

FERMENTATION

By

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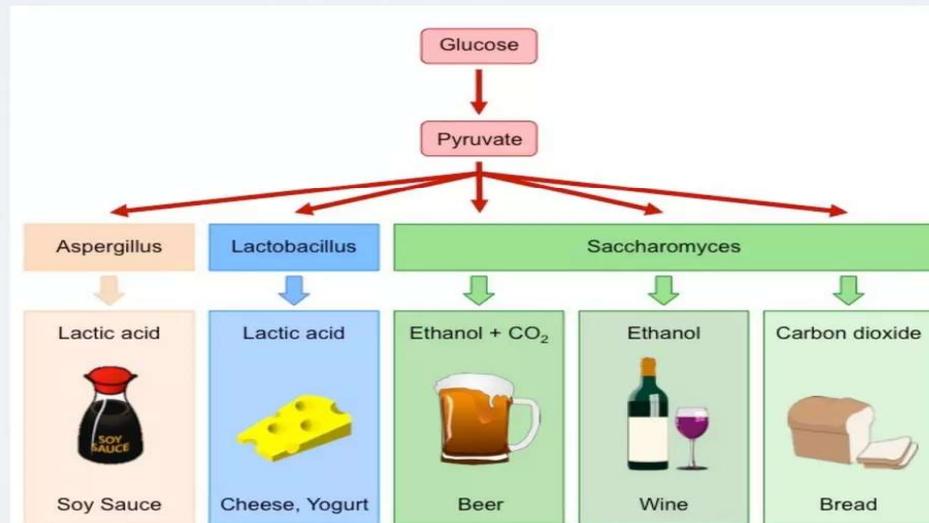
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INTRODUCTION

- The science of Fermentation is called ZYMOLOGY.
- Fermentation means any process that converts larger molecules into smaller and simpler molecules using micro-organisms I.e. it is a metabolic anaerobic process that converts Sugars(larger molecules) to acids , gases , alcohol or any metabolic by-product.
- Thus in biotechnology Fermentation is a process in which micro-organisms are grown in large quantities by providing them favourable conditions which converts Organic complex substances to simpler compounds.
- Fermentation also occurs in oxygen starved muscle cells , as in case of Lactic acid Fermentation.

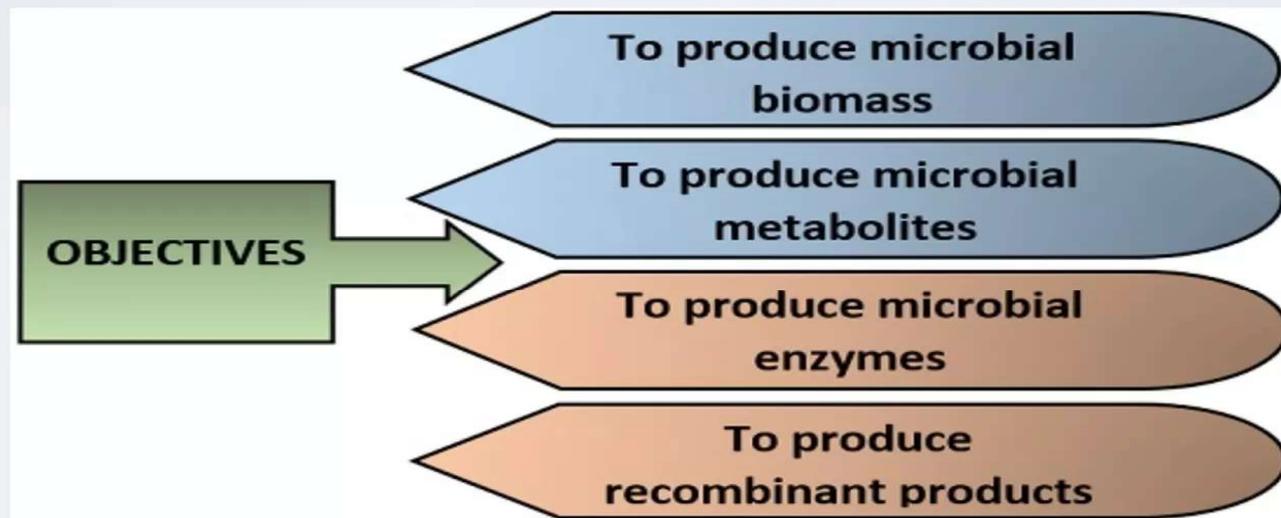


Production of Fermented Foods by Bacteria and Yeast (*Saccharomyces*)

OBJECTIVES OF FERMENTATION PROCESS

The major objectives of fermentation are:

- To produce large amount of microbial cells or Biomass (Large microbial colonies).
- To produce microbial enzymes(e.g- proteases ,invertase etc).
- To produce microbial metabolites (e.g-microbial metabolite of Glucose are Lactic acid , Ethanol etc.
- To produce recombinant products(e.g- productions of several vaccines).



Objectives of fermentation process

BASIC STEPS OF FERMENTATION PROCESS AND GENERAL REQUIREMENTS

The basic steps for fermentation process are:

1. **Formulation of fermentation media:** it is chemically a cell culture media which is a mixture of molecules used for cellular metabolism and growth. e.g. Carbon source, energy source, oxygen, nitrogen etc.
2. **Sterilisation:** sterilisation of fermentation media, Fermenters and other equipments.
3. **Inoculum formation:** production of an active , pure culture In sufficient quantity to inoculate into the production vessel.
4. **Fermentation:** growth of the micro-organism in the production fermenter under optimum conditions for formation of by-products.
5. **Extraction and purification** of the product.

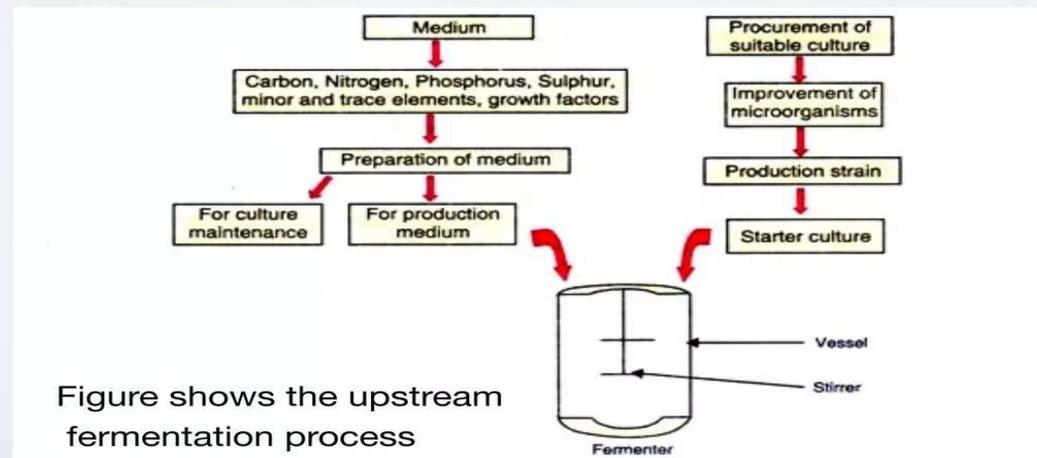


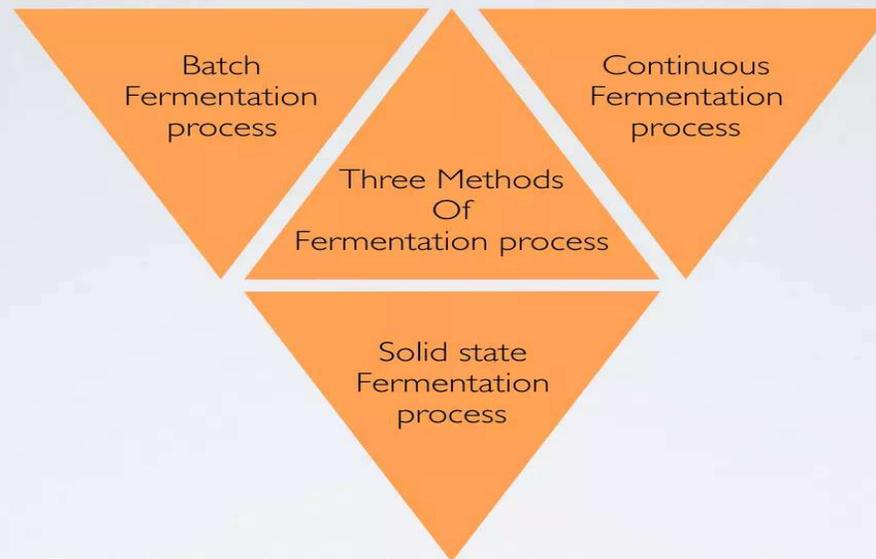
Figure shows the upstream fermentation process

BASIC STEPS OF FERMENTATION PROCESS AND GENERAL REQUIREMENTS

The general requirements for fermentation process are:

- 1. Fermentation medium**
- 2. Inoculum**
- 3. Fermenter**
- 4. Sterilisation**
- 5. Growth of organism**
- 6. Extraction and purification**

METHODS OF FERMENTATION PROCESS



- **Batch fermentation:** Batch culture is a closed culture system which contains limited amount of nutrients.
- **Continuous fermentation:** it is open culture system where nutrients are continuously added to fermenter and product is continuously removed at same rate.
- **Solid state fermentation:** the fermentation medium is in the solid state , here the micro-organisms are grown on solid substratum in the absence or near absence of free water.

STUDY OF MEDIUM

Fermentation medium:

- Medium with nutritional and hormonal requirements of cells in optimum concentration.
- Small modifications in the medium could change cell line stability, product quality, and yield.

Composition of media:

=>Carbon source-dextrin, glucose, starch etc

=>Nitrogen source-yeast extract, soybean meal, cotton seed meal etc.

=>Inorganic salts-ammonium salts, ammonium nitrate etc.

=>Vegetable/Animal fat-soybean oil, linseed oil etc.

=>Growth regulators - Vitamin B12

=>Trace elements-zinc, copper etc.

=>Buffers-to maintain pH eg calcium carbonate etc

=>Oxygen etc

Inoculum:

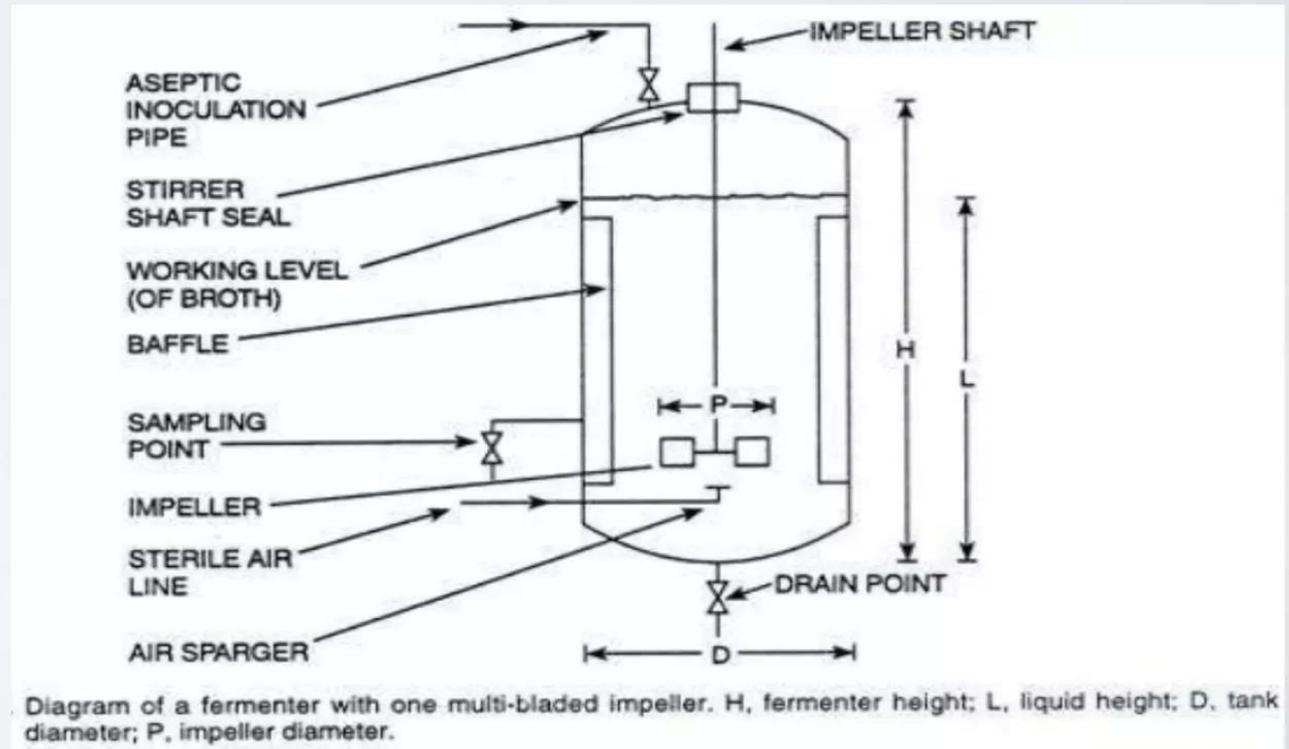
- It is the pure bacterial cell culture that is introduced to the medium .
- The cell then grows in the medium , conducting metabolism.

EQUIPMENTS

Fermenter:

- A Fermenter is a device that accomplishes the fermentation process by the help of certain microorganisms, and thus it also refers as “**Biofermenter** or **Bioreactor**“. it is equipped with all the elements that are necessary to carry out the commercial production of substances like antibiotics, enzymes, beverages etc. in many industries.

BASIC DESIGN OF FERMENTER



EQUIPMENTS

S.No.	Part	Purpose
1	Top plate	cover (made of steel)
2	Clamp	top plate compressed onto vessel using clamp
3	Seal	separates top plate from vessel (glass) to prevent air leakage
4	Vessel	glass, jacketed, steel with ports for various outputs, inputs, probes etc
5	Drive motor	used to drive mixing shaft
6	Drive shaft	mixes the medium evenly with its impeller
7	Marine impeller	for plant tissue culture
8	Baffles	prevent sedimentation on sides and proper mixing
9	Sparger	air supplier / after filtration via membranes – ensures efficient dispersal – by attached to impeller
10	Exit gas cooler	like condenser remove as much moisture as possible from exhaust
11	Inoculation needle	port to add inoculum
12	Feed pumps	regulates the flow rates of additives (medium, nutrients) variable speed
13	Peristaltic pumps	fixed speed pumps – used for continuous sampling
14	Syringe pump	using a syringe – mostly used in batch
15	Exit gas analysis	CO ₂ analyzer, O ₂ analyzer, mass spectrometer
16	Sample pipe	through which samples are drawn
17	3 way inlet	to insert different probes

VARIOUS COMPONENTS OF AN IDEAL FERMENTER WITH THEIR PURPOSES

EQUIPMENTS

Specific parts of fermenters are:

1-Body: It is important to select the material that can withstand repeated steam sterilisation cycles .

e.g. Glass : advantages-

- gives smooth surface
- Non toxic
- Corrosion proof
- Easy to examine the interior part of vessel during fermentation process.
- Steel: As per AISI(American Iron and Steel Institute) -Steel alloys are those containing less than 4% chromium while Stainless steel are those containing more than 4% chromium.
- For making of fermenter body mostly stainless steels are preferred
- Steel coated with glass/phenolic epoxy can also be used.

2- Seal: to seal top plate and vessel to prevent air leakage.

Types are :

- Gasket seal- can be used to seal glass and metal
- Lip seal- for joining of glass to metal
- O-ring seal-for joining of metal to metal or glass to metal.

EQUIPMENTS

3-Agitator: (Impellers)

The agitator is required for mixing of bulk fluid , gas phase mixing , air dispersion, oxygen transfer etc to maintain uniform environment.

Types of agitator are as follows:

- a)
 - Mounted to a shaft through a bearing in the lid
- b)
 - Driven by an external power source or direct drive
- c)
 - Direct drive - action varied by using different impeller blades
- d)
 - Spining of medium in circular direction

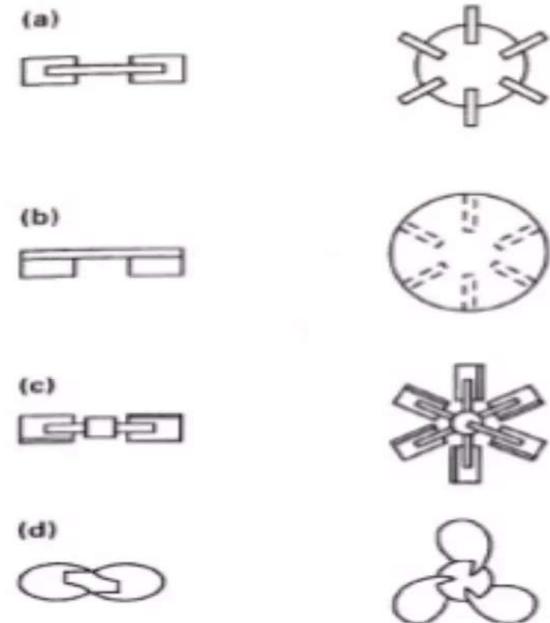
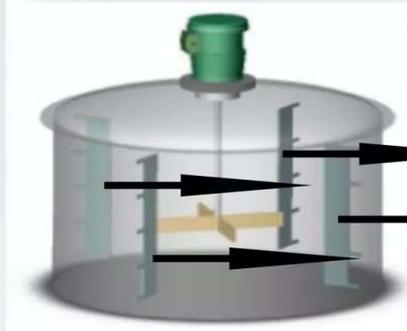


FIG. 7.9. Types of agitators: (a) disc turbine; (b) vaned disc; (c) open turbine, variable pitch; (d) marine propeller (Solomons, 1969).

EQUIPMENTS

4-Baffles: There are normally 4 baffles incorporated into of vessel of fermenter of all sized to prevent vortex (swiril effect) or to improve proper aeration efficiency. Baffles are metal strips which are radially attached to the fermenter wall.



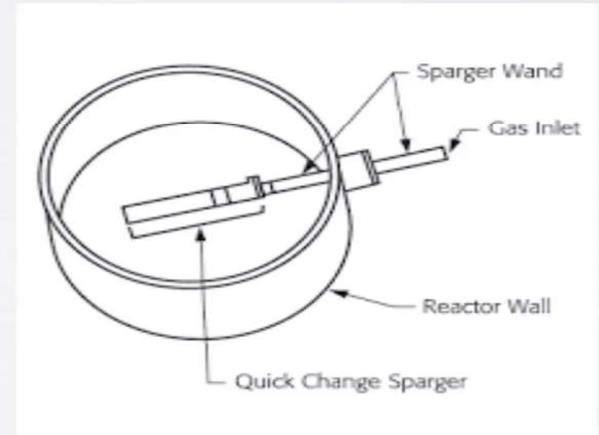
Four baffles in the fermentation vessel

5-Spargers: The air introducing device in the fermenter is the Spargers. The different types of sparers are:

- Orifice spargers
- Porous spargers
- Nozzle spargers



Sparger giving out air forming bubbles in the liquid

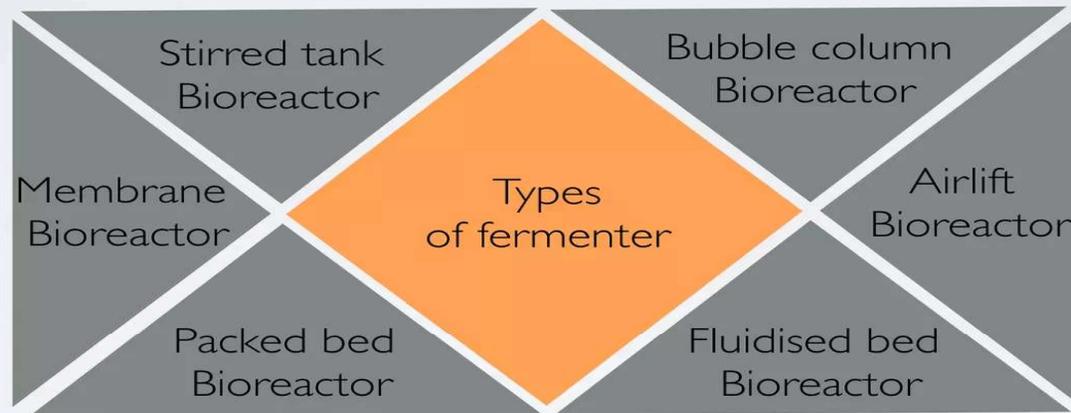


Sparger device

TYPES OF FERMENTER

Continuous Stirred tank bioreactor: In the CSTR, with one or extra agitator, fermentation broth is continuously stirred and the composition is identical and homogeneous to that for outgoing flow into the reactor. Application of the CSTR is constant flow of the gas throughout the liquid that normally contains a diluted solution of basic nutrients for the organism to develop and survive on.

Airlift bioreactor: Airlift fermenter is also recognised as tower reactor. An airlift fermenter vessel is cylindrical in shape in which air is presented at the bottom of receptacle which arises through the column to the medium. It capable of using for free or together with immobilised cells that are suitable for bacteria, yeast, fungi and animal and plant cells. These type of the fermenter does not have agitation system, but contents in the vessel is agitated with the air introduced from the bottom.

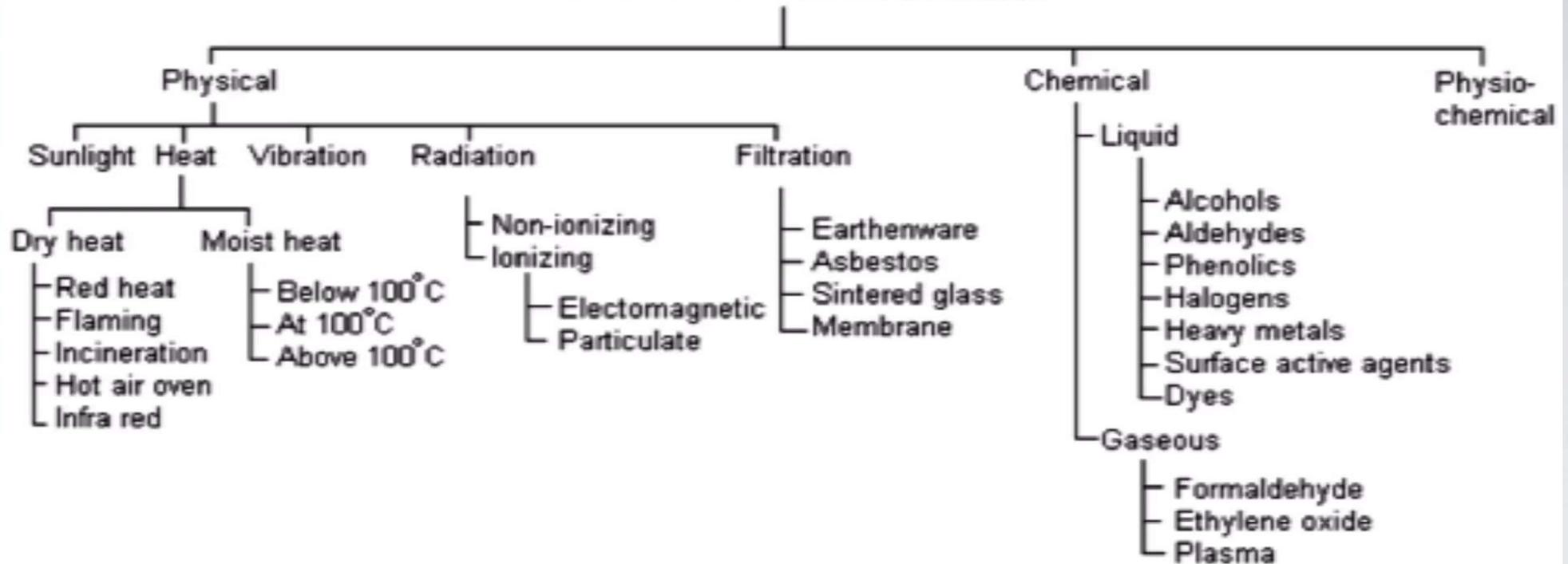


Packed bed bioreactor: Packed bed bioreactor is well known as fixed bed bioreactor. It is normally used in wastewater engineering with biofilms attached.

Fluidised bed bioreactor: This type of reactor contains packed bed with small size particles which moves the fluid. The minor particle size accelerates higher rate of oxygen transfer, mass transfer, higher mixing rates and nutrients to the cells. FBB has many advantages over other type of reactor so in current years use of this type of reactor has increased. Fluidized bed reactor are developed for the biological system has cells as biocatalyst that are three phase system (liquid, solid and gas).

STERILISATION METHODS

Methods of sterilization/ disinfection



- Heat sterilisation is the most commonly used for sterilisation of media or vessels.
- Medium is sterilised at high heat and high pressure .
- Medium can be sterilised by Filtration, Radiation, chemical treatment or heat.

STERILISATION METHODS

- **Heat:** For sterilization, the type of heat, time of application and temperature required to ensure destruction of all microorganisms must always be considered. Endospores of bacteria are the most thermo-resistant of all cells so their destruction usually guarantees sterility.
- **Boiling:** Boiling is done at $>100^{\circ}\text{C}$ for 20-30 min. It kills everything except for some endospores. To kill endospores and therefore perfectly sterilize the solution, very long boiling is required.
- **Autoclaving:** Autoclaving is the process of using steam under pressure in an autoclave or pressure cooker. It involves heating at 121°C for 15-20 min under 15 psi pressure and can be used to sterilize almost anything.
- **Dry Heat (Hot Air Oven):** The process involves heating at 160°C for 2 hours or at 170°C for 1 hour. It is used for glassware, metal and objects that will not melt.
- **Sterilization in industry-scale fermenters (or bioreactors)** is more complex. Steam is used to sterilize fermentation media sterilisation is the most commonly used for sterilisation of media or vessels.

AERATION PROCESS

- Aeration refers to the process of introducing air to increase oxygen concentration into liquids.
- Aeration may be performed by bubbling air through liquid, spraying the liquid or by agitation of liquid.
- Sparger is a device which is installed into the vessel to introduce air into the fermentation mixture.
- The structural components of aeration are: the agitator and sparger.
- The majorities of fermentation processes are aerobic and, therefore, require the provision of oxygen. Therefore, a microbial culture must be supplied with oxygen during growth at a rate sufficient to satisfy the organism's demand.
- The agitator (impeller)-The main aim of the agitator is to provide a homogeneous environment all over the fermenter. It is also used for mixing of different phases, oxygen and heat transport.

AERATION PROCESS

- The aeration system (sparger) -A sparger is a tool used for introducing air into the fermentation medium.
- Three basic types of sparger
 - 1. The porous sparger,
 - 2. The orifice sparger (a perforated pipe) and
 - 3. The nozzle sparger (an open or partially closed pipe).
- Porous Sparger :The porous sparger is mainly used for laboratory scale. It is made up of sintered glass, ceramics or metal.
- Orifice Sparger :In small stirred fermenters the perforated pipes were arranged below the impeller in the form of crosses or rings (ring sparger).
- Nozzle Sparger :Most modern mechanically stirred fermenter designs from laboratory to industrial scale have a single open or partially closed pipe as a sparger to provide the stream of air bubbles.

STIRRING PROCESS

- Mixing is an essential part of fermentation processes.
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- There are two sources of fluid-mixing energy in a fermentation process; mixing impellers provide circulation of fluids through the tank, and the expansion and velocity of air passing through a fermentor imparts fluid motion.
- The major steps in fermentation ,includes the stirring or mixing as major process like:
 - — Gas-liquid steps- physical dispersion of air in tank or oxygen transfer from gas to liquid
 - — Liquid-Solid steps-oxygen transferred from liquid to solid which effects the growth of organism.
 - — Blending steps-Blending of solids and liquids throughout entire vessel. And homogenous mixing of oxygen throughout vessel.
 - — Heat transfer.