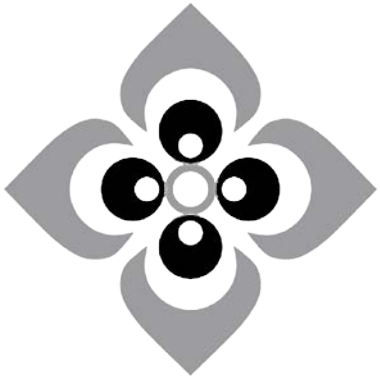

Consortium for Educational Communication

Module
on
Fermenters And Its Types

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TEXT

INTRODUCTION

A fermenter (bioreactor) is a closed vessel with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along-with their products. It is also called as heart of fermentation or bioprocess technology. A fermenter is used for commercial production in fermentation industries and is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value. Fermenters are extensively used for food processing, fermentation, waste treatment, etc.

History

The first large scale (above 20 litre capacity) fermenter for the production of yeast was first used by De Beeze and Liebmann (1944). But it was during the First World War, a British scientist named Chain Weizmann (1914-1918) developed a fermenter for the production of acetone. Since importance of aseptic conditions were recognised, hence steps were taken to design-and construct piping, joints and valves in which sterile conditions could be achieved and manufactured when required. For the first time, large scale aerobic fermenters were used in central Europe in the year 1930's for the production of compressed yeast (de Becze and Leibmann, 1944). The fermenter consisted of a large cylindrical tank with air introduced at the base via network of perforated pipes. In later modifications, mechanical impellers were used to increase the rate of mixing and to break up and disperse the air bubbles. In the year 1934, Strauch and Schmidt patented a system in which the aeration tubes were introduced with water and steam for cleaning and sterilization. In 1943, when the British Govt. decided that surface culture was inadequate, none of the fermentation plants were immediately suitable for deep fermentation. The first pilot fermenter was erected in India at Hindustan Antibiotic Ltd., Pimpri, Pune in the year 1950.

THE COMPONENT PARTS OF A FERMENTATION ROCESS

Regardless of the type of fermentation (with the possible exception of some transformation processes) an established process may be divided into six basic component parts:

- The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter.
- The sterilization of the medium, fermenters and ancillary equipment.
- The production of an active, pure culture in sufficient quantity to inoculate the production vessel.
- The growth of the organism in the production fermenter under optimum conditions for product formation.
- The extraction of the product and its purification.
- The disposal of effluents produced by the process

BASIC ATTRIBUTES OF A FERMENTER FOR MICROBIAL OR ANIMAL CELL CULTURE

The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product. In designing and constructing a fermenter a number of points must be considered:

1. The vessel should be capable of being operated aseptically for a number of days and should be reliable in long-term operation and meet the requirements of containment regulations.
2. Adequate aeration and agitation should be provided to meet the metabolic requirements of the micro-organism. However, the mixing should not cause damage to the organism.
3. Power consumption should be as low as possible.
4. A system of temperature control should be provided.
5. A system of pH control should be provided.
6. Sampling facilities should be provided.
7. Evaporation losses from the fermenter should not be excessive.
8. The vessel should be designed to require the minimal use of labour in operation,

harvesting, cleaning and maintenance.

9. Ideally the vessel should be suitable for a range of processes, but this may be restricted because of containment regulations.

10. The vessel should be constructed to ensure smooth internal surfaces, using welds instead of flange joints whenever possible.

11. The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant or plant to facilitate scale-up.

12. The cheapest materials which enable satisfactory results to be achieved should be used.

13. There should be adequate service provisions for individual plant.

Construction of Fermentors

In fermentations with strict aseptic requirements it is important to select materials that can withstand repeated steam sterilization cycles. On a small scale (1 to 30 dm^3) it is possible to use glass and/or stainless steel. Glass is useful because it gives smooth surfaces, is non-toxic, corrosion proof and it is usually easy to examine the interior of the vessel. Two basic types of fermenter are used:

1. A glass vessel with a round or flat bottom and a top flanged carrying plate .The large glass containers originally used were borosilicate battery jars. All vessels of this type have to be sterilized by autoclaving. The largest practical diameter for glass fermenters is 60 cm.

2. A glass cylinder with stainless-steel top and bottom plates these fermenters may be sterilized in situ, but 30 cm diameter is the upper size limit to safely withstand working pressures (Solomons, 1969). Vessels with two stainless steel plates cost approximately 50% more than those with just a top plate.

Design of Fermentors

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical

engineering principles are employed for design and operation of bioreactors.

A bioreactor should provide for the following:

i). Agitation (for mixing of cells and medium),

ii). Aeration (aerobic fermentors); for O2 supply,

iii). Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.

iv). Sterilization and maintenance of sterility, and

v). Withdrawal of cells/medium (for continuous fermentors).

Modern fermentors are usually integrated with computers for efficient process monitoring, data acquisition, etc. Generally, 20-25% of fermentor volume is left unfilled with medium as “head space” to allow for splashing, foaming and aeration. The fermentor design varies greatly depending on the type and the fermentation for which it is used. Bioreactors are so designed that they provide the best possible growth and biosynthesis for industrially important cultures and allow ease of manipulation for all operations.

Size of Fermentors

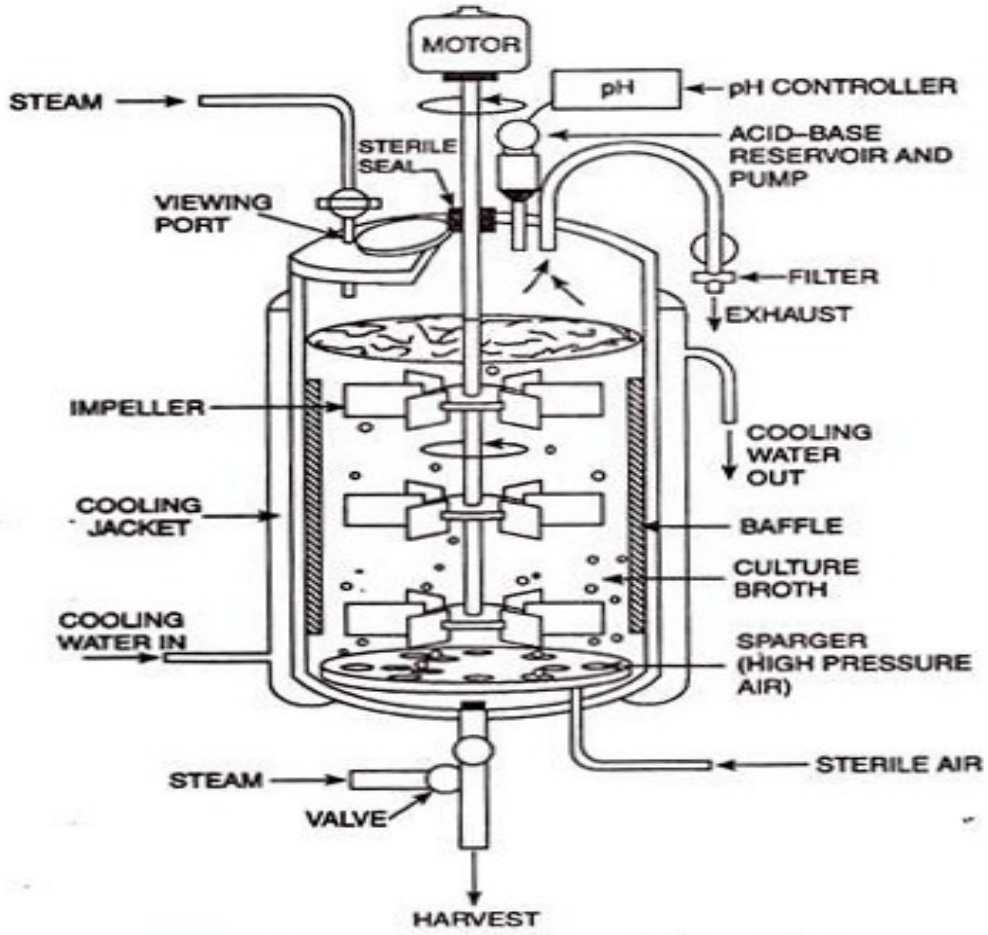
The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre or, occasionally, even more, fermentors of upto 1.2 million litres have been used. The size of the fermentor used depends on the process and how it is operated. A summary of fermentor or size of fermentor (litres) Industrial product sizes for some common microbial fermentations is given in Table.

Fermentor sizes for various microbial fermentations

Size of fermentor (litres)	*Industrial product*
1-20,000	Diagnostic enzymes, substances for molecular biology.
40-80,000	Some enzymes, antibiotics.
100-1,50,000	Penicillium, aminolycoside, antibioties, amyloses, proteases, amino acids, steriod transformations, wine, beer.
2,00,000-5,00,000	Amino acids(glutamate), wine, beer.

Industrial fermentors

Industrial fermentors can be divided into two major classes, anaerobic and aerobic; Anaerobic fermentors require little special equipment except for removal of heat generated during the fermentation process, whereas aerobic fermentors require much more elaborate equipment to ensure that mixing and adequate aeration are achieved. Since most industrial fermentation process is aerobic, the construction of a typical aerobic fermentor is the following:



An industrial aerobic fermentor (internal view)

1. Cooling Jacket

Large-scale industrial fermentors are almost always constructed of stainless steel. A fermentor is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it. The fermentor is fitted externally with a cooling jacket through which steam (for sterilization) or cooling water (for cooling) is run. Cooling jacket is necessary because sterilization of the nutrient medium and removal of the heat generated are obligatory for successful completion of the fermentation in the fermentor. For very large fermentors, insufficient heat transfer takes place through the jacket and therefore, internal coils are provided through which either steam or cooling water is run.

2. Aeration System

Aeration system is one of the most critical part of a fermentor. In a fermentor with a high microbial population density, there is a tremendous oxygen demand by the

culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium.

It is necessary, therefore, that elaborate precautions are taken using a good aeration system to ensure proper aeration oxygen availability throughout the culture. However, two separate aeration devices are used to ensure proper aeration in fermentor. These devices are sparger and impeller.

The sparger is typically just a series of holes in a metal ring or a nozzle through which filter-sterilized air (or oxygen-enriched air) passes into the fermentor under high pressure. The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium.

The impeller (also called agitator) is an agitating device necessary for stirring of the fermenter.

The stirring accomplishes two things:

(i) It mixes the gas bubbles through the liquid culture medium and

(ii) It mixes the microbial cells through the liquid culture medium. In this way, the stirring ensures uniform access of microbial cells to the nutrients.

The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermentors, more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally, the impeller should be 1/3 of the fermentors diameter fitted above the base of the fermentor. The number of impeller may vary from size to size to the fermentor.

3. Baffles

The baffles are normally incorporated into fermentors of all sizes to prevent a vortex and to improve aeration efficiency. They are metal strips roughly one-tenth of the fermentors diameter and attached radially to the walls.

4. Controlling Devices for Environmental Factors

In any microbial fermentation, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as

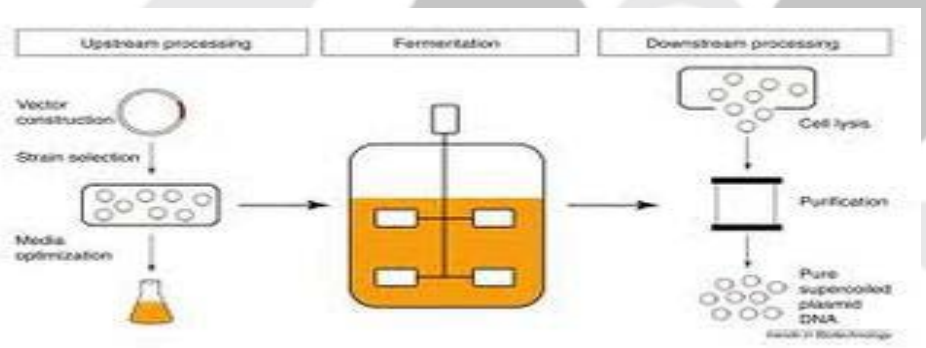
the process proceeds. For this purpose, various devices are used in a fermentor. Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cells mass, levels of key nutrients, and product concentration.

Use of Computer in Fermentor

Computer technology has produced a remarkable impact in fermentation work in recent years and the computers are used to model fermentation processes in industrial fermentors. Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition, data storage, and error-detection.

Some typical, on-line data analysis functions include the acquisition measurements, verification of data, filtering, unit conversion, calculations of indirect measurements, differential integration calculations of estimated variables, data reduction, and tabulation of results, graphical presentation of results, process stimulation and storage of data.

STAGES OF BIOPROCESSING



The entire process can be divided in three stages:

Stage I

Upstream processing which involves preparation of liquid medium, separation of particulate and inhibitory chemicals from the medium, sterilization, air purification etc. Upstream processes include selection of a microbial strain characterized by the ability to synthesize a specific product having the desired commercial value. This strain then is subjected to improvement protocols to maximize the ability of the strain to synthesize economical amounts of the product. Included in the upstream phase is the fermentation process itself which usually is carried out in large tanks known

as fermenters or bioreactors. In addition to mechanical parts which provide proper conditions inside the tank such as aeration, cooling, agitation, etc., the tank is usually also equipped with complex sets of monitors and control devices in order to run the microbial growth and product synthesis under optimized conditions. The processing of the fermentation reactions inside the fermenter can be done using many modifications of engineering technologies. One of the most commonly used fermenter types is the stirred-tank fermenter which utilizes mechanical agitation principles, mainly using radial-flow impellers, during the fermentation process.

Stage II

Fermentation which involves the conversion of substrates to desired product with the help of biological agents such as microorganisms, techniques for large-scale production of microbial products. It must both provide an optimum environment for the microbial synthesis of the desired product and be economically feasible on a large scale. They can be divided into surface (emersion) and submersion techniques. The latter may be run in batch, fed batch, continuous reactors. In the surface techniques, the microorganisms are cultivated on the surface of a liquid or solid substrate. These techniques are very complicated and rarely used in industry. In the submersion processes, the microorganisms grow in a liquid medium. Except in traditional beer and wine fermentation, the medium is held in fermenters and stirred to obtain a homogeneous distribution of cells and medium. Most processes are aerobic, and for these the medium must be vigorously aerated. All important industrial processes (production of biomass and protein, antibiotics, enzymes and sewage treatment) are carried out by submersion processes.

Stage III

Downstream processing which involves separation of cells from the fermentation broth, purification and concentration of desired product and waste disposal or recycle. Downstream processing, the various stages that follow the fermentation process, involves suitable techniques and methods for recovery, purification, and characterization of the desired fermentation product. A vast array of methods for downstream processing, such as centrifugation, filtration, and chromatography, may be applied. These methods vary according to the chemical and physical nature, as well as the desired grade, of the final product.

UPSTREAM PROCESSING

Certain organisms perform fermentation to obtain the energy they need to carry on their life processes. (Most organisms obtain the energy for these processes through aerobic respiration, in the presence of free oxygen.) Various microorganisms, including yeasts and certain molds and bacteria, obtain their energy through fermentation. Many of the fermentation processes result in products that are important in medicine, food preparation, and other fields. The specific product resulting from fermentation is determined by the type of microorganism carrying on the process and the substance in which the fermentation occurs. For example, wine is the product of yeast fermentation in fruit juice, while beer is the product of yeast fermentation in grain. Antibiotics (drugs used to fight infectious diseases) are obtained from both bacterial and mold fermentation. Fermentation by various microorganisms is used to produce substances called enzymes, which are used in many medical and industrial processes to speed up chemical reactions. Vinegar and cheese are products of bacterial fermentation. Yeast fermentation is used to make leavened bread. In this stage, the laboratory work is carried out. Micro-organisms that are most suitable for production of a particular compound are selected. Strains of the same are made so as to improve the yield as well as the quality. Media preparation is done. Depending on the biomass chosen, the media is formulated such that it is best suited for the optimal growth of the micro-organisms used. Then come the sterlisation procedures wherein the intruments that are to be used for fermentation and other stuff are ridden off contaminants like fungi, virus, bacteria etc. this ensures that the nutrients supplied in the medium are available to the desired microbes only and not to the other unnecessary microbes present, if any. A particular amount of inoculum is prepared. That is the amount of microbes that are going to be used to start the fermentation in the fermentor. The amount should be sufficient enough to begin the fermentation.

DOWNSTREAM PROCESSING

A widely recognized heuristic for categorizing downstream processing operations divides them into four groups which are applied in order to bring a product from its natural state as a component of a tissue, cell or fermentation broth through progressive improvements in purity and concentration.

1) Removal of insolubles is the first step and involves the capture of the product as

a solute in a particulate-free liquid, for example the separation of cells, cell debris or other particulate matter from fermentation broth containing an antibiotic. Typical operations to achieve this are filtration, centrifugation, sedimentation, precipitation, flocculation, electro-precipitation, and gravity settling. Additional operations such as grinding, homogenization, or leaching, required recovering products from solid sources such as plant and animal tissues are usually included in this group.

2) Product isolation is the removal of those components whose properties vary markedly from that of the desired product. For most products, water is the chief impurity and isolation steps are designed to remove most of it, reducing the volume of material to be handled and concentrating the product. Solvent extraction, adsorption, ultrafiltration, and precipitation are some of the unit operations involved.

3) Product purification is done to separate those contaminants that resemble the product very closely in physical and chemical properties. Consequently steps in this stage are expensive to carry out and require sensitive and sophisticated equipment. This stage contributes a significant fraction of the entire downstream processing expenditure. Examples of operations include affinity, size exclusion, reversed phase chromatography, crystallization and fractional precipitation.

4) Product polishing describes the final processing steps which end with packaging of the product in a form that is stable, easily transportable and convenient. Crystallization, desiccation, lyophilization and spray drying are typical unit operations. Depending on the product and its intended use, polishing may also include operations to sterilize the product and remove or deactivate trace contaminants which might compromise product safety. Such operations might include the removal of viruses or depyrogenation

FERMENTATION

A fermentation process requires a fermenter for successful production because it provides the following facilities for the process such as contamination free environment, specific temperature maintenance, maintenance of agitation and aeration, pH control, monitoring Dissolved Oxygen (DO), ports for nutrient and reagent feeding, ports for

inoculation and sampling, fittings and geometry for scale up, minimize liquid loss and growth facility for wide range of organisms. Aseptic environment or contamination is defined as protection against entry of unwanted organisms. Containment is defined as prevention of escape of viable cells from the process. Both these environment is provided by a fermenter where ever required. Contamination is applicable in all process whereas containment is necessary when pathogenic organism is used for the fermentation process. The containment level varies based on the pathogenicity of the organism used. Some organism are termed GRAS i.e., Generally Recognized As Safe. Criteria for assessment of hazardous organism are known pathogenicity of organism, virulence level, number of organisms required to initiate infection, routes of infection, known incidence of infection, local existence of vectors and reserves of microorganisms, volume of organisms used in process, techniques used for cultivation and harvesting and prophylaxis and treatment facility. Based on all the criteria if an organism is termed pathogenic the containment of the fermentation process is maintained. Good industrial large scale practice (GILSP) involves safe and highly productive organism for the process. Depending on the type of product, the concentration levels it is produced and the purity desired, the fermentation stage might constitute anywhere between 5-50% of the total fixed and operating costs of the process. Therefore, optimal design and operation of bioreactor frequently dominates the overall technological and economic performance of the process.

In any biological process, the following are unique features.

a) The concentrations of starting materials (substrates) and products in the reaction mixture are frequently low; both the substrates and the products may inhibit the process. Cell growth, the structure of intracellular enzymes, and product formation depend on the nutritional needs of the cell (salts, oxygen) and on the maintenance of optimum biological conditions (temperature, concentration of reactants, and pH) with in narrow limits.

b) Certain substances inhibitors effectors, precursors, metabolic products influence the rate and the mechanism of the reactions and intracellular regulation.

c) Microorganisms can metabolize unconventional or even contaminated raw materials (cellulose, molasses, mineral oil, starch, ores, wastewater, exhaust air, biogenic waste), a process which is frequently carried out in highly viscous, non-

Newtonian media.

d) In contrast to isolated enzymes or chemical catalysts, microorganisms adapt the structure and activity of their enzymes to the process conditions, whereby selectivity and productivity can change. Mutations of the microorganisms can occur under sub optimal biological conditions.

e) Microorganisms are frequently sensitive to strong shear stress and to thermal and chemical influences.

f) Reactions generally occur in gas-liquid -solid systems, the liquid phase usually being aqueous.

g) The microbial mass can increase as biochemical conversion progresses. Effects such as growth on the walls, flocculation, or autolysis of microorganisms can occur during the reaction.

h) Continuous bioreactors often exhibit complicated dynamic behavior. Due to above mentioned demands made by biological systems on their environment, there is no universal bioreactor.

However, the general requirements of the bioreactor are as follows:

1. The design and construction of biochemical reactors must preclude foreign contamination (sterility). Furthermore, monoseptic conditions should be maintained during the fermentation and ensure containment.

2. Optimal mixing with low, uniform shear achieved by proper designing of agitator and aerator.

3. Adequate mass transfer (oxygen) achieved by monitoring the speed of agitator and agitator

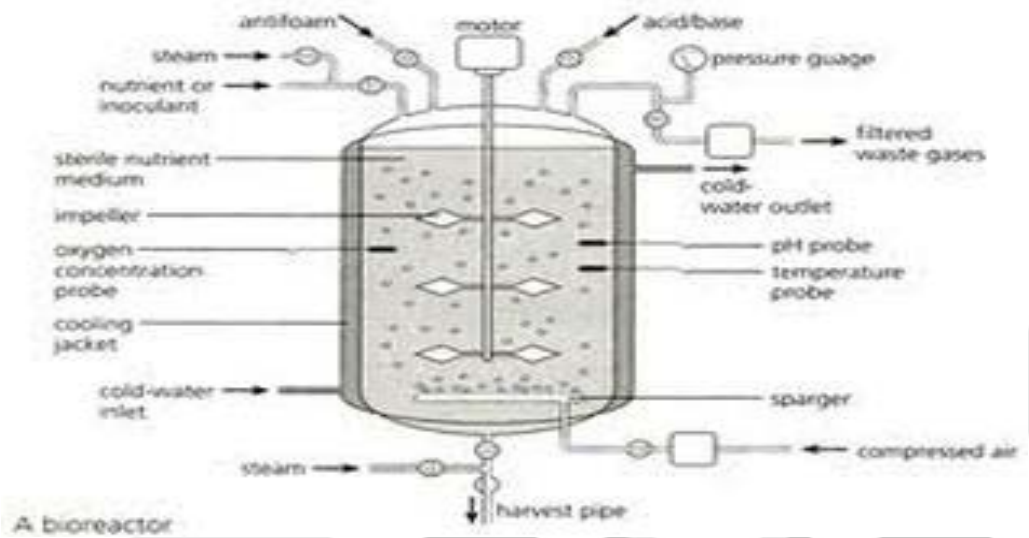
4. Clearly defined flow conditions that can be maintained by proper opening valves and monitoring devices

5. Feeding of substrate with prevention of under or overdosing by proper feed ports and monitoring

6. Suspension of solids

7. Gentle heat transfer
8. Compliance with design requirements such as: ability to be sterilized; simple construction; simple measuring, control, regulating techniques; scaleup; flexibility; long term stability; compatibility with up- downstream processes; antifoaming measures.

AN IDEAL FERMENTOR



BODY CONSTRUCTION

Construction materials differ with small scale, pilot and large scale. In small scale for vessel construction glass or stainless steel may be used. For pilot and large scale process, stainless steel (>4% chromium), mild steel (coated with glass or epoxy material), wood, plastic or concrete may be used as vessel construction material. Any vessel used should not have any corners and smooth surface is essential. The construction material must be non toxic and corrosion proof.

Glass vessel (borosilicate glass)

Type I – glass vessel round or flat bottom with top plate. It can be sterilized by autoclaving and the largest diameter is 60cm.

Type II – glass vessel flat bottom with top and bottom stainless steel plate. This type is used in in situ sterilization process and the largest diameter 30cm.

Stainless steel

Stainless steel is used as vessel construction material with the following modifications,

1. >4% chromium (atleast 10-13%) may be added

2. Film of thin hydrous oxide - non-porous, continuous, self-healing, corrosion resistance

3. Inclusion of nickel - improves engineering

4. Presence of molybdenum - resistance to halogen salts, brine, sea water

5. Tungsten, silicone - improve resistance

Thickness of vessel should be increased with scale. Side plates have lower thickness than top and bottom plates. Top and bottom plate are hemispherical to withstand pressures.

SEALING

Sealing between top plate and vessel is an important criteria to maintain airtight condition, aseptic and containment. Sealing have to be done between three types of surfaces viz. between glass-glass, glass- metal and metal-metal. There are three types of sealing. They are gasket, lipseal and 'O' ring. This sealing ensures tight joint in spite of expansion of vessel material during fermentation. The materials used for sealing may be fabric-nitryl or butyl rubbers. The seals should be changed after finite time. There are two way of sealing in O ring type simple sealing and double sealing with steam between two seals.

BAFFLES

Baffles are metal strips that prevent vortex formation around the walls of the vessel. These metal strips attached radially to the wall for every 1/10th of vessel diameter. Usually 4 baffles are present but when the vessel diameter is over $3dm^3$ around 6-8 baffles are used. There should be enough gap between wall and baffle so that scouring action around vessel is facilitated. This movement minimizes microbial growth on baffles and fermentation walls. If needed cooling coils may be attached to baffles.

AERATION SYSTEM (SPARGER)

Sparger is a device for introducing air into fermenter. Aeration provides sufficient oxygen for organism in the fermenter. Fine bubble aerators must be used. Large bubbles will have less surface area than smaller bubbles which will facilitate oxygen transfer to a greater extent. Agitation is not required when aeration provides enough agitation which is the case Air lift fermenter. But this is possible with only for medium with low viscosity and low total solids. For aeration to provide agitation the vessel height/diameter ratio (aspect ration) should be 5:1. Air supply to sparger should be supplied through filter.

There are three types of sparger viz. porous sparger, orifice sparger and nozzle sparger.

1. **Porous sparger**: made of sintered glass, ceramics or metal. It is used only in lab scale-non agitated vessel. The size of the bubble formed is 10-100 times larger than pore size. There is a pressure drop across the sparger and the holes tend to be blocked by growth which is the limitation of porous sparger.
2. **Orifice sparger:** used in small stirred fermenter. It is a perforated pipe kept below the impeller in the form of crosses or rings. The size should be ~ ¾ of impeller diameter. Air holes drilled on the under surfaces of the tubes and the holes should be atleast 6mm diameter. This type of sparger is used mostly with agitation. It is also used with out agitation in some cases like yeast manufacture, effluent treatment and production of SCP.
3. **Nozzle sparger**: Mostly used in large scale. It is single open/partially closed pipe positioned centrally below the impeller. When air is passed through this pipe there is lower pressure loss and does not get blocked.
4. **Combined sparger agitator:** This is air supply via hallow agitator shaft. The air is emitted through holes in the disc or blades of agitator.

EXIT GAS COOLER

Similar to liebig condenser, condenses the moisture from the exhaust gas in the fermenter. This removes as much moisture as possible from the gas leaving the fermenter and prevent excess fluid loss.

STIRRER GLANDS AND BEARINGS

The entry point of stirrer into fermenter may be from top to bottom or sides, mostly used from bottom so that that leaves more space for entry ports on top. There are four types of stirrer glands and bearings.

1) Stuffing box

a. sealed by several layers of packing rings of asbestos or cotton yarn-pressed against the shaft by a gland follower

b. At high speeds- packing wears – pressure should be applied to ensure tightness

c. Difficult to sterilize- satisfactory heat penetration

d. Sufficient for GILSP containment

2) Mechanical seal

a. 2 parts; i) stationary in the bearing housing, ii) other rotates on the shaft.

b. Two parts pressed together by springs or expanding bellows

c. Steam condensate use to lubricate and cool seals

d. safe for containment

e. double mechanical seal for level 2

f. at level 2 and 3, the condensate is piped to a kill tank

g. Disinfectants flushed through the seal

h. steam condensate outlet monitoring indicates any seal failure

3) Magnetic drives (some animal cell cultures)

i). shaft does not pierce the vessel

ii). two magnets- one driving, held in bearing in housing on outside of head plate and one driven, placed on one end of impeller shaft held in bearing in suitable housing

iii). ceramic magnets –magnetic power cross 16mm gap

iv). 300 – 2000 rpm rotation possible

4) Simple bush seals

Disadvantage of double seals are more difficult to assemble, difficult to detect failure of seal from normal and dead spaces and seals leading to contamination. Hence simple bush seal is preferred in some cases.

5) VALVES AND STEAM TRAPS

Addition valves

There are four types of addition valves viz.

(a) Simple ON and OFF,

(b) For coarse control,

(c) Accurate adjustment and

(d) Safety valve-flow in one direction.

Different Models of valves

1. Opening and closing, raising or lowering of blocking unit

a. Gate valve - a sliding disc move in / out of flow path by a turn of the stem

b. Globe valve - horizontal disc / plug – raised / lowered

c. Piston valve - similar to globe valve except a piston controls flow

d. Needle valve - similar to globe valve except disc replaced with tapered plug / needle

e. Check valves- Valves used to prevent accidental reversal flow of liquid or gas due to break down. There are three types – swing check, lift check, combined stop and check.

f. Pressure control valves -these types of valves are used for two purposes a) Pressure reduction b) Pressure retaining

Safety valve

There are types of safety valve by which the increase in pressure is released. They are,

a) A spindle lifted from its seating against the pressure – releases pressure

b) Bursting / rupturing of discs to release pressure

In case of releasing the gas, the escaping gas must be treated before release.

STEAM TRAPS

This steam trap is important to remove any steam condensate. There are two components viz. valve and seat assembly and open / close device. The operation of the component is based on:

i). Density of fluid: A float (ball / bucket) float in water, sink in steam. When it floats it closes and when it sinks it opens the valve

ii). Temperature of fluid: It has water / alcohol mixture which senses the change in temperature. This mixture expands in hot steam and closes the valve. When it contracts in cool water opens the valve.

iii). Kinetic effect of fluid in motion: if a low density steam is flowing it will be high velocity. Likewise high density will flow with low velocity. The conversion of pressure energy into kinetic energy control the opening and closing.

Types of Bioreactor

1. Continuous Stirred Tank Bioreactor
2. Airlift Bioreactor
3. Fluidized Bed Bioreactor
4. Packed Bed Bioreactor
5. Photobioreactor
6. Membrane Bioreactor

1. Continuous Stirred Tank Bioreactor

In Continuous Stirred Tank Bioreactor, the contents of the vessel no longer vary with time; this applies to the hold up of micro-organisms and the concentration of the components of the medium in the fermentor. Steady state conditions can be achieved by either Chemostatic or Turbido static principles. The former involves the adjustment of the flow rate of the fermentor to an appropriate and constant value and allowing the micro-organisms, substrates and biochemical product concentration to attain their natural levels. The turbid stat requires an experimental determination of the turbidity (ie, indirect measurement of microbial concentration). This thus used to control the flow rate. Both these methods have been employed in practice, though the former is obviously the simpler from every view point. The most successful continuous systems to date have been those employing yeasts and bacteria, in which the desired products are the cells or primary metabolites, compounds that form the chemical 'inventory' of a microbe, (e.g. enzymes and amino acids), or some product clearly associated with growth or energy producing mechanisms (e.g. the production of alcohol). The most widely used continuous process based on CSTF (Continuous Stirred Tank Fermentor) is the activated sludge process used in waste water treatment industry.

In continuous processing the autocatalytic (a reaction in which one of the products of the reaction increases the overall rate of a reaction) nature of microbiological reactions takes on a further significance. This is because the presence of one of the products, additional micro-organisms, enhances the overall rate of reaction. In the absence of micro-organisms no reaction can take place. Therefore, it is essential to retain at least a portion within the fermentor. It follows that if the flow rate is raised to a high value, then all the micro-organisms will be swept from the fermentor, and the conversion will cease. This phenomenon is commonly known as 'Wash-out'. if micro-organisms are fed to the fermentor simultaneously with the substrate feed, the problems associated with wash-out are abated, and the reaction proceeds normally.

Advantages of Stirred Tank Bioreactor

- Continuous operation
- Good temperature control
- Easily adapts to two phase runs

- Good control
- Simplicity of construction
- Low operating (labor) cost
- Easy to clean

2. Airlift Bioreactor

Airlift bioreactors can provide an attractive alternative to stirred tanks, particularly for bioprocesses with gaseous reactants or products. Frequently, however, they are susceptible to being limited by gas–liquid mass transfer and by poor mixing of the liquid phase, particularly when they are operating at high cell densities. This kind of fermenter works on the principle of an air lift pump. It is of two kinds:

1. Internal loop type 2. External loop type

The reactor's volume is determined by its capacity, kinetic data, and specific growth rate of the organism used. The rate of airflow of the reactor depends on the volumetric mass transfer coefficient in the reactor system. It is a uniform cylindrical cross type and has an internal loop or external loop riser configuration, diverging. The external loop riser configuration is adjustable and the change in the configuration improves the O2 transfer rate vis-a-vis mass transfer coefficient for a particular rate of airflow. This helps provide required particular dissolved O2 concentration for specific microbial system. This reactor reduces the operating cost for pumping air through the bioreactor.

Advantages

- Simple design with no moving parts or agitator for less maintenance, less risk of defects.
- Easier sterilization (no agitator shaft parts)
- Low Energy requirement vs stirred tank : Obviously doesn't need the energy for the moving parts (agitator shaft).
- Greater heat-removal vs stirred tank: At the Airlift bioreactor it doesn't need the heat plate to control the temperature, because the Draught-Tube which is inside the bioreactor can be designed to serve as internal heat exchanger. It is differ-

ence to the Stirred tank bioreactor that needs the heat coat or plate surrounding the tank to make warm bioreactor. It is clear enough that the Airlift bioreactor has greater heat-removal compare to Stirred tank.

- Very low cost

3. Fluidized Bed Bioreactor

This is a characteristic of beds of regular particles suspended in an up flowing liquid stream. If an additional gas phase is involved, there is a tendency for the particles in the bed to become less evenly distributed. There are two important features of the beds of mixed particle sizes:

(i) The increase in porosity from the bottom to the top of the bed, and

(ii) The decreased particle movement when compared with beds containing particles of constant size.

Since porosity or voidage is a measure of the local free space within a bed, it also represents a measure of the microbial hold-up when expressed as wet volume per unit bed volume. Thus, a variation in microbial hold-up is to be expected within a 'fluidised bed' fermentor on fluidisation, the smaller particles rise relative to the larger particles, and produce a situation where the smaller particles are at the top and the larger particles are at the bottom of the bed. As the smaller particles have the lowest settling velocity, the bed arranges itself, so that the smaller particles may be in the region of the highest porosity and the lowest linear velocity. The tower fermentor (developed for the continuous production of beer) is based upon these general principles (Ault et al, 1969). In this process yeast flocs are maintained in suspension by the upward movement of the nutrient medium. Moreover, any entrained particles are returned by means of a sedimentation device at the top of the tower. Essentially, the fermentor consists of a vertical cylinder with an aspect ratio (length to diameter) of approximately 10:1 At the top of the tower a separator is provided to induce the gas bubbles produced by the reaction, to coalesce and escape from the liquid phase. Within the separator there is a quiescent lone, free of the rising gas, so that the yeast may settle and return to the main body of the tower, and clear beer can be removed. Flocculent yeast (i.e. yeast capable of achieving relatively large floc sizes) is essential for an alcoholic fermentation in a PBP at acceptable flow rates, otherwise a large proportion of the

yeast would be washed out. As a result of this, an insufficient yeast concentration is maintained. A mean yeast concentration of 25 % by weight (expressed as centrifuged wet weight) is typical with values as high as 30-35% by weight at the bottom of the tower, and as low as 5-10% by weight at the top. A significant feature of the tower is the progressive and continuous fall in the specific gravity of the nutrient medium between the bottom and the top of the tower. There is an initial rapid fall at the bottom of the tower. It is followed by a slower fall over the middle and the top of the tower. This gradual fall in the specific gravity is due to the fermentation of the sugars.

Advantages of Fluidized Bed Reactor

- Uniform Particle Mixing: Due to the intrinsic fluid-like behavior of the solid material, fluidized beds do not experience poor mixing as in packed beds. This complete mixing allows for a uniform product that can often be hard to achieve in other reactor designs. The elimination of radial and axial concentration gradients also allows for better fluid-solid contact, which is essential for reaction efficiency and quality.
- Uniform Temperature Gradients: Many chemical reactions require the addition or removal of heat. Local hot or cold spots within the reaction bed, often a problem in packed beds, are avoided in a fluidized situation such as an FBR. In other reactor types, these local temperature differences, especially hotspots, can result in product degradation. Thus FBRs are well suited to exothermic reactions. Researchers have also learned that the bed-to-surface heat transfer coefficients for FBRs are high.
- Ability to Operate Reactor in Continuous State: The fluidized bed nature of these reactors allows for the ability to continuously withdraw product and introduce new reactants into the reaction vessel. Operating at a continuous process state allows manufacturers to produce their various products more efficiently due to the removal of startup conditions in batch process.

4. PACKED BED BIOREACTOR

Packed bed or fixed bed bioreactors are commonly used with attached biofilms especially in wastewater engineering. The use of packed bed reactors gained importance after the potential of whole cell immobilization technique has been

demonstrated. The immobilized biocatalyst is packed in the column and fed with nutrients either from top or from bottom. One of the disadvantages of packed beds is the changed flow characteristic due to alterations in the bed porosity during operation. While working with soft gels like alginates, carragenan etc. the bed compaction which generally occurs during fermentation results in high pressure drop across the bed. In many cases the bed compaction was so severe that the gel integrity was severely hampered. In addition channeling may occur due to turbulence in the bed. Though packed beds belong to the class of plug flow reactors in which back mixing is absent in many of the packed beds slight amount of back mixing occurs which changes the characteristics of fermentation. Packed beds are generally used where substrate inhibition governs the rate of reaction. The packed bed reactors are widely used with immobilized cells. Several modifications such as tapered beds to reduce the pressure drop across the length of the reactor, inclined bed, horizontal bed, rotary horizontal reactors have been tried with limited success.

5. Photobioreactor

Photobioreactors are used for precise phototrophic cultivation of algae and cyanobacteria. Photobioreactors are equipped with a flat-vessel design that enables bringing uniform illumination over the whole volume of cultivated culture. The Photobioreactors are currently manufactured in five standard versions differing in the volume of their cultivation vessels: 400 ml, 1000 ml, 3000 ml, 25 L, and 120 L.

Advantages of Photobioreactor

- Cultivation of algae is in controlled circumstances, hence potential for much higher productivity
- Large surface-to-volume ratio. PBRs offer maximum efficiency in using light and therefore greatly improve productivity. Typically the culture density of algae produced is 10 to 20 times greater than bag culture in which algaeculture is done in bags – and can be even greater.
- Better control of gas transfer.
- Reduction in evaporation of growth medium.
- More uniform temperature.

- Better protection from outside contamination.
- Space saving – Can be mounted vertically, horizontally or at an angle, indoors or outdoors.
- Reduced Fouling – Recently available tube self-cleaning mechanisms can dramatically reduce fouling.

6. Membrane Bioreactor

Membrane bioreactors successfully applied to various microbial bioconversions such as alcoholic fermentation, solvents, organic acid production, waste water treatment, etc. In membrane bioreactor the soluble enzyme and substrate are introduced on one side of ultrafilter membrane by means of a pump and the product is forced out through the membrane. Membrane holds back the enzyme and a good mixing in the reactor can be achieved by using a stirrer. The most widely used membrane materials include; polysulfonte, polyamide and cellulose acetate.

Advantages of Membrane Bioreactor

1. The loss of enzyme is reduced.
2. Enzyme lost by denaturation can be making up by periodic addition of enzyme.
3. Substrate and enzyme can be easily replaced.