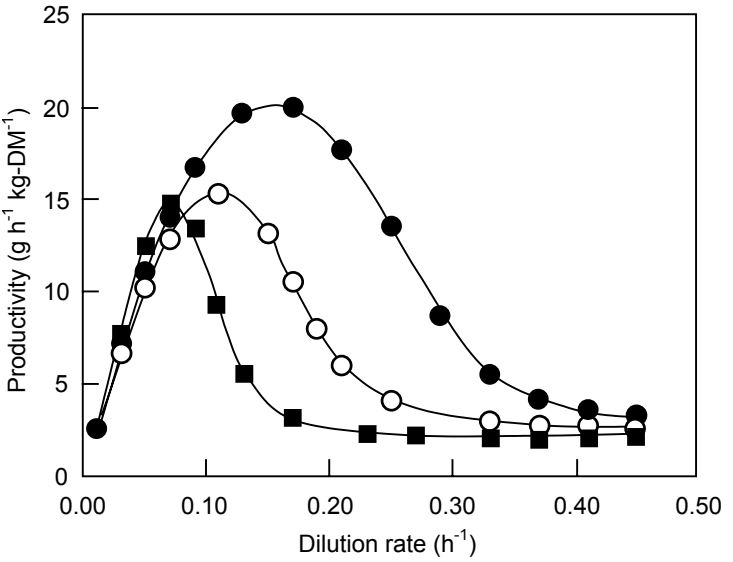

Fig. 11.10. Comparison of the performances of (●) a CSTB, (■) a CTFB ($\gamma = 0.1$), and (○) a CRDB ($\gamma = 0.1$)

- Surprisingly, the results of the perfectly-mixed CSTB are better than those the other two bioreactor types. Normally plug-flow bioreactors are better for simple reactions (Fogler 1999) but in the case in which the reaction rate increases with conversion, which could be the case for fermentation processes, which are autocatalytic, the perfectly mixed bioreactor can perform better.
- The CRDB has an intermediate behavior due to the fact that it combines perfectly-mixed flow with plug-flow. The greater the perfectly mixed component is, then the closer the performance of the CRDB will be to that of the CSTB.
- The CTFB tends to behave more and more like a CSTB as γ rises, which can be seen by comparing Figs. 11.7 and 11.10.

11.4.4 Evaluation of the Various CSSFB Configurations

Figure 11.11 shows the relationship between two important performance criteria, namely the bioreactor productivity and the biomass concentration in the product stream. The relationship is plotted for each of the three configurations of CSSFBs presented in Sects. 11.4.1 to 11.4.3, based on the results of the various simulations performed in those sections. Various points of interest are:

- The CSTB will have maximum productivity when the outlet biomass concentration is a half of X_{max}, while the maximum productivity of the CTFB occurs at greater biomass concentrations.

- The maximum productivity of the CSTB with perfectly mixed flow is 30% greater than that of the CTFB. However, the advantage of the CSTB over the CTFB becomes smaller as the recycle ratio of the CTFB is increased.
- For biomass concentrations up to 200 g kg-DM^{-1}, which is very close to the biomass concentration of 220 g kg-DM^{-1} that gives maximum productivity of the CTFB, the productivity of the CSTB is greater than that of the CTFB.
- The behavior of the CRDB is between these two ideal bioreactors. This is not surprising, because it represents a mixture of the two flow regimes. Indeed, the model of this bioreactor can represent the deviations of flow regimes from the ideal regimes assumed for the CTFB and the CSTB.

In the case of plug-flow through a tubular bioreactor, the reaction rate will be low at the entrance of the bioreactor because of the low concentration of biomass. As the solids flow through the bioreactor, the rate of the reaction will rise to a maximum level at a biomass concentration equal to $0.5X_{max}$, due to logistic growth kinetics, which cause the growth rate to decelerate as the biomass concentration rises from $0.5X_{max}$ towards X_{max}. Therefore at the exit of the plug-flow bioreactor, if the biomass concentration is close to X_{max}, the rate of the reaction will tend to be low. This means that the average reaction rate within the plug-flow bioreactor will always be lower than the maximum possible level; hence as a consequence, the overall productivity will never be as high as it would be for a CSTB in which the biomass concentration were maintained at $0.5X_{max}$.

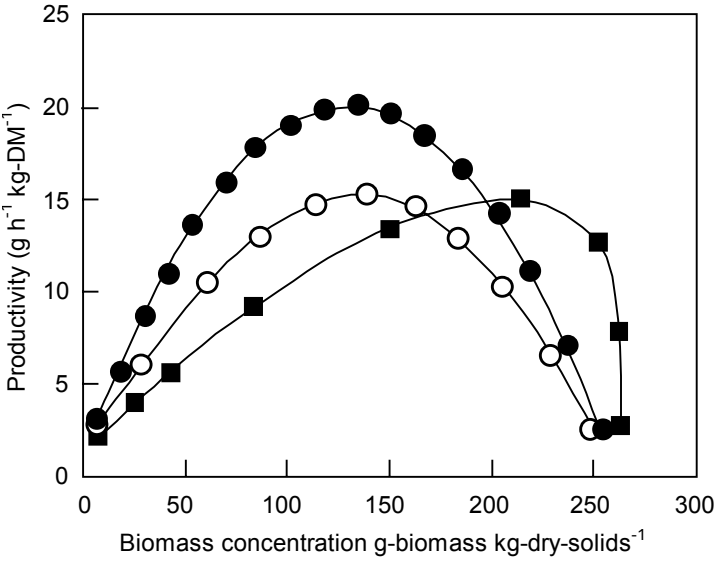

Fig. 11.11. Relation between productivity and biomass concentration in the simulation of a (●) CSTB, (■) a CTFB ($\gamma = 0.1$), and (○) a CRDB ($\gamma = 0.1$)

Finally, we should note a difference between the operation of CSTBs in SLF and SSF. In SLF it is practical to have a recycle stream for a CSTB, since it is possible to centrifuge or filter the stream exiting the bioreactor, such that the recycle stream has a higher biomass concentration than the stream exiting the bioreactor while the product stream leaving the process has a lower biomass concentration than the stream exiting the bioreactor. The important point is that in SLF it is possible to separate, at least partially, the biomass and the liquid. In contrast, in SSF the biomass in the stream exiting the bioreactor cannot be separated from the solids. If any recycling is done, then the composition of the recycle stream and the product stream leaving the process will have compositions identical to that of the stream leaving the bioreactor. Therefore, there are no advantages, in terms of productivity, in recycling solids in a CSTB. In fact, solids recycling is only useful for inoculation of the incoming fresh solids, and this will only be effective if there is efficient inter-particle transfer of biomass.

11.5 Scientific and Technical Challenges for CSSFBs

Continuous solid-state fermentation shares many of the challenges that are faced by SSF processes operated in the batch mode but it also has its own features. It has not received much attention in the literature. In order to understand the possible advantages and limitations of this mode of operation, it will be necessary to

- develop flow models that more realistically describe the flow patterns within the various designs;
- incorporate heat and mass balances into the models of continuous operation;
- recognize the fact that each particle acts much like a “batch micro-bioreactor”, this being quite different from the situation in SLF where perfect mixing is assumed down to the molecular level;
- understand the dynamic behavior of these systems, in order to develop appropriate start-up strategies for continuous operation and also to control the process, minimizing oscillations in the product quality.

Further Reading

Contains a good treatment of the general principles of operation of continuous bioreactors for submerged fermentations

Nielsen J, Villadsen J, Liden G (2003) Bioreaction engineering principles, 2nd edn. Kluwer Academic/Plenum Publishers, New York

A recent example of use of a continuous process in SSF

van de Lagemaat J, Pyle DL (2004) Solid-state fermentation: A continuous process for fungal tannase production. Biotechnol Bioeng 87:924–929