INDUSTRIAL BIOTECHNOLOGY AND ITS APPLICATIONS
SUBJECT CODE – P3AM23MT

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Antifoam agents

INTRODUCTION

- Antifoaming agents are added to prevent or counter the foam generation in the formulation.
- Generally, these agents have surface active properties and are insoluble in the foaming medium.
- These are less viscous, easily spreadable on the foamy surface, and possess affinity to the air–liquid surface where it destabilizes the foam lamellas, which rupture the air bubbles and break down the surface foam.
- Entrained air bubbles are agglomerated, and the larger bubbles rise to the surface of the bulk liquid more quickly.
- Commonly used antifoaming agents are certain alcohols (cetostearyl alcohol), insoluble oils (castor oil), stearates, polydimethylsiloxanes and other silicones derivatives, ether and glycols

TYPES OF DEFOAMERS

1. SILICONE FREE LIQUID DEFOAMERS

- Silicone free liquid defoamers are effective in water based and solvent based systems.
- Various industries buy defoamers belonging this class as they control foam regeneration, don’t cause discoloration and can be used in formaldehyde sensitive systems.
- Silicone free liquid defoamer suppliers supply these to industries such as paints, coatings, resins, rubber, photographic chemicals, adhesives, metal processing, etc.
- There are various types of silicone free liquid defoamers manufactured specially for general applications with good defoaming properties.
- Many industries buy silicone free liquid defoamers as they give the desired results.
- These range of antifoaming agents are eco-friendly and therefore safe to use, which have balanced composition, precise pH value and long shelf life.

- **2. SILICONE BASED LIQUID DEFOAMERS**

- Silicone based liquid defoamers are composed of functional additives and non-ionic surfactants.
- Various surfactants are used in the manufacture of silicone antifoams.
- Silicone based liquid defoamers constitute functional additives and non-ionic surfactants.
- These defoamers can either be aqueous, non-aqueous or both cationic, which depends on the type of surfactant used, whether it is non-ionic, amphoteric or anionic.
- Many end users buy silicone based liquid defoamers due to their surface tension altering property.
- Many industrial users buy silicone defoamers and combine it with hydrophobic solids to increase the defoaming effect.

- **3. SILICONE FREE POWDER DEFOAMERS**

- Foam can lead to decreased plant efficiency, loss of valuable products and damage of goods.
- Various industries buy silicone free powder defoamers to break the foams as well as control foam regeneration.
- Silicone free powder defoamers suppliers supply it in the dry form for the manufacture of products.
- Framing systems’ companies buy defoamers and use it in very small concentrations.
- Various industries buy silicone free powder defoamers for use in cement admixtures, wettable agrochemical powders and dry flowable agrochemicals.
- Silicone free powder defoamer suppliers supply the chemical to various industrial manufacturers, distributors and end users as they are eco-friendly and bio acceptable.
- These range of defoamers are widely bought due to their cost and work efficiency. They are stable in the framing system and storage.

- **4. SILICONE BASED POWDER DEFOAMERS**
- Silicon based powder defoamers are composed of silicone antifoam compound.
- Many industries buy silicon based powder defoamers for numerous manufacturing applications.
- Industries such as adhesives, agriculture, construction, detergents, oil well refining, paints, inks, coatings and textiles buy silicon based powder defoamers from silicon based powder defoamers suppliers.
- These silicon based defoamer formulations have excellent defoaming action and are given preference for their lasting defoaming power and stability in small concentrations.
- Silicon based powder defoamers are highly effective in controlling foam in foaming systems with less dosage.

- Fermentors – Basic functions, designs, components, body construction, aeration, agitation.

- The **fermentor** is a vessel or an apparatus which is used for the fermentation activity of the microorganisms for the commercial production of certain substances. ... The common type of **fermentors** are tower fermenter, bubble column, air-lift, fluid bed fermenter, etc.
- The **bioreactors** refers to any manufactured device or system that supports a biologically active environment. In one case, a **bioreactor** is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms.

- A fermenter is an apparatus that maintains required optimal environmental conditions for the growth of industrially important microorganisms, used in large scale fermentation process and in the commercial production of a range of fermentation products like Antibiotics, Enzymes, Organic acids, Alcoholic beverages etc.
- To provide a controlled physico-chemical environment for the growth of a pure culture or a well-defined mixed culture of microorganisms is the key function of an ideal fermenter to obtain the desired fermentation products
- An ideal fermenter maintains optimal environmental conditions throughout the process for the process organisms, added substrates and additives for a quality end product
- Saving of energy and cost effective operation is very important concern as far as fermentation economics is concern
- Many times, the terms “Bioreactor” and “Fermenter” are used synonymously. There is a very minor difference between these two

- Many times, the terms “Bioreactor” and “Fermenter” are used synonymously. There is a very minor difference between these two
- The bioreactor is used for the mass culture of plant and animal cells, while fermenter is mainly used for microbial culture
- The operational parameters and design engineering of fermenters and bioreactors are identical.

Parameters of an ideal fermenter A fermenter should

- Facilitate the growth of a wide range of organisms capable of producing a varieties of fermentation products
- Do not allow entry to any sort of unwanted microorganisms thus to provide operation free from contamination
- Maintain a specific required temperature
- Provide adequate aeration and agitation to meet the metabolic requirements of the organisms during the process to attain mass and heat transfer within the system without damaging the process organisms

- Control the pH of the culture throughout the process
- Have provision of constant monitoring and control of level of dissolved oxygen
- Allow feeding of nutrient solutions and other supplementary requirements
- Provide access points for seed culture inoculation and sampling during the process
- Reduce liquid loss from the vessel during process by cooling system
- Be capable of being operated aseptically during the tenure of the process thus fulfilling the requirements of containment regulations
- Ensure that overall process period should have power consumption, as low as possible

- Be designed in such a way that it require the minimal use of labours during production process and downstream operations (i.e. harvesting, cleaning and maintenance)
- Should be suitable for a range of processes along with the containment regulations
- Be constructed in such a way that it ensure even internal surfaces, using welds instead of flange joints whenever possible
- Should have identical geometry at different operational level (i.e. both smaller and larger vessels in the pilot or scale-up plant)
- The material from which the fermenter is made up of should be inert and capable to withstand repeated stem sterilization conditions
- Apart from all these parameters, it is very imperative to have adequate service provisions for individual plants

The important service provisions are listed below.

- Provision of compressed as well as sterile compressed air
- Provision of chilled water, cold water and hot water
- Facility to supply steam (high pressure) and steam condensate
- Provision of continuous electricity and stand-by generator
- Confine and well protected storage facilities for media components
- Availability of control and monitoring equipment for fermenters
- Provision of instrument maintenance facilities
- Facilities for extraction and recovery equipment
- Arrangement of convenience and easy excess delivery of materials
- Appropriate containment facilities
- Facility for appropriate effluents treatment of generated from the production unit
- A well-equipped workshop for minor mechanical and electrical repair under urgent situation

Functions of different parts of a fermenter Vessel:

- Size and Material
- Function of a fermenter is to carryout process under appropriate aseptic and predefined environmental conditions
- A fermentation vessel is designed in such a way that it requires minimal labour operation and maintenance
- It should have even internal surfaces with a similar geometry
- The volume capacity of the fermenter vary at different stages
- From laboratory experimental models with volume capacity of one or two litres, pilot scale fermenter up to one thousand litres
- Industrial scale fermenter are of several hundred litres capacity
- There are mainly two types of vessels base on the type fermentation process

BASIC DESIGN OF A FERMENTOR

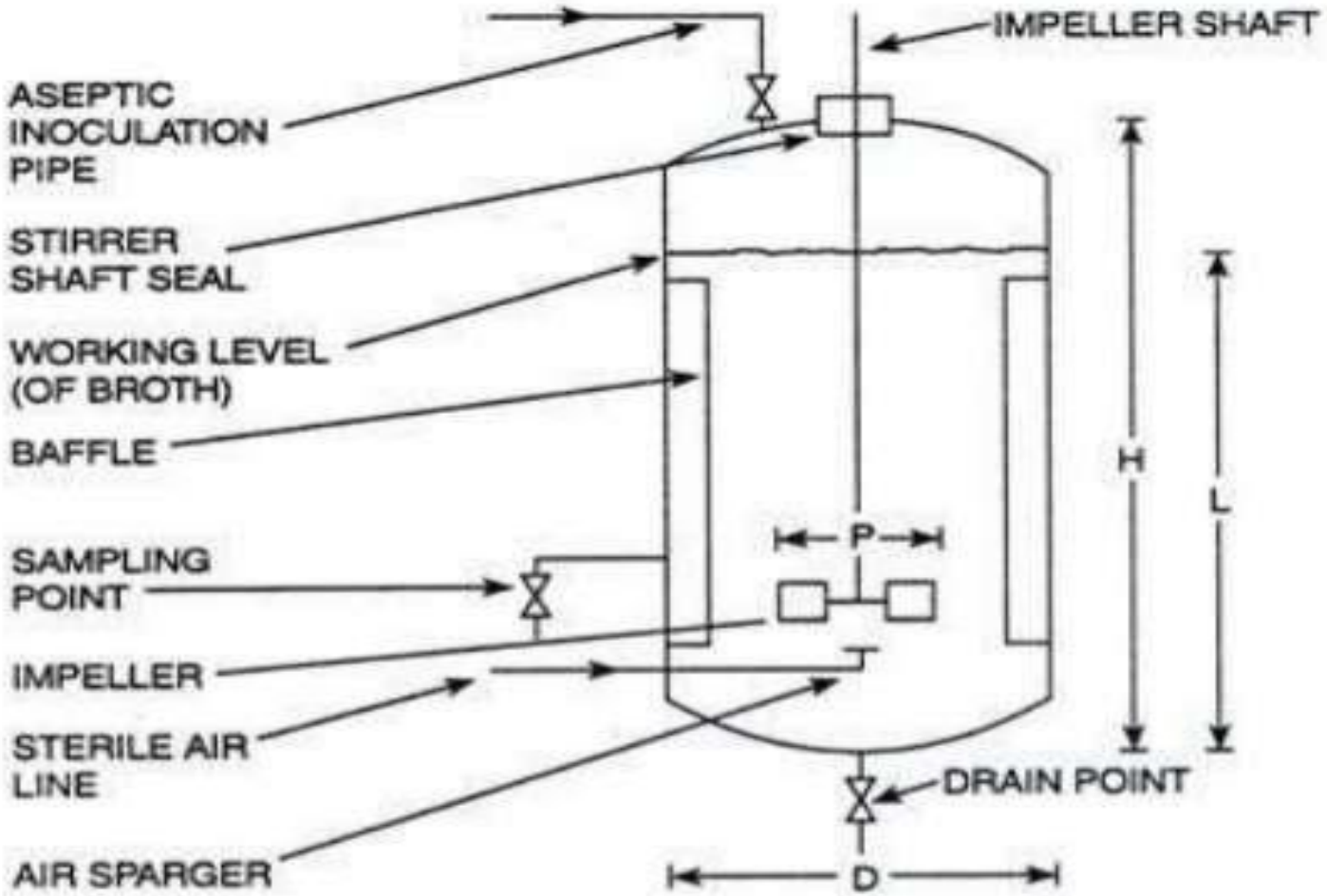


FIG. 14.3. Diagram of a fermenter with one multi-bladed impeller. H, fermenter height; L, liquid height; D, tank diameter; P, impeller diameter.

1. Small scale fermenter (Laboratory scale fermenter)

a. These are made up of glass

b. The large vessels are made up of borosilicate battery jars

c. They have a round or flat bottom and a top flanged carrying plate

d. They are smooth, non toxic and corrosion free

e. These vessels can be sterilized by autoclaving

f. It is feasible to examine the interior of the glass vessels

g. The diameter of the vessel is usually more than 50 cm

- 2. Large scale fermenter (Industrial scale fermenter)

a. As, stainless steel is the most satisfactory material, it is used to manufacture vessels of high volume

b. These vessels can be sterilized in situ

c. They can withstand high pressure and corrosion

d. Corrosion resistance property is due to the thin hydrous film on the surface of the metal

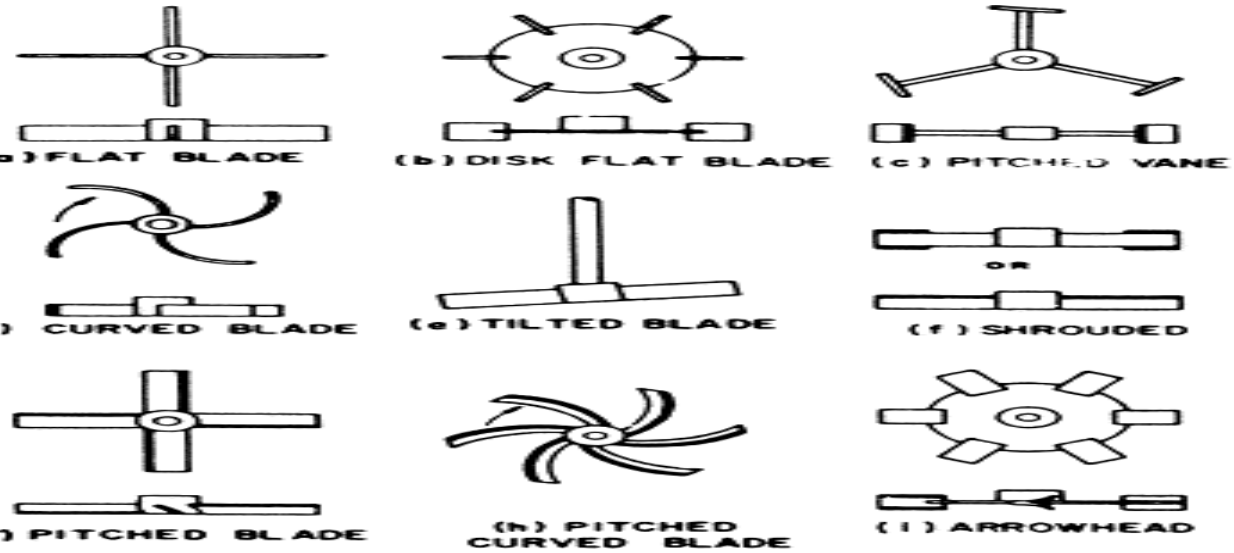
e. This film is stabilized by chromium

f. This film is continuous, non-porous, insoluble and self-heating

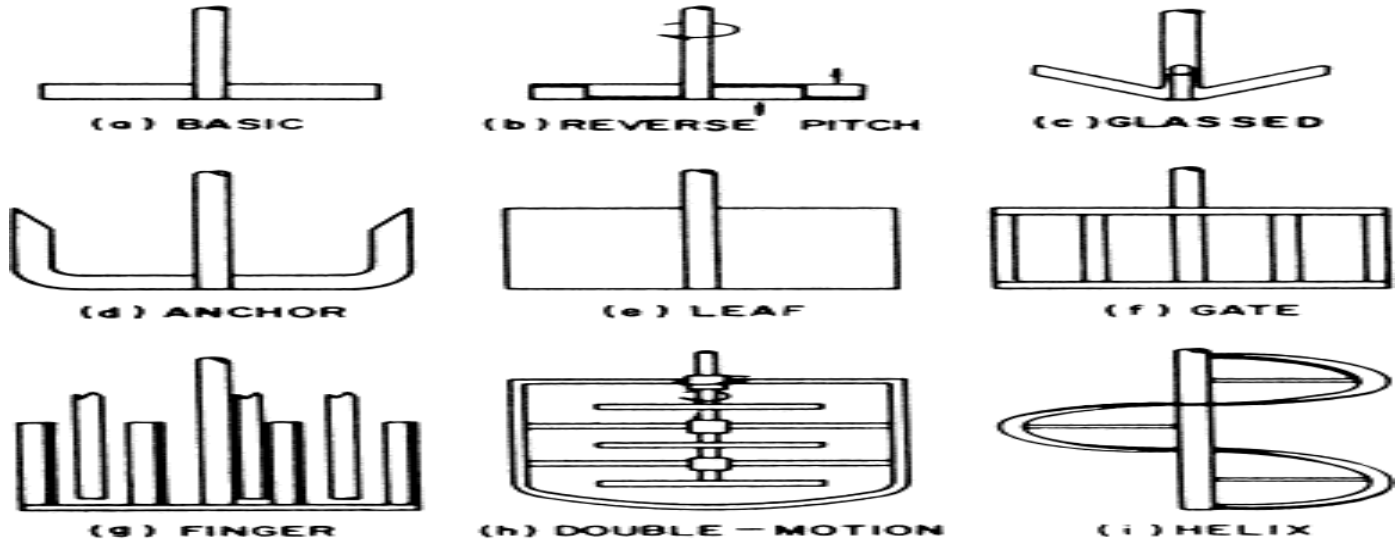
g. The corrosion resistance property of the vessel can be improved by mixing tungsten, silicone and other elements at the time of manufacturing

- Impeller (Agitator)
- This device is use for agitation (mixing up) of the medium
- Agitation creates a uniform environment in which all organisms remain in continuous contact with medium resulting in maximum up take of the nutrient.
- It also increase the air bubble path generated from the sparger (aeration device) hence more time to dissolve oxygen in the medium
- The impeller achieves a number of mixing objectives like suspension of solid particles, bulk fluid and gas phase mixing
- There are various types of impellers

1. Disc turbine
2. Vaned disc
3. Open turbine
4. Marine propeller



Turbine impeller designs.



Paddle impeller designs.

Sparger

- This device is used for aeration
- Aerobic fermentation process require sufficient oxygen to the microorganisms for metabolic requirements
- Depending on volume of medium in the fermentation vessel, different types of spargers are installed in the fermenter.
- 1. Porous sparger

a. It is made up of sintered glass or ceramics

b. It is used on the laboratory scale in non-agitated vessel

2. Nozzle sparger

a. It is a partially closed or single open pipe which provides stem to the air bubbles

b. As nozzle sparger causes a lower pressure and does not get blocked, they are used as a single nozzle

- 3. Combined sparger – agitator

a. It introduced air through a hollow agitator shaft

b. The holes are drilled in the disc, which remains connected to the base of the main shaft from where it is emitting the air bubbles

c. It provide excellent aeration in a baffled vessel

The efficiency of aeration depends on operation of agitator at a range of Revolution Per Minutes (RPM)

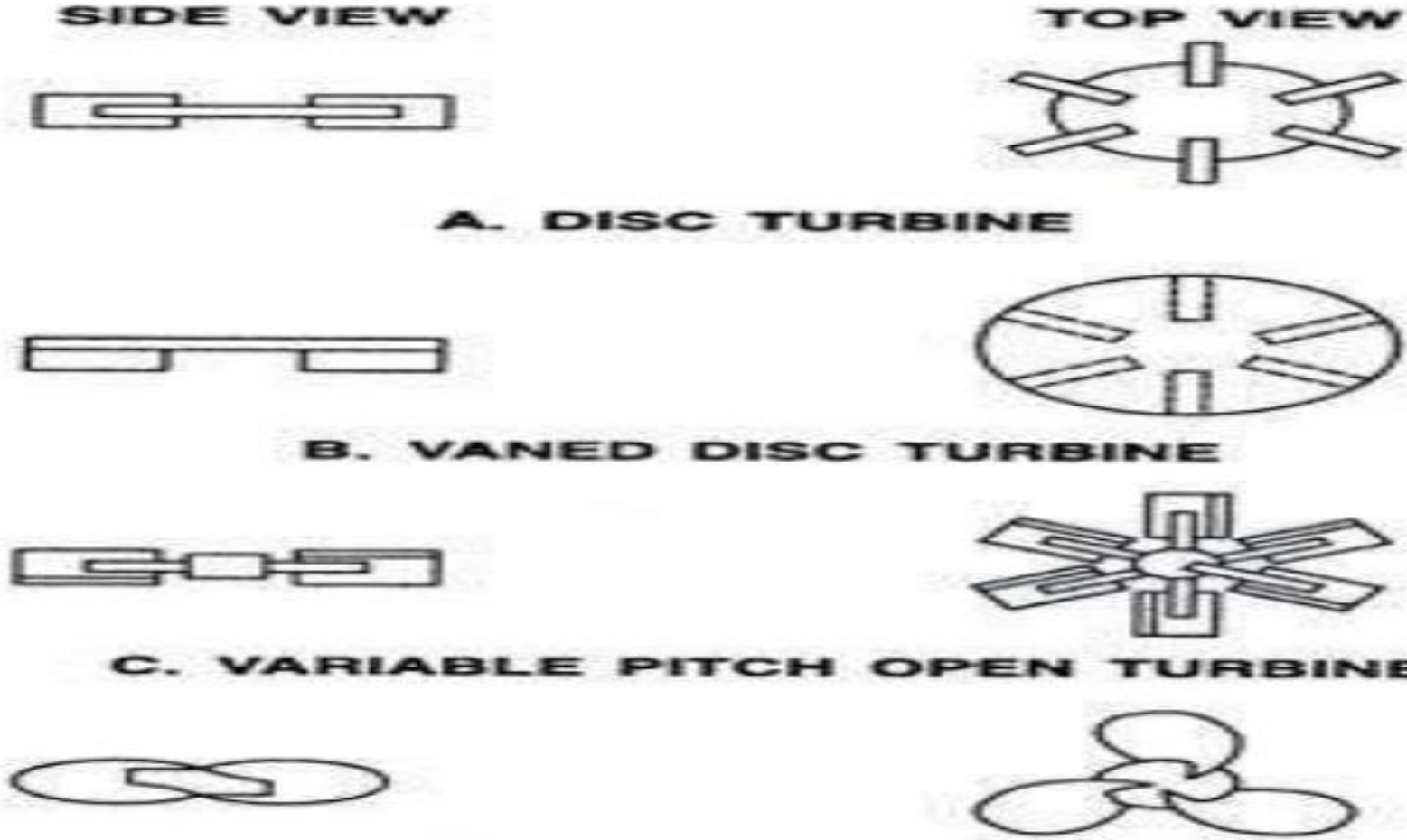


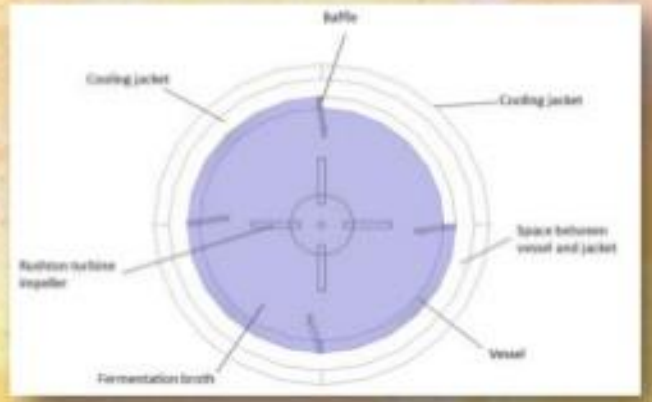
FIG. 14.1. Different types of agitators : **A.** disc turbine; **B.** vaned disc; **C.** open turbine, variable pitch; and **D.** marine propeller agitators.

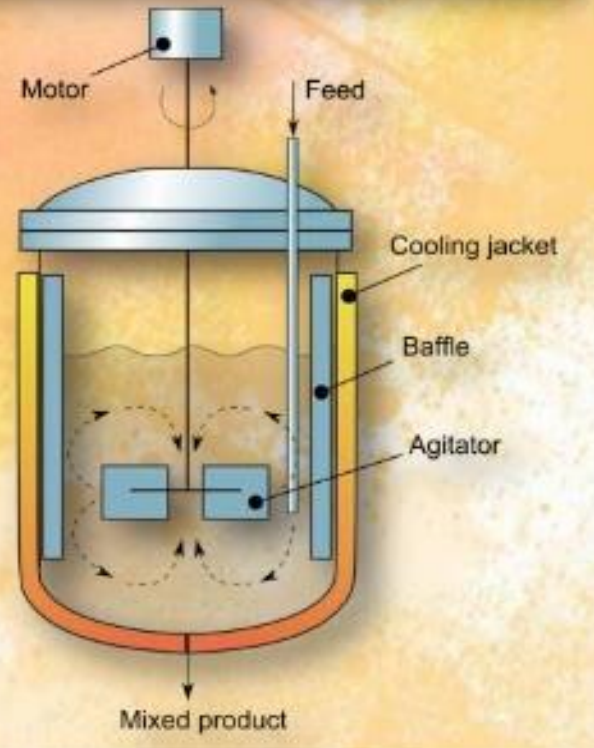
Baffels

- This device is used to avoid the vortex formation generated during the agitation of the medium
- Baffles are made up of metal strips attached 900 to the wall of fermentation vessel
- The diameter of the baffles is nearly one tenth of the vessel diameter
- The gap between the vessel wall and the baffles strips should be maintain in such a way that scouring action of the minimise microbial growth on the walls of the fermenter

BAFFLES

- Four baffles incorporated into agitated vessels of all sizes to **prevent vortex and to improve aeration efficiency**
- Metal strips roughly one-tenth of vessel diameter and attached radially to the wall
- Minimizes microbial growth on baffles and fermenter walls.





Foam control

- A medium rich in protein when subjected to agitation, it generate foam
- If the excessive foaming is not prevented, it results in the leakage of the medium from the lid of the fermentation vessel and hence lead to the contamination of the fermentation medium
- A foam sensing devised is usually installed from the lid in the fermenter, set at a definite level above the broth surface
- When the foam rises and touches the probe tip, a signal is generated in form current and pass through the circuit of the probe and gives a signal
- If the fermentation operation is automatic, then the signal triggers the pump and antifoam agent is released within seconds mechanically or robotically
- It is also possible to add antifoam manually in case the process is not automatic

- Temperature controlling (heating and cooling) devices
- Mechanical agitation and exothermic microbial metabolic activity generates heat during the fermentation process
- Endothermic microbial metabolic activity lower down the temperature of the fermentation
- To maintain this temperature, heat is to be either added to or removed from the system
- The cooling system is used to remove excess heat from the system
- Internal heating coils are used for providing heat (Note: In case of lab scale process, the fermenter is placed in thermostatically controlled bath)

- Feed ports
- Feed ports are the tubes (for Lab scale fermenter) and pipelines (for large scale fermenter) connected to the nutrient reservoir
- These tubes or pipelines are used to add nutrients and acid/alkali in the fermenter before and during the fermentation process
- They are heat sterilized in situ and /or ex situ with stem
- It is advisable to sterilize after connection has been done and before any additions are made

- Flow regulation and controlling devices (i.e. Valves)
- Five types of valves are used.

1. Safety valves

a. Any pipe layout which work under pressure are incorporated with these safety valves to

b. These valves protect the pipe layout and ensures that the pressure never go beyond the upper limit of the specified value

2. Globe valves

a. These valves do not regulate the flow of steam or water

b. They are suitable for general purposes use like completely opened or completely closed

3. Butterfly valves

a. When the diameter of the pipes is large and there is low or no pressure butterfly vales are ideal choice

b. These valves do not ensure aseptic operation

- 4. Ball valves

a. These valves are appropriate when aseptic condition is required

b. These valves can also be operated under high temperature

c. Ball valves can handle mycelia broths

5. Diaphragm valves

a. They are used for flow regulation

b. Sealing assembly

- Stirrer shaft, a device providing agitation must be sealed properly ensuring long term aseptic operation
- There are various types of sealing assembly available in the market, of which three are mainly used

1. Mechanical seal

a. Made up of a stationary part in the bearing and the rotating part on the shaft

b. A spring is used to press these two components together

c. A suitable lubricant should be apply to ensure friction free smooth rotation and control the heat generation at the point of stationary and moving parts of the seal

2. Packed gland seal

a. Several layers of rings made up of asbestos are used to seal the shaft

b. Periodical monitoring and replacement of these rings advisable to prevent the penetration of heat

- 3. Magnetic drives

a. This assembly is made up of two magnets

b. The driving magnet is held in bearing on the outside of head plate and connected to the drive shaft

c. The driven magnet is placed on one end of the impeller shaft and held in bearings on the inner surface of the top plate

Sr.	Parts of fermenter	Function
1	Impellor (agitator)	To stir the media continuously and hence prevent cells from settling down. and distribute oxygen throughout the medium
2	Sparger (Aerator)	Introduce sterile oxygen to the media in case of aerobic fermentation process
3	Baffles (vortex breaker)	Disrupt vortex and provide better mixing
4	Inlet Air filter	Filter air before it enter the fermenter
5	Exhaust Air filter	Trap and prevent contaminants from escaping
6	Rotameter	Measure flow rate of Air or liquid
7	Pressure gauge	Measure pressure inside the fermenter
8	Temperature probe	Measure and monitor change in temperature of the medium during the process
9	Cooling Jacket	To maintain the temperature of the medium throughout the process
10	pH probe	Measure and monitor pH of the medium
11	Dissolve Oxygen Probe	Measure dissolve oxygen in the fermenter
12	Level probe	Measure the level of medium
13	Foam probe	Detect the presence of the foam
14	Acid	Maintain the required pH of the medium by neutralizing the basic environment
15	Base	Maintain the required pH of the medium by neutralizing the acidic environment
16	Antifoam	Breakdown and prevent foams
17	Sampling pint	To obtain samples during the process
18	Valves	Regulation and control the flow liquids and gases
19	Control panel	Monitor over all parameters

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UNIT II: FERMENTATION MEDIA AND FERMENTOR (11)

- Fermentation media – substrates, sterilization and screening.
- Antifoam agents.
- Fermentors – Basic functions, designs, components, body construction, aeration, agitation. Sterilization of fermentor. Types of fermentor and fermentation.
- Scale up of fermentation.
- Downstream processing.

Introduction

- **Fermentation** is a metabolic **process** that produces chemical changes in organic substrates through the action of enzymes.
- In biochemistry, it is narrowly defined as the extraction of energy from carbohydrates in the absence of oxygen.
- The science of **fermentation** is known as zymology.

Types of Fermentation

- Lactic acid **fermentation**. Yeast strains and bacteria convert starches or sugars into lactic acid, requiring no heat in preparation. ...
- Ethanol **fermentation**/alcohol **fermentation**. ...
- Acetic acid **fermentation**.

Uses

- **Fermentation** is widely **used for** the production of alcoholic beverages, for instance, wine from fruit juices and beer from grains. Potatoes, rich in starch, can also be **fermented** and distilled to make gin and vodka. **Fermentation** is also extensively **used in** bread making.

Industrial Fermentation

- Industrial fermentation processes begin with suitable microorganisms and specified conditions, such as careful adjustment of nutrient concentration.
- The products are of many types: alcohol, glycerol, and carbon dioxide from yeast fermentation of various sugars; butyl alcohol, acetone, lactic acid, monosodium glutamate, and acetic acid from various bacteria; and citric acid, gluconic acid, and small amounts of antibiotics, vitamin B_{12}, and riboflavin (vitamin B_2) from mold fermentation.
- Ethyl alcohol produced via the fermentation of starch or sugar is an important source of liquid biofuel.

Design of Fermentation Media

- In a fermentation process, the choice of the most optimum micro-organisms and fermentation media is very important for high yield of product.
- The quality of fermentation media is important as it provides nutrients and energy for growth of micro-organisms.
- This medium provides substrate for product synthesis in a fermentor.

- **Fermentation media consists of major and minor components.**

1.Major components include Carbon and Nitrogen source.

2.Minor components include inorganic salts, vitamins, growth factors, anti-foaming agents, buffers, dissolved oxygen, other dissolved gases, growth inhibitors and enzymes.

- Nutrients required for fermentation media also depend upon the type of fermentation organisms as well as the type of fermentation process to be used.
- Poor choice of fermentation media might result in poor yield of output.
- Types of nutrients present in the fermentation media always determine the yield of the product.

Fermentation Media

Major components

Carbon Source
Nitrogen source

Minor components

Inorganic salts, vitamins, growth factors, anti-foaming agents, buffers, dissolved oxygen, other dissolved gases, growth inhibitors and enzymes.

- **There are two uses of fermentation media**

1.Growth media

2.Fermentation media

- **Growth medium** contains low amounts of nutrients.It is useful in creating raw material for further fermentation processes.
- **Fermentation media** contains high amounts of nutrients. It is used in creating final products using fermentation.
- For example, growth of yeast requires 1% carbon. But during fermentation of alcohol, yeast requires 12 to 13 % carbon in the medium.

- **What is the role of Fermentation Media?**
- During the fermentation process, media contains high amounts of nutrients, micro-organism and optimum conditions.
- When these micro-organisms are incubated at the desired optimum conditions, they enjoy luxurious metabolism.
- Here, the fermentation organisms become hyperactive due to presence of high quantities of nutrients, thus it results in consumption of excess nutrients and partial degradation of fermentation media.
- The waste effluents excreted by the microbes could be the desired output product of the fermentation process.

- The amount of substrate given to microbes should not reach inhibitory concentration levels because excess substrate inhibits vital enzymes and may results in death of cells.
- Also, water present in cytoplasm is important for metabolism process. If excess sugar or salt is available in the fermentation media, it would tie up cytoplasm water and may result in lack of water for metabolism and cause death of microbes, thus affecting fermentation output.
- Excess substrate may increase osmotic pressure and effect enzyme activities in a cell.
- Microbes excrete this excess substrate in the form of partially digested fermentation media.
- It is converted to an insoluble inert compound in the form of reserve food material and this reserve food material is harmless to cells.

There are two types of fermentation media used in industries.

1.Synthetic media

2.Crude media

1.Synthetic media

- Synthetic media is useful in the field of research as each and every component is chemically known and the exact composition of nutrients is predetermined.
- So, in case of synthetic media, variation in levels and concentration of nutrients can be controlled.
- Here, by experimentation with synthetic media, the effect of nutrients on growth and yield of product can be analysed.
- We can redesign the synthetic media as per our needs.
- It is very useful in controlling the growth and yield of product in a lab environment.
- We can also use it to determine the metabolic pathway used in the synthesis of products.

- With the help of radio-isotope labelling technique, we can determine the main ingredients that gets used up to create the final desired product.
- In this way, we can know the exact proportions of ingredients required for our process.
- We can optimise this process by using alternative sources of carbon or nitrogen, and creating a fermentation media which is the most optimum for our needs.
- The use of Synthetic media allows us to experiment with various sources of fermentation media in the lab as the results are accurately reproducible for a given composition.

Advantages

- a well designed synthetic media is that it lacks sources of protein and peptides.
- no foam formation, and chances of contamination are very less.
- Product recovery is easier because synthetic media contains pure components.

Disadvantages.

- A major disadvantage is the cost of media.
- The most important aspect of fermentation is that it should be economic and profitable.
- Synthetic media is never used on industrial scale because it is expensive.
- This process in only suitable for experimentation in a lab on a small scale.

2.Crude Media

- Crude media is generally used on an industrial scale for fermentation process.
- Crude media contains a rough composition of media required for fermentation.
- It gives high yield of product and contains undefined sources of ingredients.
- Crude media contains high level of nutrients, vitamins, proteins, growth factors, anti-foaming agents and precursors.
- It is important to ensure that crude media should not contain toxic substances that could effect the growth of bacteria and yield of product.

Ingredients of Crude Media

1) Inorganic nutrients

- Crude media contains inorganic salts containing cations and anion along with a carbon source.
- Sometimes, fermentation micro-organisms have a specific requirement of ions like magnesium ions, phosphates or sulphates.
- These requirements are fulfilled by addition of these ions to balance the crude media.

2) Carbon source

- Simple to complex carbohydrates can be added to media as a source of carbon. We can add different sugars like mannitol, sorbitol, organic acids, fatty acids, proteins, peptides we can choose any of these as a source of carbon. The selection of carbon source depends upon the availability as well as the cost of raw material. In most of the fermentation media, crude source of carbon is added.

1. **Simple carbohydrates** – simple sugars are semi purified polysaccharides and sugar alcohol are added. Sources of simple carbohydrates are Black strap molasses, Corn molasses, Beet molasses, sulphite waste liquor, Hydrol (corn sugar molasses), Cannery waste.
2. **Complex carbohydrates** – Source of complex carbohydrates are Starch, Corn, Rice, Rye, Milo, wheat potatoes etc. Source of starch cellulose are corn cobs, straws, wood waste, saw meal etc.

- **3) Nitrogen source**
- Salts of urea, ammonia, and nitrate can be used as a nitrogen source.
- When fermentation organisms are non-proteolytic in nature, pure form of urea, ammonia and nitrate are used as a source of nitrogen.
- When fermentation organisms are proteolytic in nature, animal and plant raw material is used; like distillery dried solubles, Casein, Cereal grains, peptones, yeast extract, hydrolysate, and soybean meal etc.

4) Growth factors

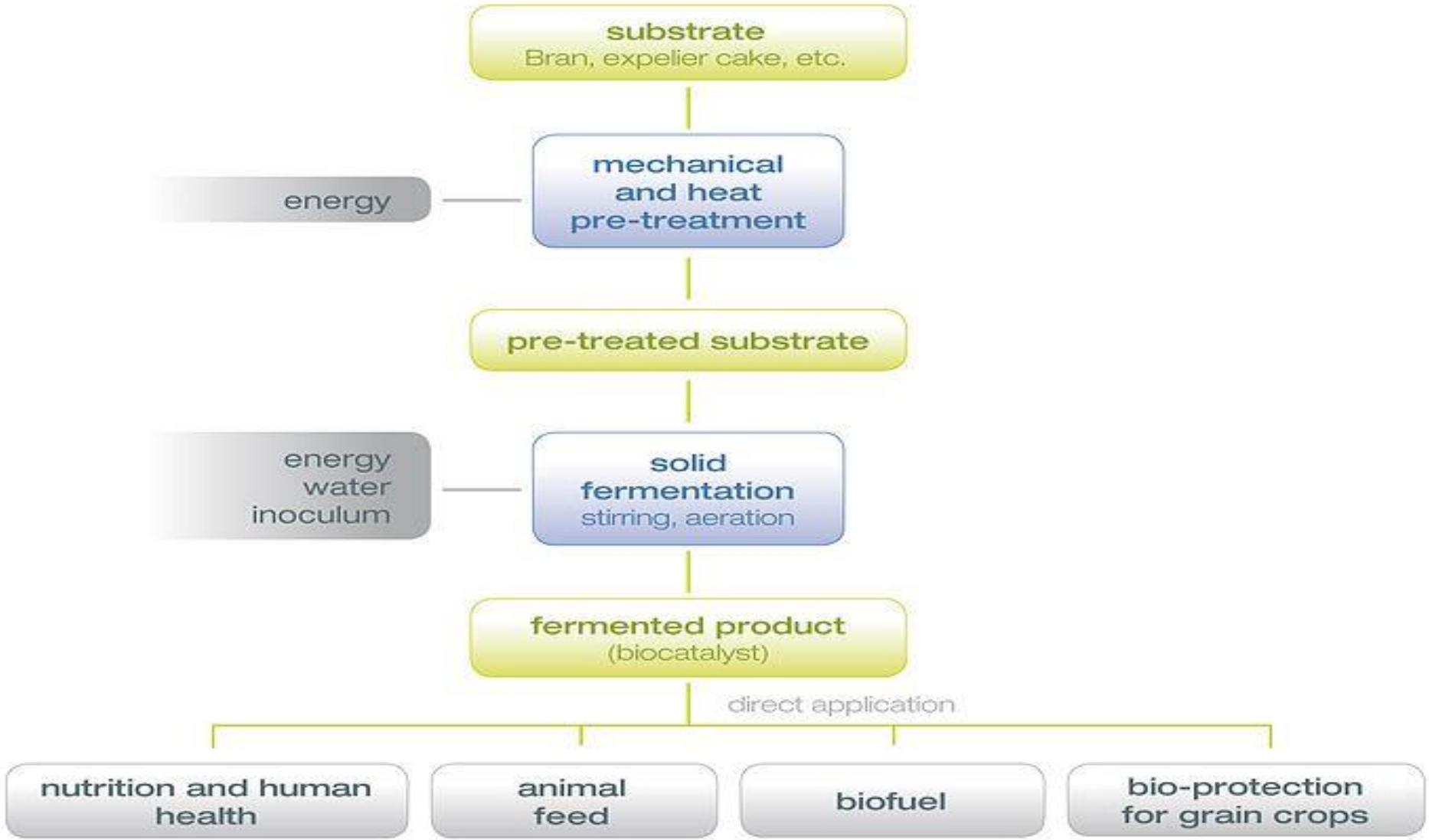
- Crude media constituents provides enough amount of growth factors so no extra addition of growth factor is required. If there is a lack of any kind if vitamins or nutrients, growth factors can be added to media.
- Examples are yeast extract, and beef extract.

5) Precursors

- Precursors are generally present in the media as crude constituents.
- Precursors are added in the fermentation media at time of fermentation as it get incorporated in the molecules of product without bringing any kind of change to the final product.
- This helps in improving yield and quality of product. Sometimes, precursors are added in pure form depending upon the need of product.
- For example, Cobalt chloride is added less than 10 ppm in fermentation of vitamin B12

6) Buffers

- Buffers are used to control drastic changes of pH. Sometimes, media components may act as buffers.
- For example, protein, peptides, mino-acids act as good buffers at neutral pH. Sometimes inorganic buffers like K_2HPO_4, KH_2PO_4, and $CaCO_3$ etc, can be added as required.
- Generally, during the fermentation process, pH changes to acidic or alkaline pH.
- The cheapest and easily available buffer is $CaCO_3$.


substrate
Bran, expelier cake, etc.
energy
mechanical and heat pre-treatment
pre-treated substrate
energy
water
inoculum
solid fermentation
stirring, aeration
fermented product
(biocatalyst)
direct application
nutrition and human health
animal feed
biofuel
bio-protection for grain crops

- STERILISATION OF FERMENTATION MEDIA

Methods for Sterilization of Media and Air

1. Sterilization of Culture Media:

- The constituents of culture media, water and containers contribute to the contamination by vegetative cells and spores.
- The media must be free from contamination before use in fermentation.
- Sterilization of the media is most commonly achieved by applying heat and to a lesser extent by other means (physical methods, chemical treatment, and radiation).

a)Heat sterilization:

- Heat is the most widely used sterilization technique. The quality and quantity of contamination (i.e., the type and load of microorganisms), composition of the media and its pH and size of the suspended particles are the important factors that influence the success of heat sterilization.

- In general, vegetative cells are destroyed at lower temperature in a short time (around 60°C in 5-10 minutes). However, destruction of spores requires higher temperature and relatively longer time (around 80°C for 15-20 minutes). Spores of Bacillus stearothermophilus are the most heat resistant. In fact, this organism is exploited for testing the sterility of fermentation equipment.

2.Physical methods:

- The physical methods such as filtration, centrifugation, and adsorption (to ion-exchangers or activated carbon) are in use. Among these, filtration is most widely used.

- Certain constituents (vitamins, blood components, antibiotics) of culture media are heat labile and therefore, are destroyed by heat sterilization.

- Such components of the medium are completely dissolved (absolutely essential or else they will be removed along with microorganisms) and then subjected to filter sterilization.

- **There are a couple of limitations of filtration technique:**
- 1. Application of high pressure in filtration is unsuitable for industries
- 2. Some of the media components may be lost form the media during filtration.
- Sometimes, a combination of filtration and heat sterilization are applied. For instance, the water used for media preparation is filtered while concentrated nutrient solution is subjected to heat sterilization. The filtered water is now added for appropriate dilution of the media. The chemical methods (by using disinfectants) and radiation procedures (by using UV rays, y rays, X-rays) are not commonly used for media sterilization.

- **Batch sterilization:**
- The culture media are subjected to sterilization at 121°C in batch volumes, in the bioreactor. Batch sterilization can be done by injecting the steam into the medium (direct method) or injecting the steam into interior coils (indirect method). For the direct batch sterilization, the steam should be pure, and free from all chemical additives (that usually come from steam manufacturing process).

- **There are two disadvantages of batch sterilization:**
- **1. Damage to culture media:**
- Alteration in nutrients, change in pH and discolouration of the culture media are common.

- **2. High energy consumption:**
- It takes a few hours (2-4 hrs.) for the entire contents of the bioreactor to attain the requisite temperature (i.e. 120°C). Another 20-60 minutes for the actual process of sterilization, followed by cooling for 1-2 hours. All this process involves wastage of energy, and therefore batch sterilization is quite costly.

- **Continuous sterilization:**
- Continuous sterilization is carried out at 140°C for a very short period of time ranging from 30 to 120 seconds. (This is in contrast to the batch fermentation done at 121°C for 20-60 minutes).
- This is based on the principle that the time required for killing microorganisms is much shorter at higher temperature.
- Continuous sterilization is carried out by directly injecting the steam or by means of heat exchangers.
- In either case, the temperature is very quickly raised to 140°C, and maintained for 30- 120 seconds. The stages of continuous sterilization process and the corresponding temperatures are depicted .
- The different stages are— exchanger, heater, heat maintenance unit, recovery of residual heat, cooling and fermenter.

- In the continuous sterilization process, 3 types of heat exchangers are used.

1. heat exchanger raises temperature to 90-1 20°C within 20-30 seconds.
2. exchanger further raises temperature to 140°C and maintains for 30-120 seconds.
3. heat exchanger brings down the temperature by cooling in the next 20-30 seconds.

- The actual time required for sterilization depends on the size of the suspended particles.
- The bigger is the size, the more is the time required.

Advantage in continuous sterilization

- about 80-90% of the energy is conserved.

- The limitation however, is that certain compounds in the medium precipitate (e.g., calcium phosphate, calcium oxalate) due to very high temperature differences that occur in a very short time between sterilization and cooling.
- The starch-containing culture media becomes viscous in continuous sterilization and therefore is not used.

- **2. Sterilization of Air:**
- In general, the industrial fermentations are carried out under vigorous and continuous aeration. For an effective fermentation, the air should be completely sterile, and free from all microorganisms and suspended particles. There is a wide variation in the quantity of suspended particles and microbes in the atmospheric outdoor air.

- The microorganisms may range from 10-2,000/m^3 while the suspended particles may be 20-100,00/ m^3. Among the microorganisms present in the air, the fungal spores (50%) and Gram-negative bacteria (40%) dominate. Air or other gases can be sterilized by filtration, heat, UV radiation and gas scrubbing. Among these, heat and filtration are most commonly used.

- **a) Air sterilization by heat:**
- In the early years, air was passed over electrically heated elements and sterilized. But this is quite expense, hence not in use these day

- **(b) Air sterilization by filtration:**
- Filtration of air is the most commonly used sterilization in fermentation industries.

- **Depth filters:**
- When the air is passed through a glass wool containing depth filters the particles are trapped and removed (Fig. 19.8). This filtration technique primarily involves physical effects such as inertia, blocking, gravity, electrostatic attraction and diffusion. Glass wool filters can be subjected to steam sterilization and reused. But there is a limitation in their reuse since glass wool shrinks and solidifies on steam sterilization. In recent years, glass fiber filter cartridges (that do not have the limitations of glass wool filter) are being used.

- **Membrane cartridge filters:**
- These are removable pleated membrane filters made up of cellulose ester, nylon or polysulfone. Membrane cartridge filters are smaller in size, simpler for operation and replacement. The most important limitation of air sterilization is that there is no filter that can remove bacteriophages. Bacteriophages are capable of crippling the industrial fermentation. e.g., bacteriophages interfere in the production of glutamic acid by Corynebacterium glutamicum.

Industrial biotechnology and it’s application

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- FERMENTER(bioreactor)

• Closed container 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.

• Is a device in which a substrate of low value is utilized by living cells or enzymes to generate a product of higher value.

• Extensively used for food processing, fermentation, waste treatment, etc.

- BIOREACTOR

• 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.

• Generally, 20-25% of fermenter volume is left unfilled with medium as “head space” to allow for splashing, foaming and aeration.

• The fermenter design varies greatly depending on the type and the fermentation for which it is used

- BIOREACTOR
- A bioreactor should provide for the following:
- Agitation (for mixing of cells and medium),
- Aeration (aerobic fermenters); for O2 supply,
- Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level etc.,
- Sterilization and maintenance of sterility, and
- Withdrawal of cells/medium (for continuous fermenters).
- Modern fermenters are usually integrated with computers for efficient process monitoring, data acquisition, etc.

SIZE OF FERMENTERS(BIOREACTOR):

- The size of fermenters ranges from 1-2-liter laboratory fermenters to 5,00,000 liters or, occasionally, even more, fermenters of up to 1.2 million liters have been used.
- The size of the fermenter used depends on the process and how it is operated.

1. CONSTRUCTION OF FERMENTERS:

• Large-scale industrial fermenters are almost always constructed of stainless steel.

• A fermenter is a large cylinder closed at the top and the bottom and various pipes and valves are fitted into it.

CONSTRUCTION OF FERMENTERS(BIOREACTOR):

• Since most industrial fermentation process is aerobic, the construction of a typical aerobic fermenter is the following:

I. Cooling Jacket:

• The fermenter 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 fermenter.

- II. Aeration system:
- Critical part of a fermenter.
- In a fermenter 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.
- Two separate aeration devices are used to ensure proper aeration in fermenter.

i. Sparger (series of holes in a metal ring)

ii. Impeller(also called agitator) 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.

- III. Baffles:

- The baffles are metal strips normally incorporated into fermenters of all sizes to prevent a vortex and to improve aeration efficiency. IV. Controlling Devices for Environmental Factors

- Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cells mass, levels of key nutrients, and product concentration.

- Use of Computer in Fermenter

- Computers are used to model fermentation processes in industrial fermenters.

- Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition, data storage, and error-detection.

TYPES OF FERMENTER:

Following are the types of fermenter

- Airlift Fermenter
- Continuous Stirred Tank Bioreactors
- Photo-Bioreactors
- Bubble Column Fermenter
- Fluidized Bed Bioreactors
- Packed Bed Bioreactors

1.Airlift Fermenter:

- In airlift fermenter the liquid culture volume of the vessel is divided into two interconnected zones by means of a baffle or draft tube.
- Only one of the two zones is sparged with air or other gas and this sparged zone is known as the riser.
- The other zone that receives no gas is called down-comer.
- The bulk density of the gas-liquid dispersion in the gas-sparged riser tends to be lower than the bulk density in the down-comer.
- consequently the dispersion flows up in the riser zone and down-flow occurs in the down-comer.

• Airlift fermenters are highly energy-efficient.

• They are often used in large-scale manufacture of biopharmaceutical proteins obtained from fragile animal cells.

• Heat and mass transfer capabilities of airlift reactors are at least as good as those of other systems.

• Airlift reactors are more effective in suspending solids than are bubble column fermenters.

• All performance characteristics of airlift -fermenter are related ultimately to the gas injection rate and the resulting rate of liquid circulation.

• The rate of liquid circulation increases with the square root of the height of the airlift device.

• Because the liquid circulation is driven by the gas hold-up difference between the riser and the down-comer. • circulation is enhanced if there is little or no gas in the down-comer.

• All the gas in the down-comer comes from being entrained in with the liquid as it flows into the down-comer from the riser near the top of the reactor.

2.Continuous Stirred Tank Bioreactors:

• A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers).

• The shaft is fitted at the bottom of the bioreactor.

• The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio.

• Several types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use.

• The air is added to the culture medium under pressure through a device called sparger.

• The sparger may be a ring with many holes or a tube with a single orifice.

• The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel.

• The bubbles generated by sparger are broken down to smaller ones by impellers and dispersed throughout the medium.

• This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

Advantages of STRs:

There are many advantages of STRs.

• The efficient gas transfer to growing cells. • Good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

3.Photo-Bioreactors:

• These are the bioreactors specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination.

• Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred.

• Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin.

Types of photo- bioreactors

a.Continuous run tubular loop

b.Multiple Parallel tube

c.Helical wound tubular loop

d.Flat panel configuration

- They are made up of glass or more commonly transparent plastic.
- The array of tubes or flat panels constitute light receiving systems (solar receivers).
- The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps.
- It is essential that the cells are in continuous circulation without forming sediments.
- Further adequate penetration of sunlight should be maintained.
- The tubes should also be cooled to prevent rise in temperature

4.Bubble Column Fermenter

- Bubble column fermenter is usually cylindrical with an aspect (height to diameter) ratio of 4-6.
- Gas is sparged at the base of the column through perforated pipes,perforated plates,or sintered glass or metal micro-porous spargers.
- O2 transfer,mixing and other performance factors are influenced mainly by the gas flow rate and the properties of the fluid.
- Internal devices such as horizontal perforated plates,vertical baffles and corrugated sheet packing,s may be placed in the vessel to improve mass transfer and modify the basic design.
- One exception is the axial mixing performance.
- For a given gas flow rate,the mixing improves with increasing vessel diameter.

4. Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid.

- The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out.
- These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs.

Fluidized Bed Bioreactors:

- For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed.
- It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state.
- Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts.
- This enable good efficiency of bioprocessing.

5.Packed Bed Bioreactors:

- A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor.
- The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature.
- A nutrient broth flows continuously over the immobilized biocatalyst.
- The products obtained in the packed bed bioreactor are released into the fluid and removed.
- While the flow of the fluid can be upward or downward, down flow under gravity is preferred.

- The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth.
- Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali.
- However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions.
- The packed bed bioreactors do not allow accumulation of the products to any significant extent.

Thank you