CH6460-BioProcess Technology

Assignment-2

Group No: 4

Members:

CS17BTECH11020 JATIN SHARMA
CS17BTECH11038 SURGAN JANDIAL
CS17BTECH11039 SYEDA RANIYA HAMEED
CS17BTECH11047 AKHIL GEORGE THOMAS
CS18BTECH11001 ABBURI VENKATA SAI MAHESH
CS18BTECH11004 BANDI CHIRANJEEVI SAI GANESH
CS18BTECH11008 DARAPANENI KRISHNA PAWAN
CS18BTECH11010 G PRAJWAL
CS18BTECH11013 GIDUTURI VISHAL SIVA KUMAR
CS18BTECH11014 GOTTIMUKKULA VENKATA SATHWIK REDDY
CS18BTECH11016 JILLELA DEEPAK REDDY
CS18BTECH11018 JUPALLY SAI NISHITH
CS18BTECH11022 KARUTURI HAVYASREE
CS18BTECH11024 KONTHAM VAMSHI KRISHNA REDDY
CS18BTECH11025 KUCHI SAIKRISHNA
CS18BTECH11033 NAVUDU JYOTHI SWAROOP
CS18BTECH11034 PERUGU SAISRIKAR
CS18BTECH11035 PONNAM SAI VARSHITTHA
CS18BTECH11037 PULIKONDA VENKATA ASISH
CS18BTECH11044 THOTA SANDEEP KUMAR
EE17BTECH11005 AMBALLA AVINASH

1. Basic Bioreactor considerations for SSF

Efficient performance of SSF bioreactors will only be achieved through:

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

The best criterion to use is the economic performance of the process while comparing different bioreactors and different operating conditions in order to be able to end up with the best system possible for my particular process. In fact, at present the only way to compare the economic performance of bioreactors would be to build and operate a full-scale version of each bioreactor and record their capital and operating costs. In the absence of sufficient information about the economics of SSF processes, the aim should then be to maximize the productivity of the bioreactor, in terms of product formation, which might be biomass or a metabolite. In other words, the criterion is the rate of production in kg of product per m^3 of bioreactor volume.

General Questions :

1. **To what degree is the microorganism, or the desired form of the final product, affected deleteriously by agitation**

   Bioreactors can either be completely static, intermittently agitated, or continuously agitated. Frequent or continuous agitation would be desirable if it were tolerated, because it aids bulk transport of heat and O2 , improving the ability to control the conditions within the bed. Further, evaporative cooling of the bed can dry it out to water activities that restrict growth, meaning that it is often desirable to add water during the fermentation. It is only feasible to add

water while the bed is being mixed. However, agitation can also affect the process deleteriously. It may damage hyphae in fungal-based processes, which might adversely affect growth and product formation. Conversely, it may be desired that the final product be knitted together by fungal hyphae, such as in the production of a fermented food, and this would be prevented by agitation. Beyond this, agitation can crush substrate particles if they do not have sufficient mechanical strength or can cause sticky particles to agglomerate, in either case producing a paste in which O2 transfer is greatly hindered. Unfortunately, the balance between positive and negative effects of agitation has not been well characterized. It will be necessary to undertake your own studies at laboratory-scale in which the performance of agitated and non-agitated fermentations is compared, with both being forcefully aerated in order to minimize transport limitations, thereby isolating agitation as the factor responsible for any differences.

2. **How fast does the organism grow and how sensitive is it, and product formation by it, to increases in temperature**

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

3. **What are the aeration requirements of the system**

   The majority of SSF processes involve aerobic growth. There are essentially two aeration options in SSF processes. One is to circulate air around the bed, but not to blow air forcefully through it. The other is to blow air forcefully through the bed. Agitation can influence the efficiency with which fresh air is delivered to the substrate particles. Note that in forcefully aerated beds the air phase plays an important role in heat removal. In fact aeration rates are typically governed by

heat removal considerations since the air flow rates required for adequate heat removal are usually more than sufficient to avoid limitations in the supply of O2 to the particle surface.

4. **The degree to which sterile operation is required**

Some SSF processes involve fast-growing organisms growing under conditions of low moisture that give the process organism a competitive advantage over contaminants. For example, in many fungal processes, the water activity is below that which is optimal for bacteria, so there are not serious problems with growth of bacterial contaminants, although fungal contaminants might cause problems. It may be possible to operate without strict asepsis. The process organism might be given sufficient advantage over any contaminants through cooking of the substrate, avoidance of gross contaminations, and the provision of a relatively pure and vigorous inoculum. However, in other cases the organism grows slowly and care must be taken to design the bioreactor for sterile operation and to operate it in such a manner as to prevent contamination. In this case it is necessary to sterilize the bioreactor before operation, to properly seal openings, to filter the inlet air and to add solutions to the bioreactor during the fermentation in an aseptic manner. The various bioreactors that have been used to date differ with respect to their ability to operate aseptically.

5. **The degree to which containment of the process organism is required**

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

6. **The desirability of continuous operation**

Continuous operation in a well-mixed bioreactor is not a useful option for SSF. In SLF the nutrients added to a continuous stirred tank reactor are

distributed throughout the bioreactor, becoming available to all the microorganisms. In SSF, any solid particles added to the fermentation would need to be colonized, a process that would take a significant period of time. Even if the particles were inoculated at the time of addition, early growth might be expected to be slow, especially in a mixed bed, and an unduly high fraction of poorly colonized substrate particles would leave in the outflow. However, continuous operation of the "plug-flow type" certainly is an option.

7. **The ease of loading and unloading and the cost of labor**

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

8. **The amount of substrate to be fermented**

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

9. **Involvement of the bioreactor in downstream processing steps**

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

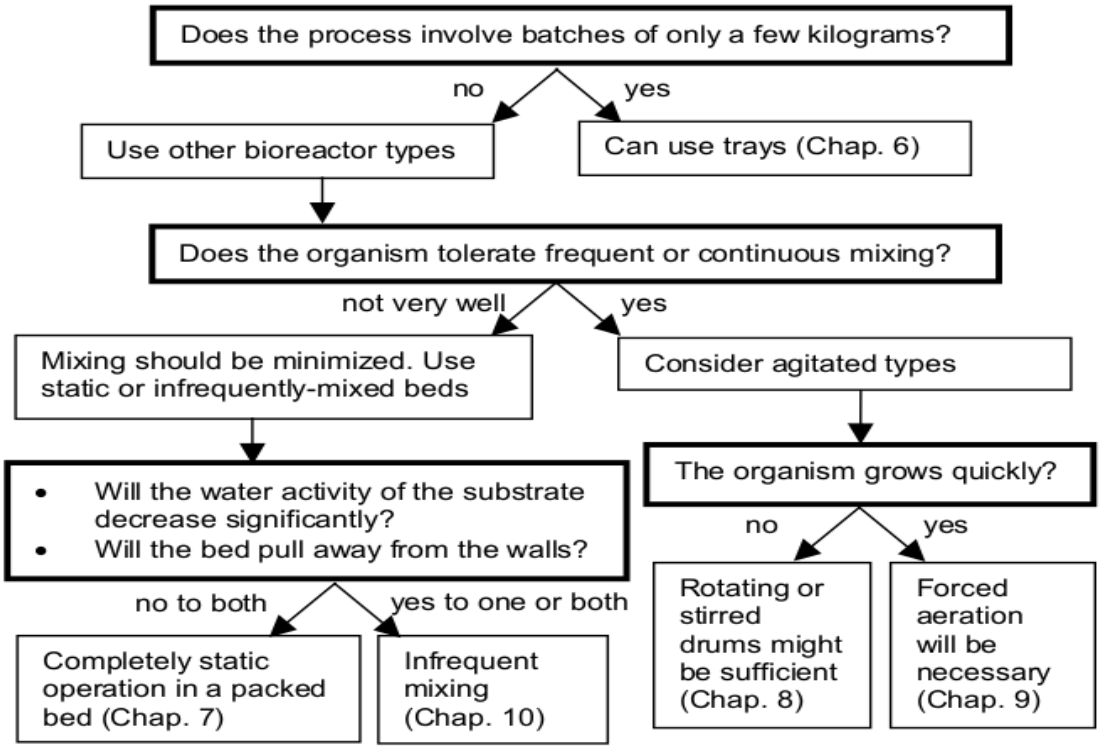


Figure : A suggested key for SSF bioreactor selection

2. Design criteria for SSF Bioreactors :

SSF bioreactor design greatly depends on the solid substrate. The bioreactor is the core of the biological process.

Materials of construction :-

The material of construction should be mechanically strong, non-toxic, corrosion resistant and less cost like Stainless Steel, Mild Steel

a) Microorganisms and culture conditions:

The strains of Aspergillus awamori and Aspergillus oryzae were refined and safeguarded in a strong sporulation medium containing 5% (w/v) entire wheat flour and 2% (w/v) agar (Sigma-Aldrich). The strains were enacted in sanitized media and brooded more than 7 days at 32 °C, at

that point protected at 4 °C. They were sub-refined on a period timespan 2 months.

b) Fungal spores for inoculum preparation:

The spores were washed by softly rejecting with a wire circle in 10.0 mL of clean 0.1% (v/v) Tween 80. Of the spore suspension, 0.5 mL was additionally moved onto the outside of 100.0 mL of a similar sporulation medium in a 500.0-mL Erlenmeyer flagon and hatched for an additional 7 days at 30 °C. After the incubation time frame, 50.0 mL of clean 0.1% (v/v) Tween 80 arrangement and a few sterile glass dots (4 mm distance across) were added to the flagon. The spores were suspended by shaking the jar delicately and gathered in one container as a spore suspension. The convergence of the spore suspension was estimated by haemocytometer.

c) Solid substrate : The substrates were kept in an impermeable compartment and put away in a room at 4 °C to use later. Wheat grain is a minimal effort buildup of the processing business, a fascinating strong substrate for SSF and was utilized, with no treatment, as a strong mode for growing A. awamori and A. oryzae. Wheat grain might be viewed as a model of modest and bountiful farming waste and have potential in creating the whole SSF process.

d) Substrate preparation:

To start with, 12.0 g wheat grain was gauged and set into isolated 250-mL jars prior to being sanitized at 121 °C for 15 min. The substrates were permitted to cool at room temperature prior to inoculating with A. awamori and A. oryzae spores and being soaked with a measure of sterile refined water to get the moisture content required for each experiment. About 1.2 × 106 spores/g substrate was immunized into the container and blended well in with a sterile spatula under aseptic conditions to consistently appropriate the spores inside the substrate.

Oxygen supply :-

Adequate supply of oxygen is required to maintain aerobic conditions. In the entire fermentation process, nothing is added into the bioreactor except oxygen (O_2)
In aerobic SmF cultivations, the oxygen supply is often the limiting factor for growth, due to low solubility of oxygen in water, while in SSF processes there is free access to atmospheric oxygen.
Oxygen supply is promoted by Mixing and aeration.

Due to low oxygen solubility in the medium, the oxygen supply or oxygen transfer rate (OTR) required may become limiting at higher cell concentrations. Insufficient oxygen supply at higher cell concentrations is mainly due to the gentle mixing and aeration required, and the resulting low mass transfer coefficients. This problem is solved by adding pure oxygen to the inlet gas stream, causing a few-fold increase in oxygen supply to be achieved.

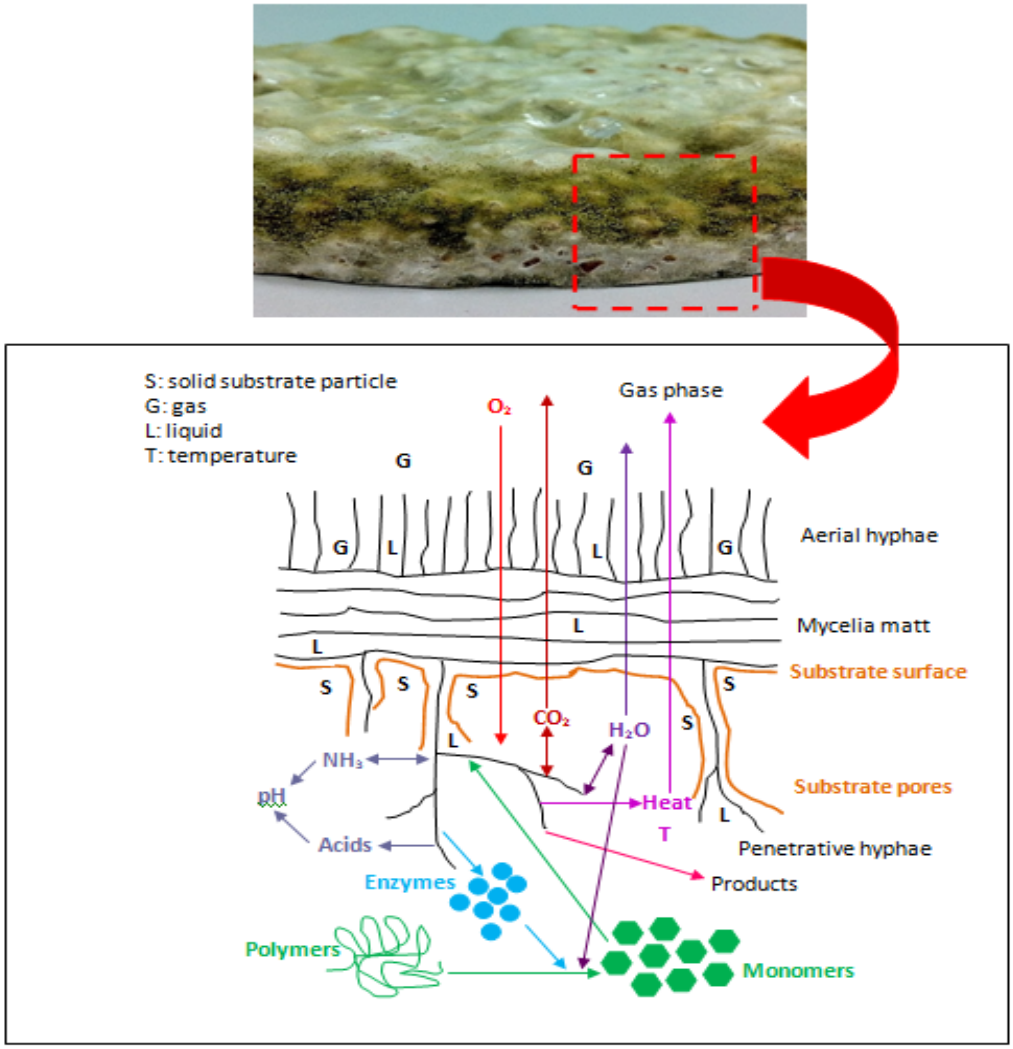
Mixing of media :-

Mixing is difficult or impossible, some microorganisms are sensitive to mixing or agitation and the growth of microorganisms is restricted by nutrient diffusion.
Mixing greatly promotes heat removal by bringing the medium into contact with the cooling surfaces within the bioreactor. However, typically mixing must be minimized in SSF bioreactors, for several reasons:

- Firstly, it requires higher energy inputs to mix the bed of solid particles within an SSF bioreactor than to mix the liquid medium in an SLF bioreactor. Secondly, the presence of internal heat transfer surfaces such as plates or coils within the bioreactor will interfere much more with the mixing of a solid bed than it will with the mixing of a liquid medium.
- Finally, a liquid medium can be mixed reasonably well without causing undue shear forces, whereas in a bed of solids in an SSF process involving a fungus, even the slightest mixing action will cause significant physical damage to the mycelium growing at the particle surface

Micro-scale Phenomenon :-

They are of great interest because they are a major disadvantage of SSF. The below figure shows the schematic of some micro-scale phenomena that happens in a SSF (SSF has 3 major phases: which are the solid, gas and liquid phase.



Such stages make the system very complex and varied. Following this theory, microorganisms have restricted access to solid substrate nutrients, oxygen, water, enzymes, and also limited removal of carbon dioxide and heat. Because of these limitations, this will lead to poor growth, reduced bioreaction rates, low productivity and poor bioreactor system efficiency. In this situation, where SSF involves the use of fungi, mass transfers in micro-scale phenomena can be classified into four categories:

1. **Intra-particle mass transfer :-**
   Inter-particle mass transfer requires oxygen transfer from the void fraction inside the solid substrate particles to the developing fungus. A very important function is played by internal oxygen concentration. In the solid substrate particle surface, oxygen first passes through the actively respiring biomass and then diffuses through the liquid phase of the substrate.
2. **Inter-particle mass transfer :-**
   The transfer of nutrients and enzymes within solid substrate particles requires intra-particle mass transfer. Here the key problem is the oxygen diffusion needed in metabolic respiration. Oxygen is consumed and there is the production of carbon dioxide, water, heat and other things. In addition, enzymes, other polymers and secondary metabolites are formed by the substrate that contains the biomass.
3. **Heat transfer :-**
   A high amount of metabolic heat is created during SSF. The quantity of heat depends on the microorganism's metabolic activity levels. Due to the solid substrate being a strong thermal conductor, heat builds up in the fermentation medium. Heat transfer into or out of the SSF system is closely related to the metabolic operation of the microorganism as well as the aeration of the fermentation system. For temperature control, heat transfer is required during SSF. High temperatures have a detrimental effect on growth and product formation. For growth and biochemical reactions, low temperatures are unfavourable. In the SSF method, the high moisture content makes it difficult to achieve successful heat transfer.
4. **Water transfer :-**
   There are 4 factors within the system during SSF that aid status and water balance. These include the amount of water in the hydrolysis step, the production of metabolic water, the absorption of intracellular water during the production of biomass, and the production of metabolic heat that causes water evaporation.
   To avoid poor SSF growth, it is necessary to maintain the water content at an optimal level . The first feature is due to dissolved solutes and capillary forces are

due to the second one. Monitoring the behaviour of water or the quality of moisture and concentration of solvent may help to maintain the water potential at the correct level for SSF

Macro-scale Phenomena:-

Macro-scale phenomena explain how efficient the SSF bioreactor system's design and operation strategy is and how they impact conditions in the local environment of the microorganism(such as provision for aeration, mixing or agitation and heat removal). 6 macro-scale phenomena refer to several situations and issues faced by SSF bioreactor systems.

1. How well bulk transport, especially oxygen, carbon dioxide, water and heat, occurs can be influenced by the movement of air into and out of the bioreactor. Usually, depending on the bioreactor type, it occurs in the headspace or interparticle spaces.
2. This can affect how efficient bulk transport is, especially inter-particle transport, if the bioreactor is operated with forced aeration. Dry forced air can result in significant losses of moisture and solid substrate drying.
3. Two major requirements need to be taken into account when running the bioreactor with agitation or mixing. Next, shear stress must be dealt with by the solid substrate particles and must not coagulate after agitation or mixing. Second, shear stress should be able to cope with the microorganisms, or it could be impaired by mixing or agitation.
4. As a result of natural conduction, convection, and diffusion, bulk transport may occur.
5. Via conduction across bioreactor walls to the atmosphere, bulk heat transport can occur.
6. Convective cooling can also occur through the surrounding walls of the bioreactor.
7. During fermentation, the physical properties of the solid substrate, such as density, porosity (void space) and stickiness, alter. Also, until it is moved from the gas phase into the liquid phase within the molecule, oxygen can only pass through diffusion.

3. Types of bioreactors:
Tray, Packed bed, Rotating drum, Air pressure pulsation and intermittent or continuously mixed reactors

Tray bioreactor:

Tray bioreactors in conventional SSF have traditionally been widely used. The use of trays is the oldest system; they are simple in nature and use unmixed beds without forced aeration of the solid substrate in static conditions. The fermentation takes place without mechanical agitation in stationary trays. To maintain the solid support, the bottom of the plate is perforated with mesh to allow natural aeration. This type of system includes only a small amount of fermentation substrate. Since only thin layers are needed to prevent overheating and to keep aerobic conditions. Alcântara et al. and Vaseghi et al. found that the substrate thickness, surface area and temperature of the chamber have had a beneficial impact on the enzyme activity and could enhance the metabolic heat and gas transfer. Solid substrates can vary in thickness. In the incubating area, where temperature and humidity are monitored for optimal development, trays are usually placed. Trays with sufficient gaps are stacked above each other. Zhang Chen et al. analysed the effects on the tray bio reactivity of two dynamic air shifts (including air pressure pulsations and air movement) and observed changes in temperature gradient.22 Zhang Chen et al. issued that the results produced a positive internal circulation of air, speeding up the heat transfer between the surface of the substrate and external air. In addition the column tray bioreactors with forced aeration were engineered by Ruiza et al. and Assamoi et Al. This allowed better control of the atmosphere in the bed due to temperature modulation and air flow speeds.

Figure 1 shows general schematic multiple tray fermenters.

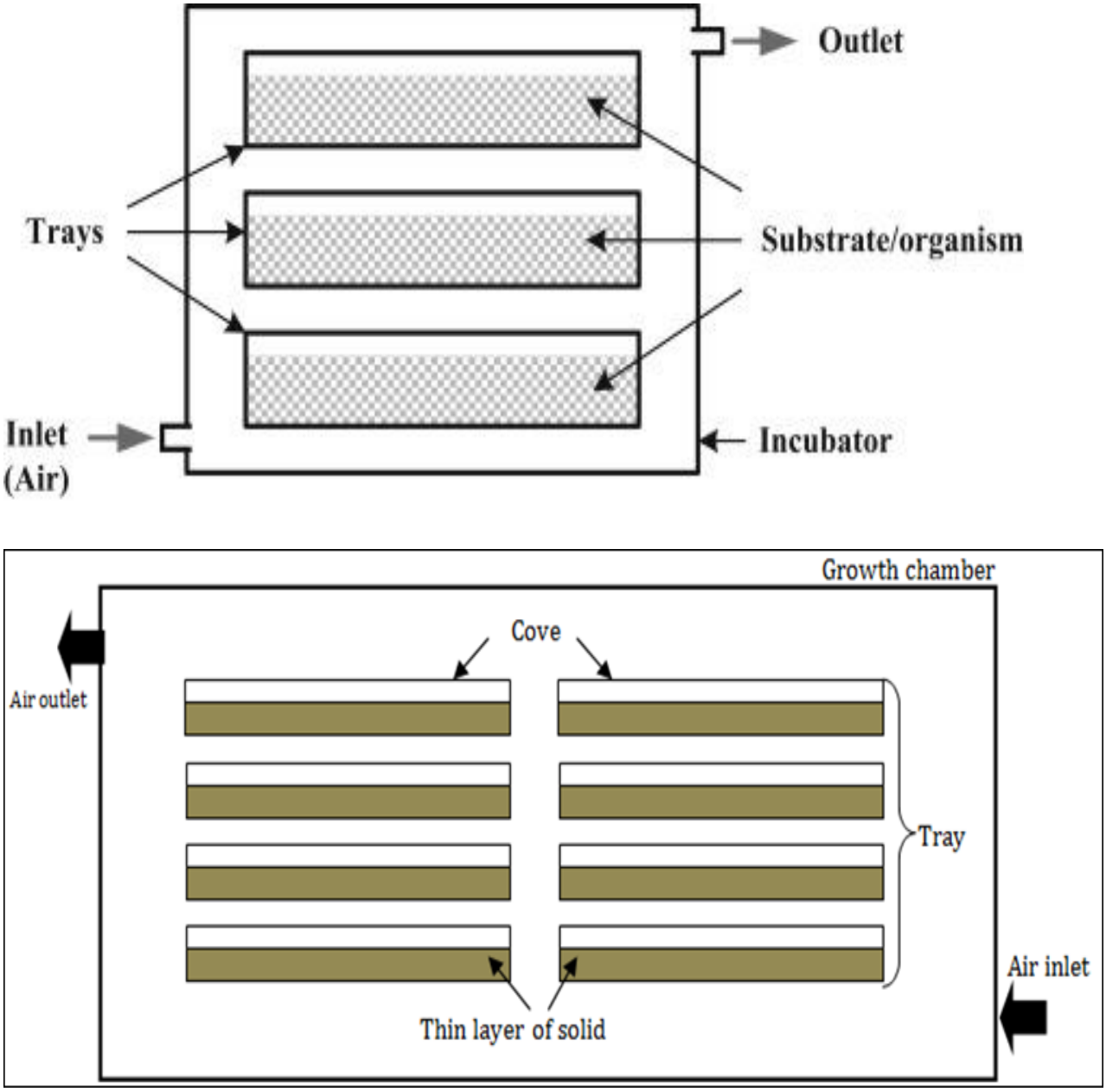


Fig 1: Schematic of tray fermentation bioreactor

Packed Bed Bioreactor:

Packed-bed bioreactors are designed from columns of glass or plastic and are composed of unmixed perforated base beds.Forced aeration is applied to the column's bottom. These systems are important for the production of products with efficient process controls, particularly for the removal of heat. In contrast to forced dry air, forced aeration using moistened air enhances fermented bed moisture gradients and temperature regulation. However, not all the heat produced during the fermentation process is removed, according to Gutiérrez-Rojas et al. They proposed an injection of cool-dry air and the replacement of moisture at various points in the packed-bed to accomplish this. Salum et al. cultivated Burkholderia cepacia LTEB11 with a mixture of sunflower seed meal and sugarcane bagasse.They found that fermentation solids could be used in a fixed packed-bed bioreactor to catalyse the ethanolysis of soybean oil to produce biodiesel (a co-solvent-free system). This method does not require costly processing steps, such as enzyme recovery and immobilisation and separation of co-solvents. It is very effective and has the ability to reduce costs through the synthesis of enzyme-catalysed biodiesel.

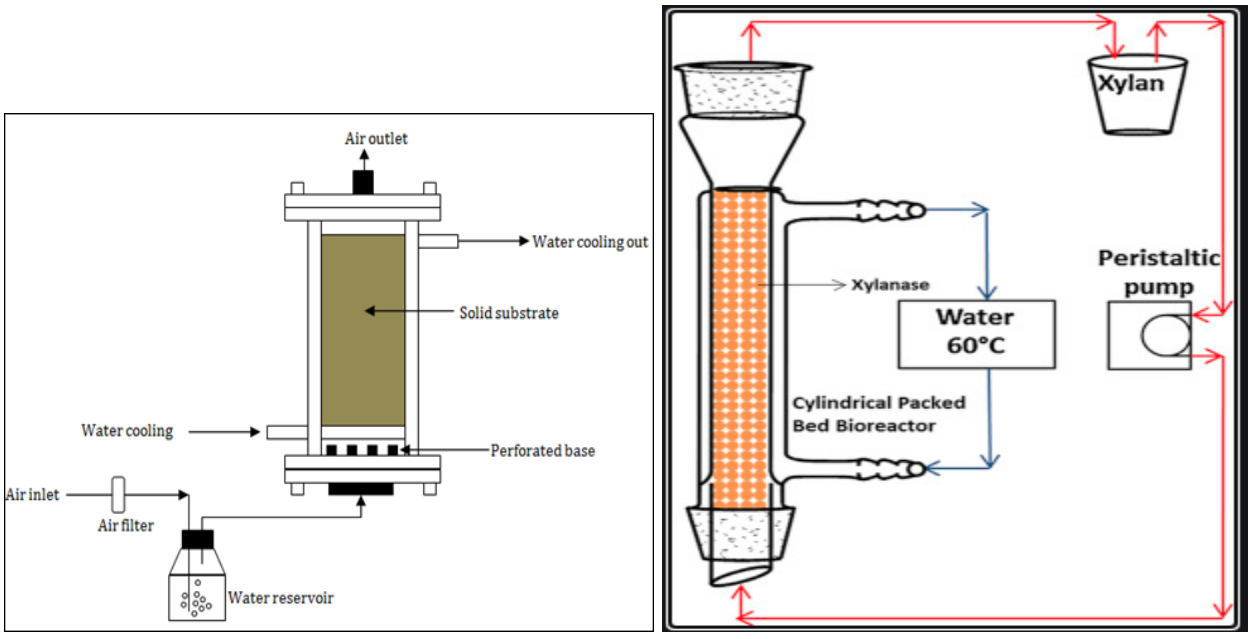


Fig 2: Schematic packed-bed fermenter in the dairy industry.

Rotating Drum Bioreactor:

Rotating drum bioreactors, working in continuous or semi-continuous mode, mix intermittently without forced aeration. A horizontal cylinder is a rotating drum bioreactor. A bed of substrate is semi-filled with the drum. It is not possible for the fermented bed to be too large and this creates effective transport of oxygen and carbon dioxide. Nava et al. on a small scale found that temperature regulation is very difficult in drum bioreactors. For various microorganisms, solid substrates are combined differently. Mixing could be continuous, mixed or intermittent. It prevents metabolic heat produced by the accumulation of microbial activity. The mixing effect on the solid substrate also depends on temperature regulation. Air may also be blown into the headspace as well. Also in forced aeration, conditioned air is passed through the bed. Metabolic heat is absorbed from the bed, transmitted to the atmosphere by diffusion or through the conductive wall in the headspace.The growth of microorganisms is found to be much more and less harmful to fungal mycelium during intermittent mixing. Continuous mixing could increase fungal mycelium damage, affecting the growth of microorganisms. When rising Aspergillus oryzae, Stuart and Mitchel observed the operative variables of the revolving drum bioreactor and they found that the rate of growth was declining, when rotational speed increased because of shear forces. Ali et al. suggested that there are two possible ways to address these problems:

1. Intermittently mixing and aeration into the headspace. Both mixing and aeration could control the temperature, moisture gradients, uniformity and oxygen concentrations.
2. Equipped with baffles on the inner wall.

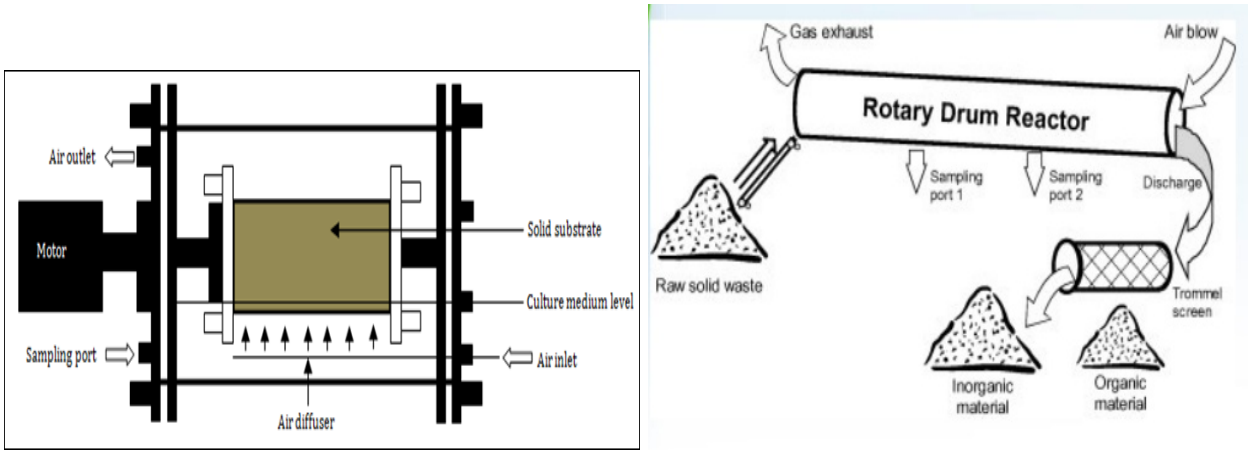


Fig 3: Schema and Example for Rotary Drum Bioreactor.

Fluidized-Bed Bioreactor:

Fluidized-bed bioreactors are usually built from a vertical chamber with a base plate perforated. Forced aeration is applied at the bottom chamber at sufficient speed to fluidize the solid substrate particles and cause mixing.There is also an agitator (clump breaker) in the bioreactor, breaking up agglomerates that can shape and settle towards the bottom. The bed is expanding, so sufficient headspace is required.The solid particle and gas mixture would behave like a liquid. This fluidized-bed bioreactor provides a good mixing behaviour of gas, solid and liquids. Evaporating water can cool the biomass. The substrate properties of gas-solid fluidized-bed bioreactors influence bioreactor effectiveness. For example, a sticky substrate will form large agglomerates (clumps) that are difficult to fluidize. In order for all to be fluidized, the solid substrates can have the same size as electrons. With size differences, some small size particles might fluidize and the large particles size might not fluidize. There is no problem with controlling the temperature and cooling the substrate bed, because high flow rates for fluidization provide a large enough convective cooling capacity and good rate of heat and mass transfer. However, mixing steps in a fluidized bed could damage the penetrative mycelium of the fungus because fungi are affected by hard force.

The design scheme of the fluidized-bed bioreactor can be seen in Figure 4.

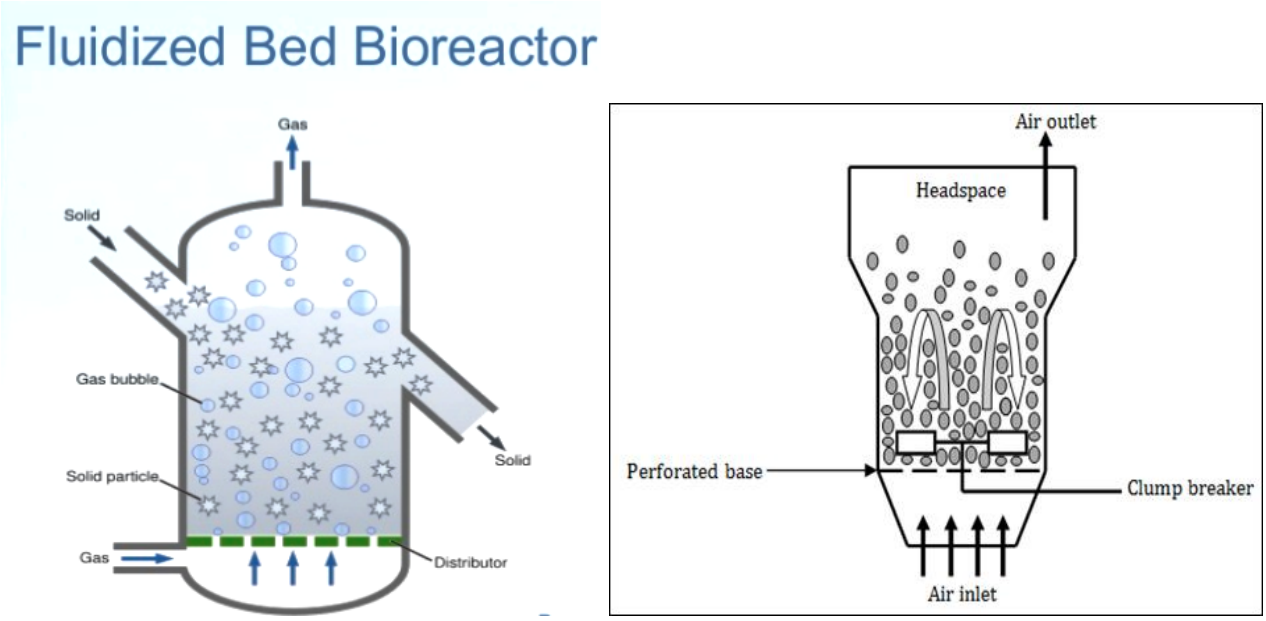


Fig 4: Fluidized-bed bioreactor

Spouted-Bed Bioreactor:

In spouted-bed bioreactors, air is only blown upwards through the central axis of the solid bed. As a consequence of this only half of the bed is fluidized and the bed can be expanded. Due to the vigorous contact between gas and solids,solids slip down the bottom of the bioreactor due to its sloped sides and solids cycle continuously. The advantage of the spouted-bed bioreactor is that it prevents particle agglomeration caused by high-speed
impacts in the spouted-bed's core region. This is sufficient for the handling of Solids that are sticky in nature, have an unusual texture or distribution of scale that can not be treated in bioreactors of fluidized beds. Spouted-bed bioreactors are also capable of treating big, coarse solid particles, differing densities and shapes associated with solid particles substrate for the SSF process. Furthermore, spouted-bed bioreactors have other advantages for mass and heat transfer. This is because stable substrates are extremely mixed in the bioreactor creating high mass and heat transfer rates.

The spouted bed bioreactor solves the problems of more common SSF carried out in tray and packed-bed bioreactors. Spouted bed bioreactor shows improved fermentation quality, including better fermentation output,product titers, yields, and productivity. When a spouted-bed bioreactor is studied with intermittent spouting with air, it achieves high production levels of both total protein and enzymes. The result was similar to a packed-bed bioreactor, but the spouted-bed had uniformity and had no issues with solids-handling. It was noticed, however the continual spouting was found to be unfavourable to this SSF, possibly because of shear impact damage to fungal mycelium during spouting.

The design scheme of the lab-scale spouted-bed bioreactor is illustrated in Figure 5.

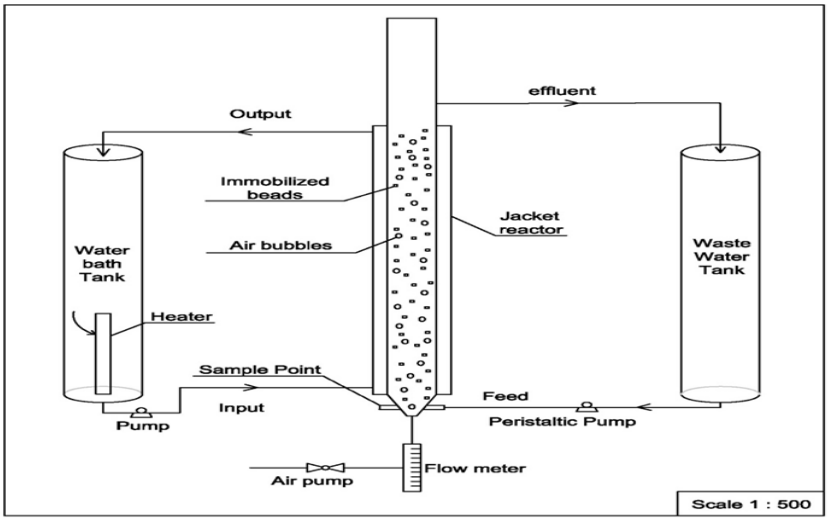


Fig 5: Lab-scale Spouted-bed bioreactor

4. SCALE-UP OF SSF BIOREACTORS:

Solid-state fermentation (SSF) systems, involving the growth of microorganísms on the moist substrate in the absence of free water, simulates the fermentatíon reactions occurring ín nature. The humid Solid substrates that are naturally polymeric and insoluble in water serve as sources of carbon, phosphorus, minerals, water, and other nutrients to provide the microorganisms with anchorage.

A closer exarninatíon of SSF processes in recent years in several research centers throughout the world has led to the realization of numerous economic and practical advantages of SSF.SSF has also been applied to bacteria and yeast cultures, and a wide range of products are being explored, íncludíng gibberellic acid, bacterial thermostable alpha-amylase, cheese flavor ethanol, tetracycline, penicillin, and the upgrading of straw.

The Importance of Scale-up exercise:

Scale-up has been scientifically described by numerous staff in several ways. Scale-up is not only a one-way mechanism involving structures of smaller to larger sizes. It also applies the opposite, usually referred to as scale-down. The latter is also essential for obtaining additional data in less-expensive ways of conducting procedures and is characterized by simplicity and effectiveness. Microbial fermentation process development is a multi-disciplinary activity involving microbiologists, biochemists, organic chemists, entomologists, and engineers with special biochemical specialization in chemical, mechanical, civil, and environmental disciplines.

Therefore, scale-up is the key link in converting a laboratory scale process to a commercial production scale. It also provides a large amount of the product that may be needed to assess the product and toxicological studies. Unsuccessful scale-up results in unnecessary time spent on laboratory-scale cost-intensive

work and also forces the withdrawal of prospects thought earlier to be potentially profitable.

Peculiarities in Scale-up exercise:

Scale-up exercises are unique to procedures. At the beginning of the work, nothing will be known about the peculiarities of a particular method, and the process may fail at the pilot plant level but work well at the production scale. Due to reverse scale effects, such peculiarities may have resulted in the loss of a variety of potentially useful processes. In other instances, the scale-up method could not demonstrate any particularity, and performance can be accomplished in a straightforward way. It is generally accepted that the design of a commercial-scale bioreactor cannot be accomplished solely by a purely theoretical approach. This may explain the relative abundance of studies on the quantitative understanding of the scale-up aspects of theoretical and chemical engineering, which, due to the basic intrinsic nature of the physicochemical phenomena, have progressed more quickly. The method of fermentation, which can be scaled up without any difficulty, is an unusual phenomenon, possibly due to the enormous increase in the volume or bulk of the medium, often 250-1000 times.

Levels in Scale-up Exercise:

Scale-up involves a series of stages, the number of which has been a subject of considerable debate. In practice, the number of stages is generally determined by the type of the process and the earlier experiences of the team concerned. The ultimate deciding factor, however, is the sufficiency of data for effective scale-up.

For SMF processes, a system of four stages with the selection of one fermenter size for each stage has been proposed by the Bank. This also appears to be applicable to the SSF system. In the modified form, these stages are:

- **Flask level:**
  50-1000 g working capacity for the selection of the culture, the method of optimization, and the experimental variables. Within a short time and at a low cost, data collection is facilitated.

- **Laboratory fermenter level:**
  Working capacity of 5-20 kg for the selection of procedures for inoculum development, medium sterilization, aeration, agitation and downstream processing, standardization of different parameters such as the rate of transfer of oxygen, the rate of evolution of carbon dioxide, the formation of biomass, commodity biosynthesis profiles; pH impact studies, aeration-agitation rates, continuous or intermittent nutrient feeding policies, control strategies and tools selection, economic assessment of the process and its commercial feasibility

- **Pilot fermenter level:**
  50-5000 kg, primarily for confirmation of data obtained from laboratory fermenters, selection of the best inoculum technique, medium sterilization, and processing strategies downstream. It promotes product market trials, its physicochemical characterization or toxicity testing, and the determination of the viability of the process.

- **Production fermenter level:**
  25-1000 tonnes for streamlining the process, which ultimately leads to a financial return on the investments made so far on process development.

At a large scale, industrial-grade chemicals are used, leading to pre-or post-purification needs, and any single set of trials might require a long time to be unpredictably expensive. The waste management and its influence on production cost in such scenarios have also to be examined deeply in accordance with the disposal limits of authority. The typical activity of process development

work remains the pilot plant experiment, and it is useful to adopt this approach when one or more of the following reasons occur:

- The operating condition and the influence of the parameters on the process must be studied in a representative unit—the passage from laboratory scale to manufacturing.
- The passage from laboratory scale to manufacturing unit constitutes a problem in the scale-up process not possible to be solved without carrying out an experiment at the intermediate size.
- An investigation on the building of by-products, corrosion, or other long term effects is required.
- The need to obtain samples in sufficient quantities for various trials and tests emerges (i.e., to verify the possibility to use the product in novel large scale applications or to show the product(s) to customers).
- It results conveniently to convince potential customers of the value of the process showing an operating pilot plant.

The interconnections between different parts and the eventual recycle of utilities, raw materials and wastes must be examined with close attention. Usually the problem connected to recovery is omitted except if it is necessary or interesting to 24examine it directly. The utilities involved have to be representative and easy to be used, so conventionally is preferable to use electricity instead of steam or fuel, and water instead of air. The scale-up problem becomes more difficult when we realize that this discussion has not explored all the potential problems and complications. Some further considerations are:

- in mixed beds, the efficiency of mixing is likely to decrease with scale;
- In some beds both convection and conduction play important roles in heat removal.

The optimum combination of these two mechanisms may change with scale. For example, in some cases conduction plays an important role in removal at small

scale, but its contribution decreases as scale increases as the surface area to volume ratio of the bioreactor decreases.

- bioreactor design will affect the ease of substrate handling, and ease of substrate
- handling may be an important consideration in the economics of the process,especially in relation to the need for manual labor.
- pressure drop and fluidization considerations may put a limit on possible airflow rates
- sensitivity of the microorganism to damage by mixing may put a limit on the frequency with which the bed can be mixed;increases in bed heights may have side effects, such as the deformation of particles at the bottom of the bed, affecting interparticle void fractions, or even crushing the particles.Given this complexity, we are only likely to achieve the maximum possible efficiency in large-scale bioreactors if we understand the phenomena that combine
- to control bioreactor performance and if we use quantitative approaches to the scale-up problem.

5. CHALLENGES IN DESIGN OF SSF BIOREACTORS

Agitation:

This is one of the most important parameters, especially in aerobic fermentation, as it ensures a homogeneous temperature and gaseous atmosphere and provides an interfacial gas-liquid region for the conversion of gas to liquid as well as liquid to gas. Agitation also facilitates the transfer of surface mass and heat and the uniform distribution of nutrients that are introduced incrementally during fermentation. It must be stressed that in many aerobic SSF procedures, such as tray fermentations performed in static reactors, agitation is not used.

Heat Removal:

For an SSF Bioreactor, heat removal is a major challenge. It is more difficult to remove waste metabolic heat from a bed of solids(where the inter-particle phase is occupied by air) than from a continuous aqueous phase. This is due to

- More thermal conductivity and heat capacity(thermal properties of continuous aqueous state) of the liquid water than the bed of moist solids with inter-particle air.
- In general, heat removal can be done by mixing it greatly, where the medium gets in contact with the cooler objects within the bioreactor. But in SSF bioreactor, the mixing should be minimized due to
    - The requirement of higher energy input to mix the bed of solid particles in SSF bioreactor than to mix the liquid medium of SLF bioreactor.
    - The presence of internal heat transfer surfaces such as plates or coils within the bioreactor will interfere much more with the mixing of a solid bed as in SSF than it will with the mixing of a liquid medium as in SLF
    - A liquid medium can be mixed reasonably well without causing undue shear forces, whereas in a bed of solids in an SSF process involving a fungus, even the slightest mixing action will cause significant physical damage to the mycelium growing at the particle surface

Aeration:

The aeration requirement is fulfilled on a laboratory scale by stirring the culture flask, while forced aeration is used in large-scale fermentations. Not only does aeration contain oxygen, but it also extracts carbon dioxide, other volatile metabolites and heat from the fermenter at the same time. Thus the rate of aeration is determined by factors such as the microorganism's growth requirements, the development of gaseous and volatile metabolites and the evolution of heat.

pH Control:

It is difficult to measure and regulate the pH in the SSF system because in the absence of free water, there are no pH electrodes capable of measuring the pH of moist solids. In static SSF systems, mixing small amounts of acid or alkali with the bulk of the solids would also be extremely problematic.

Medium Sterilization:

There are many issues with medium sterilization on a wide scale, such as temperature profiles, physicochemical changes in the medium, thermal degradation of critical nutrients, toxic compound formation and nutrient damage due to the impact of scale factors.

Large Scale Inoculum Development:

In most fermentation processes, the inoculum is typically used at a high ratio for the production of secondary metabolites, with the goal of achieving the desired product level in a short period of time. Similarly, most SSF processes require the use of a high inoculum ratio, but the goal is to avoid contamination during fermentation in this case. As a result, the processing of inoculum in large quantities becomes a separate unit process in large-scale fermentations and is typically accomplished by the use of a series of capacity-increasing inoculum fermenters. This in fact, needs some changes to the medium of growth and the cultural parameters. Test tubes, petri dishes and culture bottles, for example, used on a laboratory scale for the growth of inocula on the surface of agar media, are practicable for the development of inoculum on a larger scale and suitable liquid crops are used. In cases where the inoculum is grown in a liquid medium on a laboratory scale, the same composition of the liquid medium can be used in larger fermenters, but adjustments such as

agitation and aeration of the medium are necessary. The metabolic condition of the inoculum cells as a result.

Bioreactor Design:

The difficulty of heat removal from large-scale SSF bioreactors has two consequences for bioreactor design:

- Evaporation may occur as a result of temperature rises in the bed, and in some cases, it may in fact be promoted deliberately, given that it is one of the most effective heat removal mechanisms. However, continued evaporation can dry the bed out to water activities low enough to restrict growth. Therefore the maintenance of the water activity of the bed becomes a consideration that guides design and operation.
- Given that in many SSF bioreactors the air phase plays a central role in heat removal and that the aeration rates needed in order to remove heat at a reasonable rate are more than sufficient to ensure a reasonable O_2 supply to the surface of the particles, O_2 supply is typically a minor consideration (except for Group I bioreactors, i.e., static beds without forced aeration).

Bioreactor design would be simple if all you needed to do was to obtain good performance in a laboratory-scale bioreactor and then simply construct a geometrically identical larger version of this bioreactor. However, this is impossible to achieve.

- The aim of the bioreactor is to control the conditions within the bed, such as the temperature and water activity, at the optimum values for growth and product formation.
- However, the growth of the organism causes deviations from the optimum conditions in its immediate surroundings, through the release of waste metabolic heat and the consumption of O_2, amongst other processes.
- In operating a bioreactor, we are limited to manipulating external operating variables.
- The effects of the operating variables on the conditions within the bioreactor,

such as the bed temperature, are not direct. Between the manipulation that we make in the operating variable (for example, changing the temperature at which the air enters a forcefully aerated bioreactor) and any particular position in the bed, we have various transport phenomena. For example, to arrive at mid-height within a packed-bed bioreactor, the inlet air firstly has to pass through half of the bed, and the temperature of that air will have risen from the inlet value by the time it reaches the middle of the bed, due to the heat transfer that occurred over the intervening distance. This will decrease its ability to cool the middle of the bed (in fact, this phenomenon is the basis of the axial temperature profile for the forced aeration of static beds)

- The importance of these transport phenomena increases as the distance over which transport must occur increases. This distance typically increases as the size of the bioreactor increases.

Condition in Local Environment:

It is important to understand that the conditions in the local environment depend on the balance between the changes caused by the microorganism and the transport phenomena that arise to counteract these changes. For example, the local temperature sensed by the organism (and which will affect its growth) depends on the balance between the rate of waste metabolic heat production and the rate of conduction of energy away to regions in which the temperature is lower. If the rate of waste heat production is higher than the rate of conduction, then the local temperature will rise, which of course occurs during the early periods of the fermentation when the growth rate is accelerating.

The temperature in the local environment of the organism depends on the balance between heat generation and heat removal. This example is given in the context of a fermentation carried out within a tray, where the main heat removal mechanism in the bed is conduction. The “local environment” of interest is at mid-height in the bed.

- Whether the temperature in the local environment remains constant,

increases or decreases will depend on the balance between the rate of metabolic heat production (which is proportional to the growth rate) and the rate of heat removal by conduction to the bed surface (which is proportional to the temperature gradient across the substrate bed).

- Due to the change in the rate of production of waste metabolic heat as the growth rate changes, the temperature in the local environment changes over time. During early growth the rate of waste heat production increases. This causes the temperature to increase until the rate of heat removal once again equals the rate of heat production. However, since growth continues to accelerate, the rate of heat production continues to rise, so the local temperature must continue to rise in order to continue to increase heat removal. Later during growth, as the growth rate and therefore the rate of heat production decreases, the local temperature decreases.

CONCLUSION:

In countries whose biotechnological potential abilities depend not only on the reuse of agro-industrial residues but also value addition to the agriculture commodities like palm kernel cake, palm tree wastes, forest wastes, tea wastes, tapioca, and sago wastes, fruit waste, municipal solid waste, etc. are produced in huge quantities annually, which have low value in the market. SSF could be perfected for value-addition and utilization of these products and their residues to boost the economy of the nation. Hence the research on SSF to develop commercial processes with techno-economic feasibility is worth continuing. The intricacies in solid-state fermentation technology are to be understood clearly through modeling, the kinetics of growth of microbes, control of parameters, etc, and finally, scale-up and commercialization of SSF processes are essential for establishing the SSF technologies to apply in divergent areas.

REFERENCES:

1. An overview of Engineering Aspects of Solid State Fermentation
2. Design aspects of SSF as applied to microbial bioprocessing
3. Operating characteristics of SSF bioreactor with air pressure pulsation

4. Design aspects of SSF as applied to microbial bioprocessing
5. Bioreactors in solid state fermentation technology: Design, applications and engineering aspects