FERMENTORS: TYPES, FUNCTIONS, DESIGN AND CONTROL

23.1 Introduction

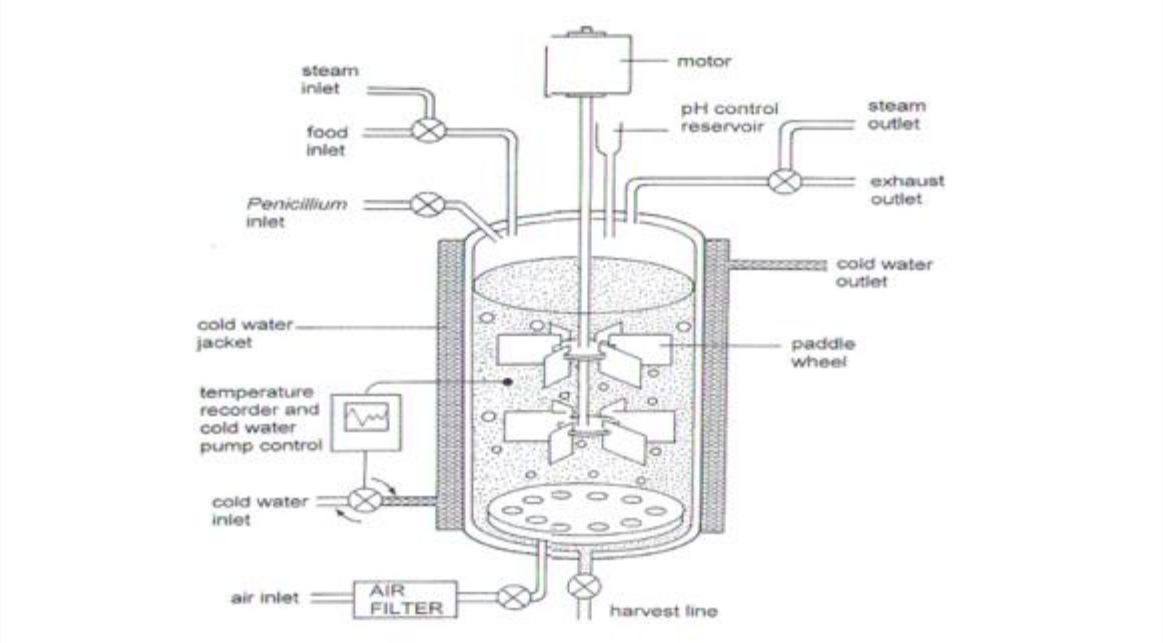
The reliable operation of a fermentation system to achieve process objectives depends on two factors: the fermentor design and the fermentation process.

The design should consider from the outset such factors as plant scheduling, space constraints, relationships between fermentor productivity and throughput rates of downstream equipment, utilities requirements, overall labor requirements and operating versus capital costs. Consistency, safety, cost, and compliance with statutory requirements usually are of prime concern for production equipment.

23.2 Fermentor and Bioreactor

In recent times, however, a fermentor is simply an optimal environment for bacteria and / or fungi to grow in, and the cultivation of said organisms will yield a desirable substance.

A bioreactor is a vessel in which is carried out a chemical process which involves organisms or biochemically active substances derived from such organisms. Bioreactors are commonly cylindrical, ranging in size from some liter to cube meters, and are often made of stainless steel. In brief bioreactor can be considered as a large scale operation whose volume/capacity ranges to several litres. Bioreactor is a system used for the growth and maintenance of a population of mammalian or insect cells whereas Fermentor is a system used for the growth and maintenance of a population of bacterial or fungal cells.



23.2.1 Bioreactor configuration

23.2.1.1 Design criteria

Reactor design and scale up considerations are driven by the need to provide the organism optimal conditions for producing the desired product uniformly in the reactor.

a) Mechanical aspects

Mechanical design aspects are important for the successful operation of any fermentation

plant. Some practical aspects of vessel design are:

1. Space requirements: The vessel dimensions must be chosen to meet plant space limitations. Poor choice of equipment sizes can cause inordinately high building costs.

2. Transportation: Shop built vessels are usually less expensive and of higher quality than field built vessels.

3. Special heads: A hemispherical bottom gives better mixing and fewer shears than does a standard dished head.

b) Process aspects

The fermentation process guidelines commonly employed without proper consideration of the process aspects do very little to promote good design. These include:

1. Aeration rate: The airflow rate to the fermentor must be generally one volume of air per liquid volume per minute (1 vvm).
2. Impeller tip speed: The tip speed of a fermentor impeller must not exceed 7.6 m/s.
3. Arrest of fermentative metabolism: At the end of fermentation, the fermentor broth must be cooled immediately and stored at 4°C, to arrest the fermentative metabolism.
4. The maximum production rate cell mass theory: Process optimization is achieved by obtaining very high cell mass concentrations at very high growth rates.
5. Oxygen transfer rate: The consequences of increasing OTR by increasing air flow rate and agitation could lead to foaming, increased gas holdup, higher gas velocities, higher vessel pressure, and oxygen enrichment.
6. Heat transfer rate: Heat transfer usually is the limiting constraint for highly aerobic large scale fermentors.

c) Jackets and coils

Jackets are used for the circulation of steam and cooling water during the heating and cooling cycles of sterilization of the fermentor. Since microbial reactions are exothermic, the heat produced during fermentations leads to a rise in the temperature of the broth, necessitating the need to maintain the temperature at the optimal value. Normally, steam is used as the heating fluid; and water, chilled water, or chilled brine is used as cooling fluids. The contact surface area of the jacket with the fermentor should be maximal and the pressure drop of the circulating fluids in the jacket should be minimal for better process performance.

d) Safety codes

The vessel must be fabricated in accordance with the Standard Code for unfired pressure vessels and tested at design conditions to insure that the vessel can withstand all forces generated under the specified operating conditions. If operation is required at high operating pressures, one should consider ways to minimize the metal thickness to allow the use of cold rolled sheet rather than plate. This results in better heat transfer, a better interior finish, and a lower price.

e) Material of construction

Stainless steel is the more commonly employed material for the fabrication of biotechnology equipment. The selection of the right steel quality in biotechnology is based on a compromise between material costs, availability, and the physical and chemical requirements of the process. The low carbon steels, SS 304L and SS 316L, are known worldwide as standard steels. Generally, vessels used in biological processes are fabricated with 316 or 316L steel. The vessels widely used in food technology or harvest storage tanks are fabricated with a cheaper and less corrosion resistant steel of grade 304 or 304L.

The selection of a vessel material for fabrication should take into consideration:

1. Sensitivity of the organism, particularly eukaryotic cells
2. Extent of vessel corrosion on exposure to fermentation media and utilities
3. Aseptic operation requires use of SS316, SS316L, SS304, or SS304L.

f) Baffles

Baffles are usually welded to the interior of the vessel. Baffles usually take the form of metal strips, roughly one tenth of the vessel diameter in width, extending vertically down the height of the vessel and attached radially to the wall. Removable baffles mean unsealed joints. Baffles should be set off from the wall, to minimize solids build up and to simplify cleaning. Slots between the baffle and the vessel wall prevent the formation of dead spots. The provision of baffles increases the turbulence in the liquid and results in a more efficient utilization of power.

g) Sparger

A device for introducing air below the liquid level in the fermentor vessel is called a sparger. The use of spargers with very small orifices is more efficient than a single orifice delivering the same volume of air. However, aerobic fermentations, e.g., penicillin, produce large quantities of mycelium and mechanical agitation must be used in order to ensure adequate dispersion of air and other nutrients in large scale fermentations. As the degree of agitation is increased, the relative efficiencies of the various types of spargers tend to converge, and at high agitation levels, all spargers give approximately the same performance. The types of spargers in general use may be classified as nozzle spargers, orifice spargers, and porous spargers.

Sparger

h) Nozzles and manways

Nozzle design must take into consideration the following:

1. To ensure aseptic connections to external piping,
2. In order to facilitate free draining
3. For proper and safe cleaning of the nozzles, protrusions inside the vessel should be as minimal as possible.
4. Addition of feed through nozzles to the fermentor to take care that the added liquids do not dribble down the interior surfaces.
5. The use of manways obviates the need for full opening heads on larger vessels.

i) Piping and valves

Piping materials: The most commonly used piping materials for biotechnology plants, in order of usage, are stainless steel, thermoplastics (polypropylene, polyethylene, polyvinylidine fluoride), carbon steel, copper, iron, glass, and lined pipe (glass and plastic liners).

Valves used on sterile lines have given cause for thought for a considerable time. For robust industrial processes such as alcohol fermentation, or even for yeast cultivation, the use of the standard type of gate valve is normally acceptable.

j) Steam locks

A good steam lock assembly should have the following features:

1. Elimination of the chances of dead spots with inefficient sterilization
2. Thorough steaming of sterile lines to be done during non usage to avoid contamination
3. Lines from feed addition tanks should be able to be steamed through their entire lengths at any time after they are connected to the assembly
4. Steam locks to be checked for leakage of steam during steaming
5. Self draining of condensate through steam traps
6. Easy cleaning and maintenance of the steam lock assembly

k) Welds and joints

Vessel welds of high quality are required to adhere to code purposes, ensure maximum smoothness and cleanability, and to minimize corrosion problems. Welding for aseptic fermentors should be carried out under an inert gas shield to minimize oxidation and flux residue, and create smoother, pit free welds.

l) Surface treatment and finish

The surface treatment of a vessel is required for any surface that comes in contact with the product. It is imperative that all stainless steel surfaces are treated and cleaned in a way that prevents corrosion under the operating conditions. Stainless steels are corrosion resistant due to the formation of a microscopically thin, invisible chromium oxide layer, which occurs on clean metal and polished surfaces only. The three main surface treatment methods used are mechanical, chemical, and electrochemical.

m) Agitation system design

Typical agitation equipment consists of the prime mover (usually an electric motor) coupled to the shaft through a reduction gear. Impellers and baffles are fitted to the shaft and vessel, respectively, to give the desired liquid motion. The shaft may enter from the top, side or bottom, and is usually fitted with a mechanical seal at the vessel wall. The number and location of impeller units depends on the vessel. In a smooth walled tank, the liquid swirls round in the same general direction as the agitator. As the impeller speed is increased, a vortex is formed and the liquid level at the wall is raised above the average liquid level. This is normally undesirable for the following reasons:

1. Power is wasted in holding up the liquid at the wall

2. The relative speed of the impeller to the liquid is reduced

3. Slight radial movements of the vortex cause the liquid to swirl unevenly, and undesirable side thrusts are set up

23.3 Biofermentor Controls

Bioreactor or fermentation processes are the core manufacturing process in the biotech industry. Implementation delays and process upsets can result in the loss of millions of dollars in revenue through lost product and downtime. Because the bioreactor is such a critical component, getting it into production as quickly as possible and keeping it running, are essential to the profitability of a biotech operation. During implementation, many end users strive to reduce the I/O footprint of their control devices, since bioreactors use a wide-range of varied signals. Analog I/O points measure pressure, temperature and bring in flow rates for buffer and media. Discrete I/O controls peristalsic/ metering pumps and valves. Additionally, analytical probes control pH, dissolved oxygen and conductivity. Throughout the process, bioreactors need to maintain precise control speed in the agitator to minimize shear. If the agitator creates too much turbulence, the microorganisms being grown may be torn apart. High rates of oxygen flowing through the sparge tube and improper agitator design can add to the problem of shear.

23.3.1 Temperature control

The temperature control system is designed using PID control algorithms. The liquid temperature is sensed and compared it with the desired temperature to form the error signal. The error signal is processed using control algorithms to produce the desired output to the heater driver. The feed back control system operates on the heating system to maintain the temperature at the required

set value by reducing the error towards zero. Continuously variable controllers are designed to produce power to the heater proportional to the error signal. As the measured value approaches its desired value, the power fed to the heater progressively reduced. PID controllers are most commonly used controllers in temperature control.

23.3.2 pH control

In real life, bioreactors actually use on-off control for pH. The two position control system is designed; so that the element controlling reagent addition is always set in one of two positions, either fully open or fully closed. The important consideration in pH control is hold time, which is required to provide time for neutralization reaction to go to completion.

SUBMERGED FERMENTOR SYSTEM AND THEIR TYPES

24.1 Introduction

The fermentor is the heart of any biochemical process in which microbial, mammalian, or plant cell systems are employed for the economic production of fermentation products. A properly designed fermentor should be used to provide an aseptic, controlled environment to facilitate optimal growth and product formation of a particular cell system.

24.2 Stirred Tank Bioreactor (STR)

The most commonly used bioreactor for industrial applications is the conventional stirred tank reactor (STR). The STR offers the advantages of high oxygen transfer rates required for high biomass productivity coupled with low investment and operating costs, which form the basis for any successful aerobic fermentation process. STRs typically have height to diameter ratios of 1:3 to 1:6. The agitator may be top driven or bottom driven depending on the scale of operation and other operational aspects. The choice of impeller depends on the physical and biological characteristics of the fermentation broth. Usually, a ring-type sparger with perforations is used to supply air to the fermentor. Baffles are provided to avoid vortex formation and improve mixing. Most fermentation processes use complex medium ingredients like corn steep liquor, molasses, and soybean flour as inexpensive nutritional sources (for carbon and nitrogen), supplemented with vital growth factors (amino acids, proteins, and vitamins) . The high turbulence imparted by the impellers in an STR can result in foaming due to the presence of proteinaceous substrates. Although chemical antifoaming agents (silicone or polypropylene glycol) can be added to control the foam, these can have detrimental effects on microbial growth and product recovery.

In order to overcome this, mechanical methods of foam suppression such as rakes on the stirrer shaft mounted above the critical level have also been adopted. The emphasis on asepsis of the bioreactor, right from the end of the sterilization cycle to the end of the fermentation, has led to the maintenance of a minimum positive pressure in the fermentor to ensure sterility. A most important aspect of sterility is the point of contact between agitator shaft and vessel, which can be effectively sealed with a lubricated double mechanical seal. The sampling devices and injection ports must be contained in steam sterilizable closures.

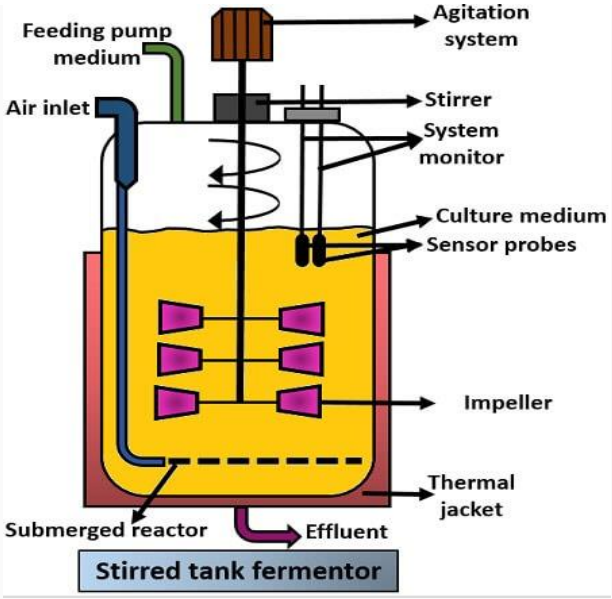


Fig. 24.1 Conventional stirred tank reactor (www.googleimages.com)

A Few important types are briefly described below

24.2.1 Stirred tank reactors

In these reactors, mechanical stirrers (using impellers) are used to mix the reactor to distribute heat and materials (such as oxygen and substrates)

24.2.2 Bubble column reactors

These are tall reactors which use air alone to mix the contents

24.2.3 Air lift reactors

These reactors are similar to bubble column reactors, but differ by the fact that they contain a draft tube. The draft tube is typically an inner tube which improves circulation and oxygen transfer and equalizes shear forces in the reactor.

24.2.4 Fluidized bed reactors

In fluidized bed reactors, cells are "immobilized" on small particles which move with the fluid. The small particles create a large surface area for cells to stick to and enable a high rate of transfer of oxygen and nutrients to the cells

24.2.5 Packed bed reactors

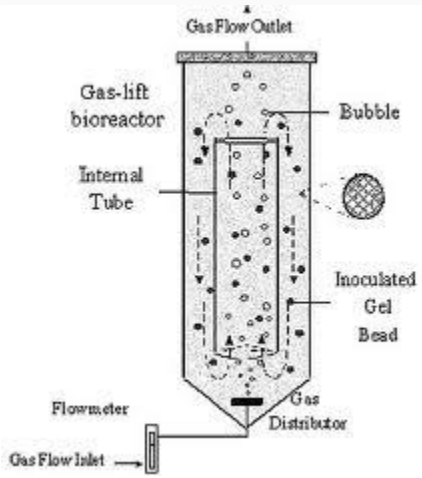
In packed bed reactors, cells are immobilized on large particles. These particles do not move with the liquid. Packed bed reactors are simple to construct and operate but can suffer from blockages and from poor oxygen transfer.

24.2.6 Flocculated cell reactors

Flocculated cell reactors retain cells by allow them to flocculate.

24.2.7 Air lift bioreactor

For fermentations that have low shear and energy requirements, an air lift reactor can be useful. The amount of air required for the fermentation process is usually sufficient to act as the sole source of liquid mixing. In this process, air pumped from the bottom of the reactor creates buoyant, bubbles, which exert a drag on the surrounding fluid. A riser and a "down comer" inside the bioreactor impose a circulating fluid pattern of movement, which provides for oxygenation and mixing of the fermentation broth. The bottlenecks associated with large scale air lift bioreactors are inadequate sterilization, higher capital investment, and aeration requirements. Since mixing in an air lift is solely caused by aeration, the power required for fluid circulation and dispersion can be higher than that needed by an agitator in a stirred tank bioreactor.



24.2.8 Fluidized bed bioreactor

In the last few decades, there has been a significant increase reported in the application of fluidized bed reactor systems. These have been mainly used for cells that have been immobilized onto particulate matter. This has the advantage that a high density of particles can be used, and that the flow velocity required for the fluidization can be achieved independently of the reactor throughput. The main advantages of a fluidized bioreactor system as observed in ethanol production from *S.cerevisiae*) are superior mass and heat transfer characteristics, very good mixing between the three phases, relatively low energy requirements, and low shear rates (which makes a fluidized bed reactor suitable also for shear sensitive cells such as mammalian and plant cells). Fluidized bed reactors have been used with cells adsorbed inside the carrier, made either of glass or of ceramics. The upward feed flow rate in a fluidized bed bioreactor is high enough to provide fluidization of carriers, resulting in improved mixing properties and medium distribution; but this can also induce carrier abrasion and damage. In addition, fluidization of glass and ceramic carriers may require high medium flow rates that could result in higher pumping costs and eventually cell leakage. Gas liquid solid fluidized bed bioreactors have been employed for production of ligninolytic enzymes, treatment of wastewater from refineries, and raw wastewater.

In this type of bioreactor, no mechanical agitation is provided, but the medium can be manually agitated *in situ* or it can be transferred into a kneading machine and reloaded into the basket. The majority of mammalian cells need a solid surface such as a microcarrier or a packed bed upon which to grow. The growth of cells on microcarrier beads depends directly on the surface available for growth up to the point where the microcarrier particles reach sufficient concentration to inhibit the cells and thus reduce cell yield. The toxicity of the support can cause long lag phases, death of the cells in the early stages of development, and limited cell yields. Microcarrier bioreactor systems (Figure 24.3) have been used for cultivation of human fibroblast cells to produce cell mass and in the production of interferon. A great advantage of microcarriers is the high surface area for cell growth provided under low shear conditions, while still allowing conventional fermentor equipment to be used. However, bead to bead and bead to impeller collisions, and hydrodynamic shear forces, may cause reduced viability.

24.2.9 Membrane bioreactor

Membrane bioreactors comprising hollow fiber systems have been developed and tested for the growth of mammalian and plant cells, and for the immobilization of bacteria, yeast and enzymes. Hollow fiber reactors have been used in the enzymatic hydrolysis of cellulose, penicillin, starch, haemoglobin, protein synthesis, and the culture of plant cells and mammalian cells. The advantages of using a hollow fiber reactor for microbial systems include high density of cell growth, using a perfusion system for simultaneous separation of product and biomass, and biocatalyst regeneration. However, a major disadvantage is the difficulty in monitoring and controlling the growth and metabolism of the culture. Other process constraints associated with microbial hollow fiber reactors are low oxygen transfer rates at high cell density and blockage, and rupture of the membranes due to excessive growth. The accumulation of toxic products in the hollow fiber might also inhibit the metabolic activity of the cell system.

24.2.10 Photobioreactor

Microalgae have been used successfully, with high productivity compared to higher plants. The high productivity in these systems is due to the high biomass produced in the bioreactor. Microalgae have been used for preparation of vitamins, pigments, antioxidants, and fatty acids, and as feed for aquaculture. The cultivation techniques employed are open systems and closed or semiclosed outdoor photobioreactors. The common photobioreactors used are tubular-type and plate-type reactors The cyanobacterium *Spirulina platensis* has been studied in batch and continuous photobioreactors under varying conditions of incident light energy and nutrient limitations.

SOLID FERMENTOR SYSTEM AND THEIR TYPES

25.1 Introduction

The main difference between submerged and solid-state fermentations is the amount of free liquid in the substrate. Solid-state fermentations (SSF) exhibit a poor conductive gas phase between the particles as compared to submerged fermentation. The presence of a wide variety of SSF matrices in terms of composition, size of solid substrate, mechanical resistance to air flow, porosity, and water holding capacity renders bioreactor design and control more difficult for the regulation of two important parameters, namely temperature and water content of the solid

medium. Other factors that influence the bioreactor design are fungal morphological characteristics, resistance to mechanical agitation, and degree of asepsis required for the fermentation process.

25.2 Categories of Bioreactor

Two categories of bioreactor exist for the SSF processes:

(i) At laboratory-scale, using quantities of dry solid medium from a few grams up to few kilograms, (ii) at pilot and industrial-scale, where several kilograms up to several tons are used. The first category comprises many designs, more or less sophisticated, while the second category, which is used mainly at industrial level, is markedly less varied .

However, based on similarities in design and operation, SSF bioreactors can be divided into groups on the basis of how they are mixed and aerated

Group I

Bioreactors in which the bed is static, or mixed only very infrequently (i.e., once or twice per day) and air is circulated around the bed, but not blown forcefully through it. These are often referred to as “tray bioreactors”.

Group II

Bioreactors in which the bed is static or mixed only very infrequently (i.e., once per day) and air is blown forcefully though the bed. These are typically referred to as “packed-bed bioreactors”.

Group III

Bioreactors in which the bed is continuously mixed or mixed intermittently with a frequency of minutes to hours, and air is circulated around the bed, but not blown forcefully through it. Two bioreactors that have this mode of operation, using different mechanisms to achieve the agitation, are “stirred drum bioreactors” and “rotating drum bioreactors”.

Group IV

Bioreactors in which the bed is agitated and air is blown forcefully through the bed. This type of bioreactor can typically be operated in either of two modes, so it is useful to identify two subgroups.

Group IV

A bioreactors are mixed continuously while **Group IVb** bioreactors are mixed intermittently with intervals of minutes to hours between mixing events. Various designs fulfill these criteria, such as “gas-solid fluidized beds”, the “rocking drum”, and various “stirred-aerated bioreactors”.

25.3 Laboratory Scale SSF Bioreactor

Small scale SSF equipment can be classified as those without forced aeration and agitation to include Petri dishes, jars, widemouth Erlenmeyer flasks, Roux bottles and roller bottles, and those incorporating continuous agitation of the solid medium such as a rotating drum bioreactor, a perforated drum bioreactor and a horizontal paddle mixer. The former are easy to operate in large numbers and commonly used for the screening of substrates or microorganisms for research purposes, while the latter offer the advantage of temperature control due to continuous agitation.

25.4 Industrial Scale SSF Bioreactor

Industrial scale SSF bioreactors can be built with or without aeration. Those without forced aeration can exhibit limitation of heat and mass transfer as the fermentation progresses, changing the properties of the microorganism involved, particularly in light of associated complexities like heat build up and inadequate oxygen transfer. However, with aeration strategies like circulation of air around the substrate layer or passing air through the substrate layer, these limitations are reduced to a certain extent.

25.4.1 SSF bioreactor without forced aeration

On an industrial scale, this bioreactor is generally a tray fermentor. The trays containing the solid medium are stacked in tiers and placed in humidity and temperature controlled chamber. This technology has the limitations of not conforming to asepsis conditions, and of high labour requirements. However, it is easily scaled up by the incorporation of additional trays.

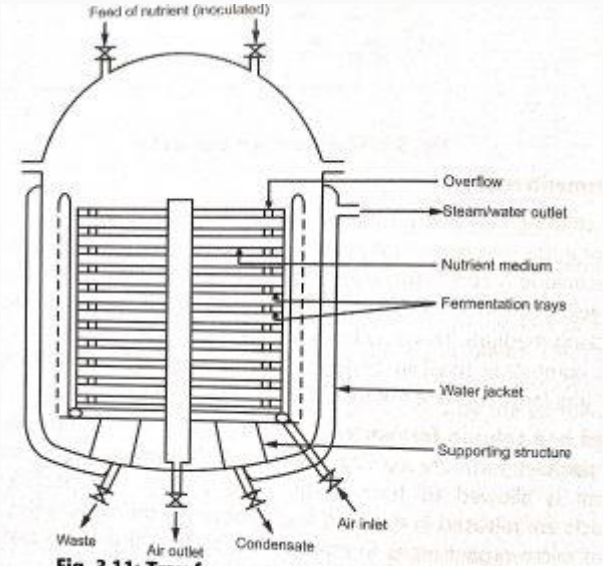


Fig. 3.11: Tray fermenter as a continuous process

24.4.2 SSF bioreactor with forced aeration and no mixing

In this type of bioreactor, no mechanical agitation is provided, but the medium can be manually agitated *in situ* or it can be transferred into a kneading machine and reloaded into the basket. However, this type of device without agitation is limited by the metabolic heat produced. Considerable temperature gradients can exist within the substrate bed. As the majority of the heat

is removed and water is evaporated by forced aeration, the bed dries out, reducing fermentation efficiency. Periodic water addition in the form of spray is required to maintain the moisture content at desired levels.

25.4.3 SSF bioreactor with continuous mixing and forced aeration

A rotating drum bioreactor with continuous mixing maximizes the exposure of each substrate particle to a thermostatic air circulating unit in the headspace. A large reactor, capable of handling 10 kg of steamed wheat bran as substrate, has been reported. Large scale use of unagitated SSF is limited by the difficulty in maintaining temperature during the fermentation. However, in a rotating drum bioreactor, efficient heat transfer is possible by convective and evaporative cooling. As the scale of fermentation increases, evaporative cooling becomes significant, because the ratio of the heat produced to the surface area available for convection decreases. The inherent difficulties encountered in the operation of solid-state fermentation systems on a large scale has led to new developments aimed at improving the efficiency of the fermentation process.