**National Education Policy 2020 (NEP-2020)**

**E-Content for of M.Sc. 3rd sem Botany**

**(Elective Course-1: Industrial Microbiology Course Code: BOT3003-EL1)**

**Unit-I "Bioreactor or Fermenter"**

**developed by**

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**Bioreactors**

Bioreactors are the heart of bioprocesses. Any bioprocess technology will be successful if the process is scaled up without any unsurmountable problem. Reactor design is one the aspects which should be taken care with utmost care. Besides the reactor design, the technology part of the bioprocess such as upstream, fermentation and downstream processes are the main unit operations which must be optimized for the economic production of bioproduct. In this lecture the design of bioreactor is discussed with a reference to various modes of operation such as batch, semi batch and continuous. A bioreactor provides a controllable environment enabling the biological, biochemical, and biomechanical requirements to manufacture bioproducts. As the bioreactor aims to create a desired biological product, it is important to closely monitor the reaction parameters like pH, temperature, agitation, aeration, internal and external mass transfer, heat transfer, fluid velocity, shear stress etc. The effects of such reaction variables on biological cultures and analyzing the other parameters such as oxygen transfer coefficient, specific oxygen uptake rate, substrate utilization rate, product formation rate is very much needed. Sophisticated and sound bioreactor design with unique performance characteristics is essential in production of useful biotechnological products from natural and genetically modified cell system. Bioreactors differ from conventional chemical reactors to the extent that they support and control biological entities. As the organisms are more sensitive and less stable than chemicals, bioreactor system must be robust enough to provide a higher degree of control over process upsets and contaminations. The paper discusses the bioreactor design and various types of bioreactors, which are useful for industrial operations.

**Selection of the bioreactor**

• Characteristics predetermined by the nature of the organism

• Characteristics predetermined by the properties of the medium

• Characteristics predetermined by the parameters of the biochemical process

• Characteristics predetermined by the site of installation Characteristics predetermined by the nature of the organism

• The size and shape of the cells also have a considerable influence on the type of reactor and its operation. Spherical cells are usually smaller and less sensitive to shear than filamentous organism.

• Spherical cells need a higher degree of dispersion of air than filamentous mycelia. High dispersing forces may cause damage, sensitive to shear,

• Small dimension ensures a high surface-to-volume ratio and a high rate of uptake of substrate and oxygen, therefore rapid growth.

• Filamentous organism only grows at the end of the threads. This leads to a low rate of growth and to a low oxygen demand

• Agglomerates of cells have a low surface-to-volume ratio, a low rate of uptake of substrate, and a low rate of growth.

• These agglomerates are often sensitive to shear stress. On increasing the mechanical stress, the same organism may form pellets. This leads to a marked reduction in the apparent viscosity of the medium. Characteristics predetermined by the properties of the medium

• The choice of the culture medium exerts a pronounced influence on the choice of reactor. The physical properties of the substrates are different: gaseous (e.g. methane), liquid (methanol, ethanol) soluble solids (glucose, lactose) and insoluble solids (cellulose, peanut meal etc).

• In the case of the substrates showing inhibition or repression of growth, the process is carried out either in “fed batch” or in a “continuous culture”.

• The use of heat-sensitive components of the medium requires special measures and separately sterilized.

• The small bubbles formed by effective gassing devices grow very rapidly by coalescence if they come into a region of low energy dissipation

• In media with coalescence-enhancing properties (more coalescence means more foam formation and more antifoams are used) the rate of energy dissipation must be as uniform as possible throughout the reactor

• A high apparent viscosity is always associated with a non-Newtonian behavior. It is due to the higher concentration of substrate, secretion of viscous products, morphology of the organism

• Any formation of foam during cultivation interferes considerably with the operation of the plant.

• The out let filter of the gas may be blocked with the cells-containing foam and is not desirable. Characteristics predetermined by the site or location

• The choice of setting up of the fermentation industry depends on the production site

• Raw material supply and costs (availability sugar in the form of starch, molasses, sugar syrup etc.)

• Trading facilities for the products and raw materials.

• Availability of qualified of manpower.

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• Market features (stable sales-single product plant; variable sales-flexible plant). Scale up

• Scale up means increasing the scale of operation. From laboratory scale to pilot scale and from pilot scale to plant scale.

• Increase in scale means increase in volume and increase in the associated problems of process scale up.

• It should be the task of biochemical engineers to scale up the process to any scale without losing process yield. Environmental parameters

• The increase in scale may change the environment for the organism. The different environmental parameters are:

• Nutrient availability

• pH

• Temperature

• Shear condition

• Dissolved CO2 concentration

• Dissolved O2 concentration

• Foam formation All the above parameters are affected by agitation and aeration Things that change on Scale-Up

• Heat transfer

• Quality of mixing

• Shear (agitator and impeller tip speed)

• Superficial air velocity, i.e. flooding tendency

• Time of inoculum transfer

• Time to set the fermentation

• Culture age and stability

• Hidden auxotroph

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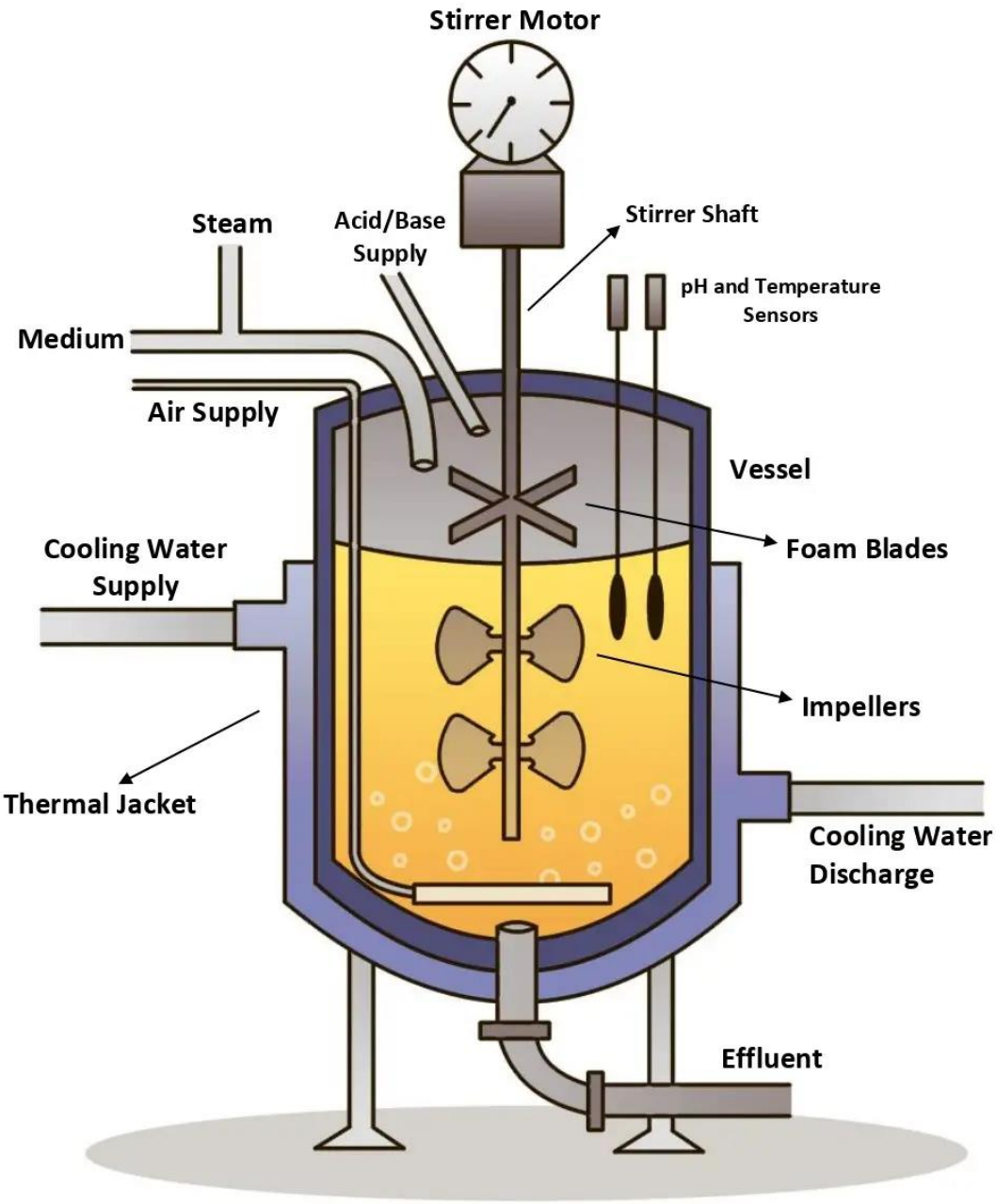


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**BIOREACTOR DESIGN ENGINEERING-I**

**BIOREACTOR DESIGN ENGINEERING-II**

**BIOREACTOR**

Vessel or tank in which whole cells or cell-free enzymes transforms raw materials into biochemical products and/or less undesirable by-products. These are also termed a Fermenters. The basic function of a Bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product that may be - cell biomass, - a metabolite, - or bioconversion product.

**Ideal bioreactor should be capable of**:

• High Biomass concentration

• Maintain sterile conditions

• Efficient power consumption

• Efficient Oxygen Transfer to media

• Effective Temperature control.

• Correct shear conditions

• Sampling facilities

**Types of BIOREACTOR/Fermenters**

- Batch – Most are batch process, which are closed systems where there are no additions following inoculation, apart from acid or alkali for pH control or air input.
- Fed-batch – Extra nutrients are added as the fermentation progresses.
- Continuous – An open system where fresh medium is continuously added and culture is simultaneously removed at the same rate, resulting a constant working volume.

**Aseptic operation**

It is a very important and foremost design aspect. Almost every fermentation reaction requires aseptic environment to carry on, otherwise contamination from unwanted microbes ruins the reaction. Every component of fermenter that is in contact with outer environment should not be in direct contact with inner vessel and if it is in direct contact then there should be provision for regular sterilization when it is used. e.g. Sampling ports and addition ports. Aseptic environment should be maintained in the fermenter till the fermentation is over and product is recovered aseptically.

**Media and vessel sterilization**

Pilot-scale and industrial fermenters can be sterilized empty and then they are filled with sterile medium or can also be sterilized with the media in it. Smaller fermenters can be filled with medium and the two are sterilized together in an autoclave/in situ sterilization.

**Design of a Fermenter**

• In a fermenter H/D ratio, L/D ratio, P/D ratio, values of Z, Y, W and V are very important design parameters.

H/D ratio normally lies between 1.5 to 3.

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L/D ratio lies between 1 to 2.5.

P/D ratio lies around 0.75.

Values of Z, Y, W, V lies around 50 to 100% of value of P.

**Basic Fermenter Design Criteria**

(i). Nature of microbial (or mammalian, plant tissue) cell;

(a) Hydrodynamic characteristics

(b) Mass and Heat Transfer

(c) Kinetics

(d) Genotype and Phenotype

(ii). Environmental Control and Monitoring of the process

(a) pH, temperature, dissolved oxygen etc.

(b) Asepsis and avoidance of contamination

(iii). Process factors;

(a) Effect on other unit operations

(b) Economics

(c) Potential for scale-u

**Bioreactors-Types**

There are 3 groups of bioreactors currently used for industrial production;

Non-Stirred, Non-Aerated

Non-Stirred, Aerated

Stirred, Aerated

Aeration (Sparger) Some fermentations operate anaerobically. But majority are aerobic and require the provision of large quantities of normally sterile air or oxygen. A sparger may be defined as a device for introducing air into the liquid in a fermenter.

Types of sparger

Porous sparger

Orifice sparger (a perforated pipe)

Nozzle sparger (an open or partially close pipe)

Mechanical Seal

Seals the system against contamination through agitation system. Two Parts: one part is stationary in the bearing housing and the other rotates on the shaft. The two components are pressed together by springs or expanding bellows. The two meeting surfaces have to be precision machined. The moving surface consists a carbon-faced Unit and the stationary unit a satellite-faced stainless steel.

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**Magnetic Drive**

It consists of two magnets: one driving and one driven. Driving magnet is held in bearings in a housing on the outside of a head plate and connected to a drive shaft. Driven magnet is placed on one end of the impeller shaft and held in bearings in a suitable housing on the inner surface of the head plate. In magnetically coupled drive, the impeller shaft does not pierce the vessel.

Bottom Driven Magnetically Coupled Lab Fermenter

A typical bioreactor used for microbial fermentations:

Laboratory scale bioreactors with liquid volumes of less than 10 litres are constructed out of glass. For larger reactors, stainless steel is used.

**Headspace volume**

A bioreactor is divided in a working volume and a head-space volume. The working volume is the fraction of the total volume taken up by the medium, microbes, and gas bubbles. The remaining volume is called the headspace. Typically, the working volume will be 70-80% of the total fermenter volume. This value will however depend on the rate of foam formation during the reactor. If the medium or the fermentation has a tendency to foam, then a larger headspace and smaller working volume will need to be used.

**Agitation System**

The function of the agitation system is to provide good mixing and thus increase mass transfer rates through the bulk liquid and bubble boundary layers. It provides the appropriate shear conditions required for the breaking up of bubbles. The agitation system consists of the agitator and the baffles. The baffles are used to break the liquid flow to increase turbulence and mixing efficiency. These are metal strips roughly one tenth width of the vessel diameter which are attached radially to the wall. These are needed to prevent vortex and to improve aeration efficiency. Commonly 4 baffles are incorporated into agitated vessels of all sizes.

**Axial flow impellers**

Axial flow mixing is considerably more energy efficient than radial flow mixing. They are also more effective at lifting solids from the base of the tank. Axial flow impellers have low shear properties. The angled pitch of the agitators coupled with the thin trailing edges of the impeller blades reduces formation of eddies in the wake of the moving blades. These are used for mixing shear sensitive processes such as crystallization and precipitation reactions. They are also used widely in the culture of animal cells. Their low shear characteristics generally makes them ineffective at breaking up bubbles and thus unsuitable for use in aeration of bacterial fermentations.

**Agitator design and operation Radial flow impellers - Rushton turbine**

The most commonly used agitator in microbial fermentations is the Rushton turbine. Like all radial flow impellers, the Rushton turbine is designed to provide the high shear conditions required for breaking bubbles and thus increasing the oxygen transfer rate. The Rushton turbine has 4 or 6 blades which are fixed onto a disk. The diameter of the Rushton turbine should be 1⁄3 of the tank diameter.

Top entry and bottom entry impellers

**Top entry impellers:**

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The impeller shaft can enter from the bottom of the tank or from the top. A top entry impeller ("overhung shaft") is more expensive to install as the motor and the shaft will need to be structurally supported.

**Bottom entry impellers:**

A reactor with bottom entry impeller however will need higher maintenance due to damage of the seal by particulates in the medium and by medium components that crystallize in the seal when reactor is not in use. Bottom entry agitators tend to require more maintenance than top entry impellers due to the formation of crystals and other solids in the seals.

**Bioreactor - Oxygen delivery system:** It consists of

A compressor

Inlet air sterilization system

An air sparger, Exhaust

(exit) air sterilization system

**Bioreactor- Oxygen delivery system-Air Compressor**

Oxygen delivery system – Sparger

Oxygen delivery system –Effect of impeller speed

Slow impeller speed Fast impeller speed

The bubbles will not be sheared into smaller bubbles and will tend to rise directly towards the surface

Smaller bubbles will be generated and these bubbles will move with throughout the reactor increasing the gas hold up and bubble residence time

Oxygen delivery system - Air flow rates are typically reported in terms of volume per volume per minute or vvm.

**Sterilizations of the air**

For larger laboratory scale fermenters (up to 1000 litres), pleated membrane filters housed in polypropylene cartridges are used.

**Exhaust-Condenser**

In small reactors, the exit air system will typically include a condenser.

Foam control system

By mechanical breaker or by antifoam agents

Foam is typically detected using two conductivity or "level" probes

When the upper-level probe is above the foam level, no current will pass between the level probes and the antifoam pump remains turned off.

When the upper-level probe is immersed in the foam layer, a current is carried in the foam. This causes the antifoam to turn on.

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**Temperature control system**

The heating/cooling requirements are provided by the following methods:

Laboratory scale reactors Pilot and production scale reactors heating electric heaters by Steam generated in boilers.

Cooling requirements by: Tap water or produced by refrigerated water baths, cooling towers or refrigerants such as ammonia.

**pH control system**

**Measuring & Control Equipment**

There are a number of control elements (or equipment) that are necessarily used in a fermentation plant to provide an effective Process like.

**Controls**

1. Ball valve
2. DiaphramValves
3. Pneumatic valves
4. Steam Trap
5. Air Flow Regulator
6. Pressure Relief Valve

**Measuring Devices**

1. Air Flow Meter
2. Steam flow meter
3. Water Flow meter

**Sensing Devices**

1. Pressure Sensor
2. pH Sensor
3. Dissolved Oxygen Sensor
4. Temperature Sensor

**Ball Valve:** These are generally ON-OFF type manually operated valves and are used to allow or prevent the flow of water, air, and steam when required.

**Diaphragm Valves:** Diaphragm valves (or membrane valves) consists of a valve body with two or more ports, a diaphragm, and a "saddle" or seat upon which the diaphragm closes the valve. The valve is constructed from either plastic or steel. These valves can be manual or automated. Their application is generally as shut-off valves in process systems within the food and beverage, pharmaceutical and biotech industries.

**Non-Return Valve (NRV)**

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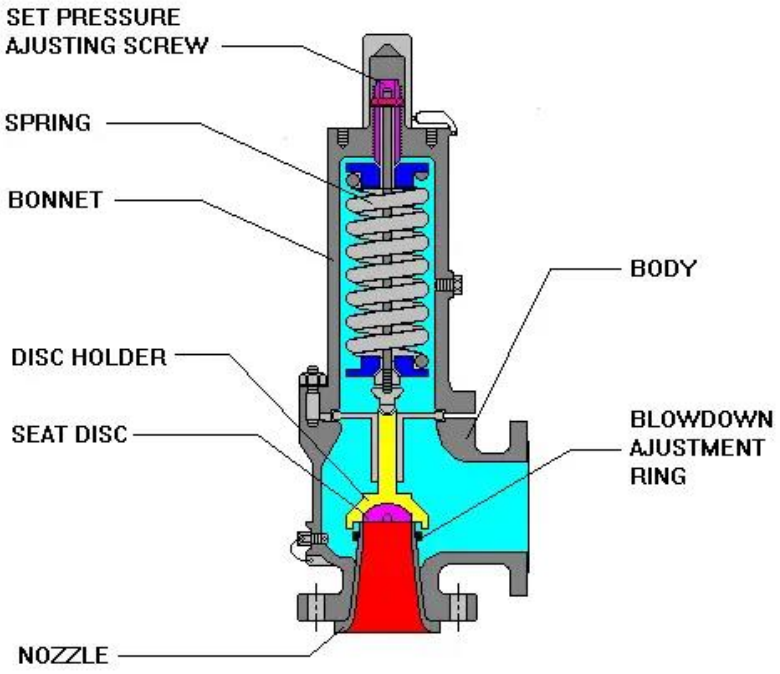
This valve is mainly used to provide the backpressure to the fluid flowing through it.

**Pressure Relief Valve (PRV)**

PRV is a safety device that relieves in case of overpressure in a vessel or piping. The generic terms are pressure relief valve (PRV) or pressure safety valve (PSV).




**Solenoid Valve (SOV)**

Solenoid Valve is an electromechanical valve for use with liquid or gas. The valve is controlled by an electric current through a solenoid coil. Their tasks are to shut off, release, dose, distribute or mix fluids .

**pH Electrode**

pH Electrode is nothing but a sensor that transmit the pH value to the pH transmitter.

**DO Electrode**

An oxygen sensor, or lambda sensor, is an electronic device that measures the proportion of oxygen ($O_2$) in the gas or liquid being analysed.

**Electrical Motor**

Electrical motor is an electrical device which converts the electrical power into mechanical rotation of shaft.

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**AC Drive**

A variable-frequency drive (VFD) is a system for controlling the rotational speed of an alternating current (AC) electric motor by controlling the frequency of the electrical power supplied to the motor. A variable frequency drive is a specific type of adjustable-speed drive.

**Instrumentation and Control**

The success of a fermentation process is highly dependent on environmental factors. The fermenter needs to be able to control such factors as temperature, pH, and dissolved oxygen levels.

**PLC -Programmable Logic Controller**

A programmable logic controller (PLC) or programmable controller is a digital computer used for automation of electromechanical processes.

Pilot Scale fermenter

Pilot Scale Fermenter Side View

Pilot Scale Fermenters Dosing Vessels

Laboratory Fermenter (Autoclavable Type)

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Vinegar Acetator Unit

Top Flange Production scale fermenter

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