FERMENTERS

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Fermenters/Bioreactors

- A fermenter/bioreactor is a specially designed vessel which is built to support the growth of high concentration of microorganisms.
- It must be so designed that it is able to provide the optimum environments or conditions that will allow supporting the growth of the microorganisms.
- 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.

Fermenters

- A bioreactor consists of a complex system of pipes, fittings, wires, and sensors.
- With the aid of on-line monitoring and diagnosis tools, it is now possible to detect many things that can go wrong during the process.
- Bioreactor operation mode is classified in: batch processes, fed-batch and continuous processes.
- Normally these operations mode are used in submerged or liquid fermentations or during cell culture

Fermenters

- 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.
- 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.

The general requirements of the bioreactor

- The vessel should be robust and strong enough to withstand the various treatments required such as exposure to high heat, pressure and strong chemicals and washings and cleanings.
- The vessel should be able to be sterilized and to maintain stringent aseptic conditions over long periods of the actual fermentation process.
- The vessel should be equipped with stirrers or mixers to ensure mass transfer processes occur efficiently.
- It should have sensors to monitor and control the fermentation process.
- It should be provided with inoculation point for aseptic transfer in inoculum.

… **The general requirements of the bioreactor**

- Sampling valve for withdrawing a sample for different tests.
- Baffles should be provided in case of stirred fermenter to prevent vertex formation.
- It should be provided with facility for intermittent addition of an antifoam agent.
- In case of aerobic submerged fermentation, the tank should be equipped with the aerating device.
- Provision for controlling temperature and pH of fermentation medium.
- Main hole should be provided at the top for access inside the fermenter for different purposes.

Construction Material

- It is important to select a material for the body of the fermenter, which restricts the chances of contamination.
- Moreover, it needs to be non-toxic and corrosion free.
- **Glass** is a material that provides a smooth surface inside the vessel, also non-toxic in nature and corrosion-proof.
- Due to the transparency, it is easy to examine the inside of the vessel.
- However, it is difficult to handle glass as a pilot-scale fermenter.
- Another non-toxic, corrosion-proof material, stainless steel, was used.
- According to Americal Iron and Steel Institute, steel contains more than 4% chromium is standardized as **stainless steel**.
- However, the minimum amount of chromium required to protect the steel from corrosion depends on the corroding agent present in a specific environment.
- In a pilot-scale fermenter normally the steel contains around **10-13% of chromium**.
- In many cases **nickel** is also mixed in high concentration with the chromium to make the steel more corrosion resistant and it also provides engineering advantages.
- Now-a-days, stainless steel fermenters are mostly used for industrial production.

Basic Feature of Fermenter Design

- The basic feature of bioreactor include agitation system, foam control, oxygen delivering system, headspace volume, sampling port, temperature and pH control system, sterilization system and lines for charging & emptying the reactor.

Table 1. Different parts of Fermenter and its function (Jagani *et al.* 2010; Kaushik *et al.* 2014)

Parts of Fermenter	**Function**
Impellor (agitator)	Continuous stirring of media, distribution of the oxygen throughout the system and prevent cells from settling down
Sparger (Aerator)	Introduce sterile air or oxygen to the media in the aerobic fermentation process
Baffles (vortex breaker)	Provides better mixing by disrupting the vortex formation
Temperature probe	Measure and monitor temperature change in the medium during the fermentation process
Cooling Jacket	maintains the temperature of process
pH probe	Evaluate and monitor the change in pH of the medium

Table 1. Different parts of Fermenter and its function (Jagani *et al.* 2010; Kaushik *et al.* 2014)

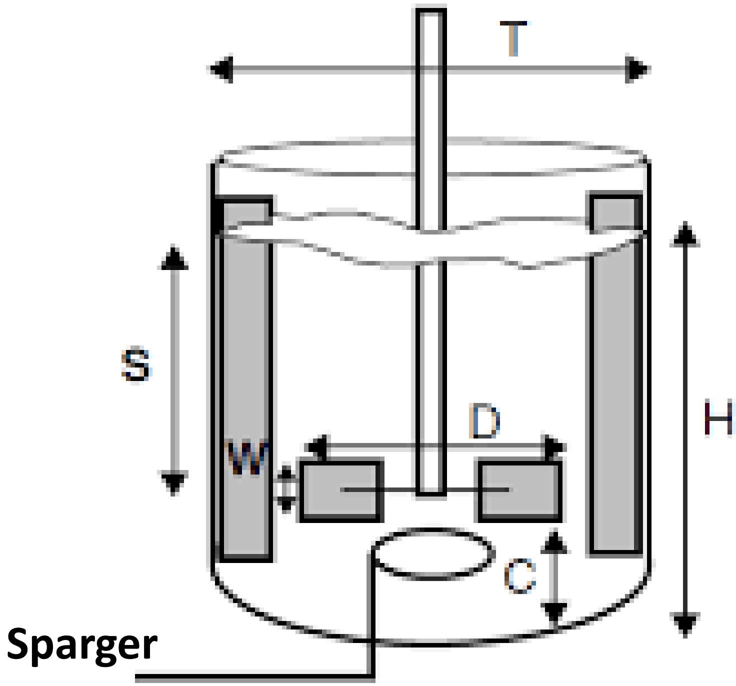
Parts of Fermenter	Function
Level probe	Measure the level of medium
Foam probe	Detect the presence of the foam
Acid	Neutralizes the basic environment and maintain the pH
Base	Neutralizes the acidic environment and maintains the required pH
Antifoam	Breakdown and avoid foam occurrence in the medium
Sampling point	To acquire samples throughout the process
Valves	Regulate and limit the flow of liquids and gases
Control panel	Monitor all parameters in the system
Inlet air filter	Filtration of air before entering the fermenter
Exhaust air filter	Prevention of contaminants from escaping
Rotameter	Measure flow rate of liquid or air
Dissolve oxygen probe	Measure dissolve oxygen in the fermenter
Pressure gauge	Measure internal pressure of the fermenter

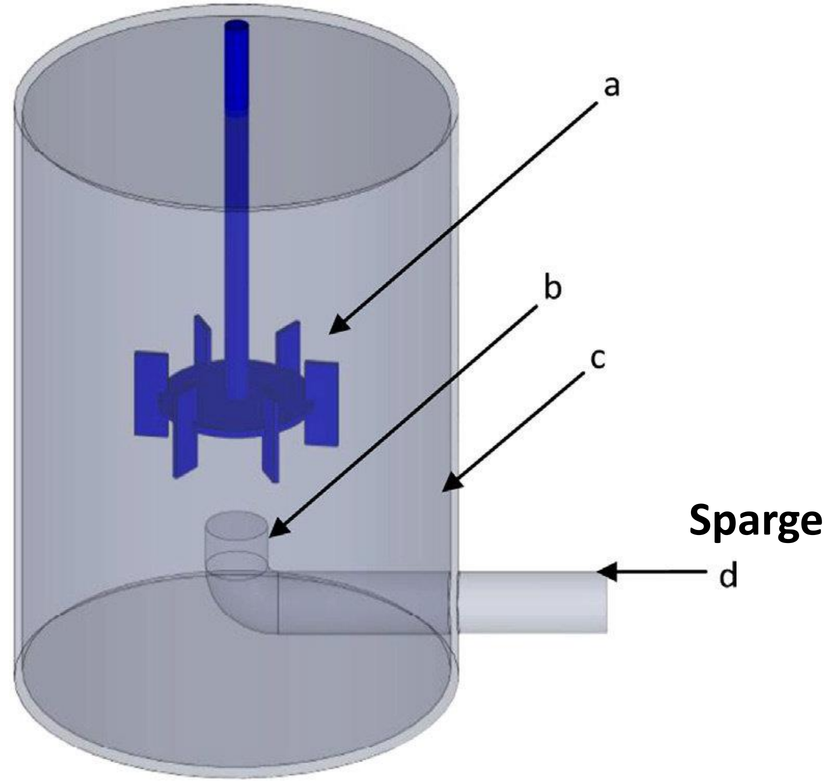
Agitator (Impeller)

- The objectives of the impeller used in fermenters are bulk fluid and gas mixing, air dispersion, heat transfer, oxygen transfer, suspension of solid particles, maintain the uniform environment inside the vessel, etc.
- Impellers involved in breaking the air bubbles produced in a liquid medium.
- There are mainly three types of agitators used in industrial-scale bioreactors
- ***Disc Turbine*****:** It consists of a disc with a series of rectangular vanes connected in a vertical plane around the disc.
- ***Vaned disc*****:** In this case, the rectangular vanes are attached vertically to the underside of a disc.
- ***Variable Pitch open turbine*****:** This type of agitator lacks disc and the vanes are directly connected to a center shaft.

The aeration system (sparger)

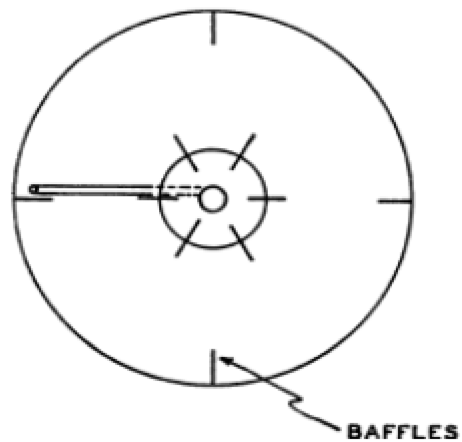
- A sparger is a device that introduces air into the liquid medium in a fermenter.
- These are of three main types:
- ***Porous Sparger:*** It is made up of sintered glass, ceramics or metals' and are mostly used in laboratory-scale bioreactors.
- As it introduces air inside a liquid medium, bubbles formed are always 10 to 100 times larger than the pore size of the aerator.
- A major disadvantage of using porous sparger is that microbial growth may occur on the pores which hamper the airflow.
- ***Orifice Sparger:*** Perforated pipes are used and attached below the impeller in the form of a ring.
- The air holes are mostly drilled under the surface of the tubes.
- Orifice spargers were used to a limited extent in yeast manufacture, effluent treatment and production of single-cell proteins.
- ***Nozzle Sparger:*** This is used in industrial-scale fermenters.
- It contains a single open or partially closed pipe as an air outlet.
- The pipe needs to be positioned below the impeller.
- The design helps to overcome troubles related to sparger blockage.


T
S
W
D
H
C
Sparger


a
b
c
Sparger
d

Baffles

- There are four baffles that are present inside of an agitated vessel to prevent a vortex and improve aeration efficiency.
- Baffles are made up of metal strips roughly one-tenth of the vessel diameter and attached to the wall.
- The agitation effect is slightly increased with wider baffles but drops sharply with narrower baffles.
- After installation of the baffle there a gap between them and the vessel wall which facilitates scouring action around the baffles and minimizes microbial growth on the baffles and the fermenter wall.
- Baffles are often attached to cooling coils to increase the cooling capacity of the fermenter.



Stirrer glands and bearings

- These stirrer shafts play an important role to seal the openings of a bioreactor.
- As a result, it restricts the entry of air from outside.
- There are several types of seals used for this purpose, which are following:
- ***The Stuffing Box:*** The shaft is sealed by several layers of packing rings of asbestos or cotton yarn which is pressed against the shaft by gland follower.
- ***The Mechanical Seal:*** The seal is divided into two parts, first is the stationary bearing housing and the second rotates on the shaft.
- These two parts are pressed together by springs.
- ***Magnetic Drives:*** This type of seals helps to counter the problem originated by the impeller shaft which is going through the top or bottom of the fermenter plate.
- The magnetic drive is made up of two magnets.

Temperature Control

- During the fermentation process heat can be produced mainly in two ways:
  - microbial biochemical reactions
  - mechanical agitation.
- In small scale production vessel the amount of produced heat is negligible.
- Therefore, extra heat is provided by hot bath or internal heat coil or heating jacket with a water circulation system or silicon heating jacket.
- The silicon heating jacket consists of silicon rubber mats with heating wires and it is wrapped around the fermenter.
- In the case of pilot-scale fermenters, it is not possible to use silicon jackets due to large size.
- In such cases, an internal heating coil is used for providing extra heat while cold water circulation helps to remove excess heat.

Foam control

- The problem often encounters in fermentation is foaming.
- When foaming becomes excessive, there is a danger that filters become wet resulting in contamination, increasing pressure drop and decreasing gas flow.
- Foam can be controlled with mechanical foam breaker or the addition of surface active chemical agents, called anti foaming agents.
- Mechanical foam breaker available is “turbosep”, in which foam is directed over stationary turbine blades in a separator and the liquid is returned to fermenter.
- Foam is also controlled by addition of oils.
- However, excessive oil additions may decrease the product formation.
- Antifoam oils may be
  - synthetic, such as silicones or polyglycols, or
  - natural, such as lard oil or soybean oil
- Either will substantially change the physical structure of foam, principally by reducing surface elasticity.
- Industrial antifoam systems operate automatically from level-sensing devices.

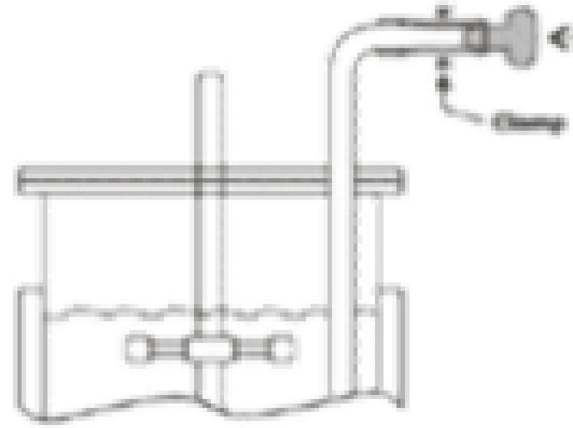
pH control sensors

- All types of fermenters are attached with a pH control sensor which consists of a pH sensor and a port to maintain the pH inside of the fermenter.
- pH alteration can lead to death of the organism which leads to product loss.
- Therefore, it is a crucial instrument for a fermenter and needs to be checked regularly.

Valves and steam traps

- Valves attached to fermenter are used to controlling the flow of liquids and gases in a variety of ways.
- The different valves available are :
  - gate valves,
  - globe valves,
  - piston valves,
  - needle valves,
  - plug valves,
  - ball valves,
  - butterfly valves,
  - pinch valves,
  - diaphragm valves,
  - check valves,
  - pressure control valves,
  - safety valves
  - steam traps
- Depending upon fermentation type and requirements these valves are chosen in designing bioreactor with good productivity.

Sampling port



- The sampling construction should be such so that measures for preventing non-sterility before and after the sampling be avoided.
- In the sites of the infection origin, sterilization should be performed promptly with alcohol or steam.
- A bladder made of silicone or a similar material is placed into the sampling pipe, and its end is stopped with a clamp.
- Thereby, it is sterilized together with the bioreactor vessel, and it remains in such a state until the sampling.
- When sampling, the clamp is removed, and the bladder is also pulled down.
- With the sample's discharge, the pipe's end is immediately washed with alcohol.
- Another drawback of this method is a hampered possibility of choosing the sample's amount.
- Keofitt sampling device is available for sampling from bioreactor.

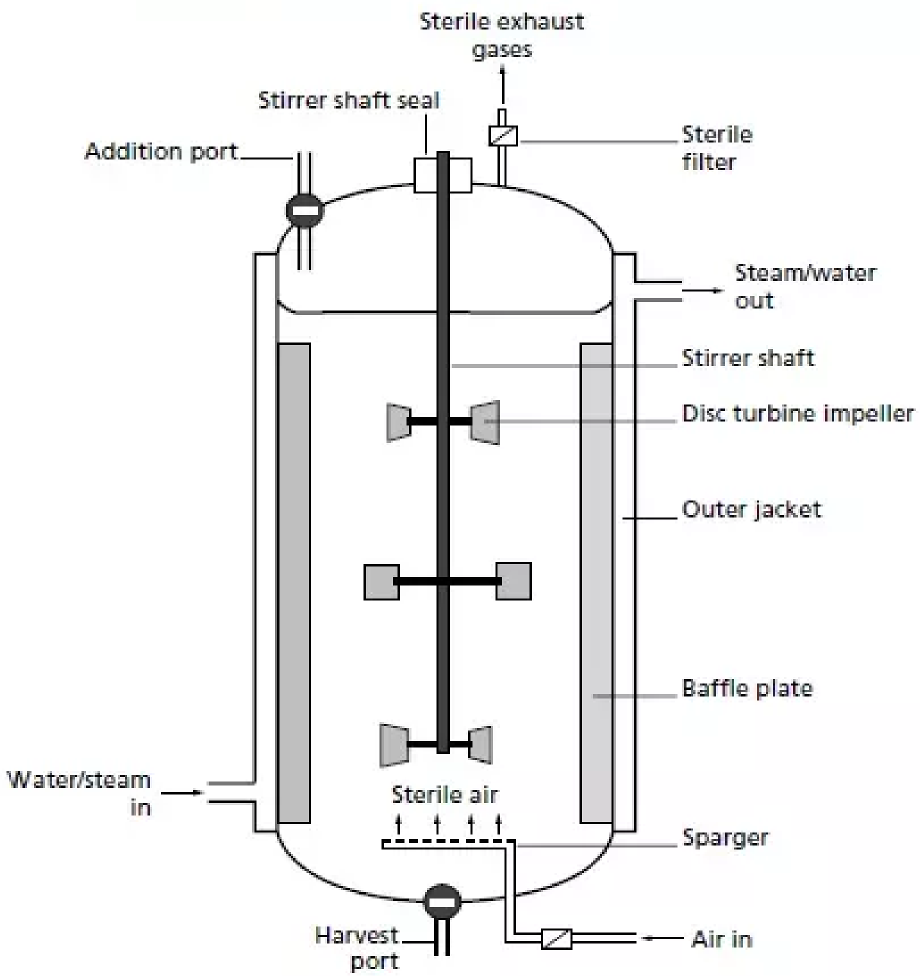
Types of Fermenters

- Stirred Tank Bioreactor
- Airlift Bioreactor
- Bubble Column Bioreactors
- Fluidized Bed Bioreactor
- Packed Bed Bioreactor
- Photobioreactor
- Membrane Bioreactor

Stirred Tank Bioreactors

- The stirred-tank bioreactor consists of a vessel, pipes, valves, pumps, agitator, shaft, impeller and a motor.
- Sparger is mostly used to add air to the culture medium under pressure.
- The impellers serve as a gas distributor throughout the fermentor and also break down the larger bubbles for a uniform distribution.
- The motor power the bioreactor which helps in mixing cultures and also there are sensors that can detect temperature, pH, dissolved oxygen, glucose, lactic acid, ammonia, ammonium ion, and other parameters in the culture medium.
- The typical decision variables are: type, size, location and the number of impellers; sparger size and location.
- These determine the hydrodynamic pattern in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc.

Stirred Tank Bioreactors



Stirred Tank Bioreactor

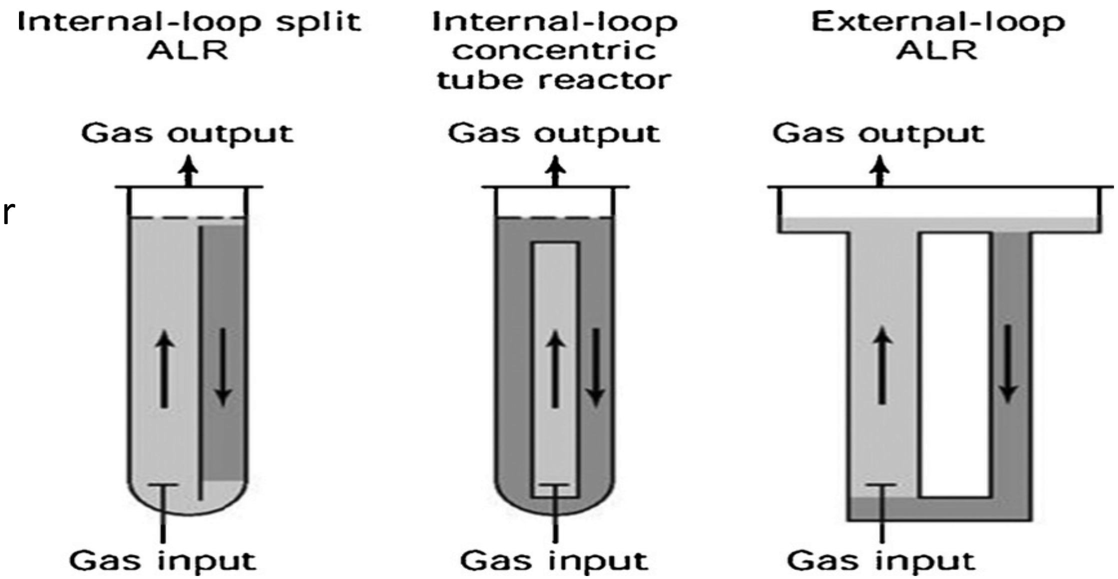
- **Advantages**
- The efficient gas transfer to growing cells
- Good mixing of the contents
- Flexible operating conditions
- Commercial availability of the bioreactors.
- **Disadvantages**
- Their mechanic agitation produces shear stress which may harm the cultured cells. but this can be overcome by changing the shape and diameter of the impeller blade or adding bovine serum albumin or dextran.
- Foaming can also cause a problem but again can be solved by antifoaming agents.

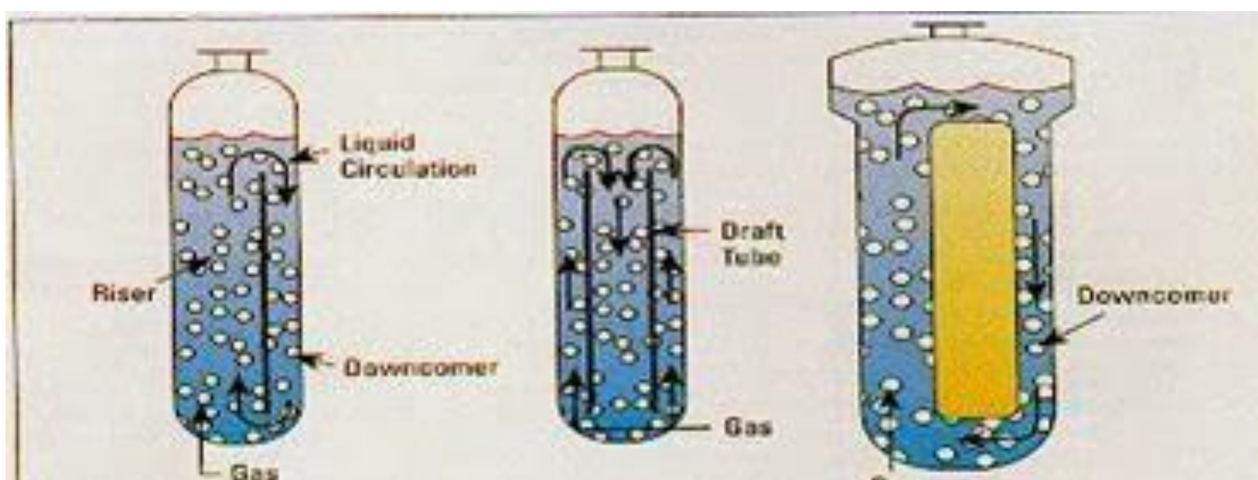
Airlift Bioreactor

- In airlift fermentor 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 fermentors are highly energy-efficient and 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, and airlift reactors are more effective in suspending solids than are bubble column fermentors.
- All performance characteristics of airlift-fermentor are related ultimately to the gas injection rate and the resulting rate of liquid circulation.

Types of Air-lift Bioreactor

- Two types
  - Internal loop reactor
    - Internal-loop split reactor
    - Internal loop concentric tube reactor
  - External loop reactor





Air Bioreactor Advantages

Airlift Bioreactor Advantages

- It produces very little shear stress, less friction
- It requires fewer efforts to construct the bioreactor.
- It is cost-efficient
- Less energy is required

Airlift Bioreactor Disadvantages

- High Pressure is required in this system
- No shaft is present which helps as a foam breaker which creates a major drawback.

Bubble Column Bioreactors

- It is simple to construct and operate.
- It consist of a cylindrical vessel with a ratio of 4:6 (height to diameter).
- The upper section of the fermenter is often widened to provide proper gas separation.
- The gas is spranger into liquid by the means of spranger and hence, adequate level of mixing is obtained.
- The liquid phase can be delivered by batch or continuous mode, which can either be countercurrent or concurrent.
- They have very low maintenance cost and very little space for maintenance.
- It is widely used for waste water treatment, production of enzymes, proteins and antibiotics.

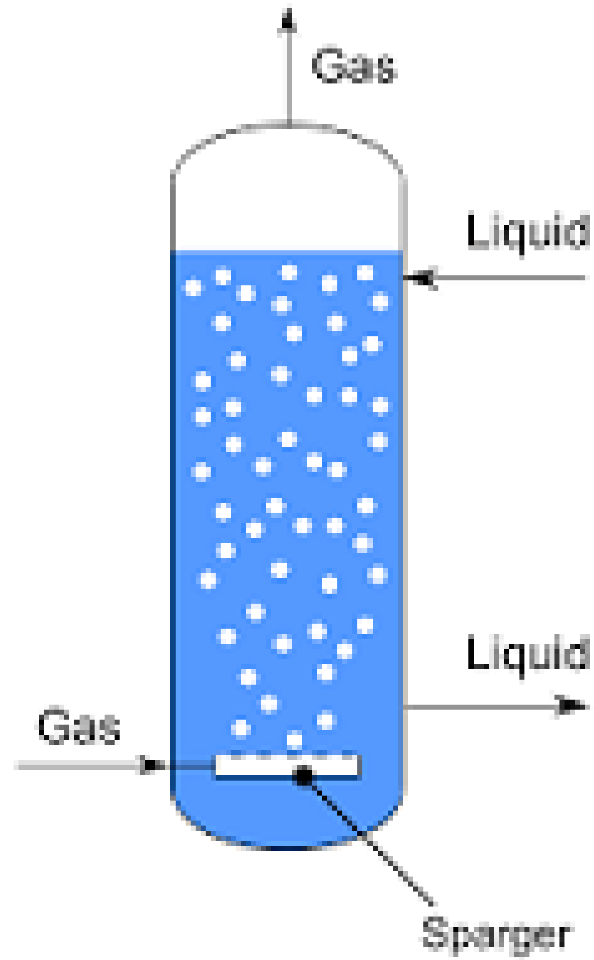
... Bubble Column Bioreactors

Advantages

- It acquires good heat and mass transfer
- Easy to operate
- Low maintenance

Disadvantages

- Bad mixing is a major drawback which adversely affects product conversion.

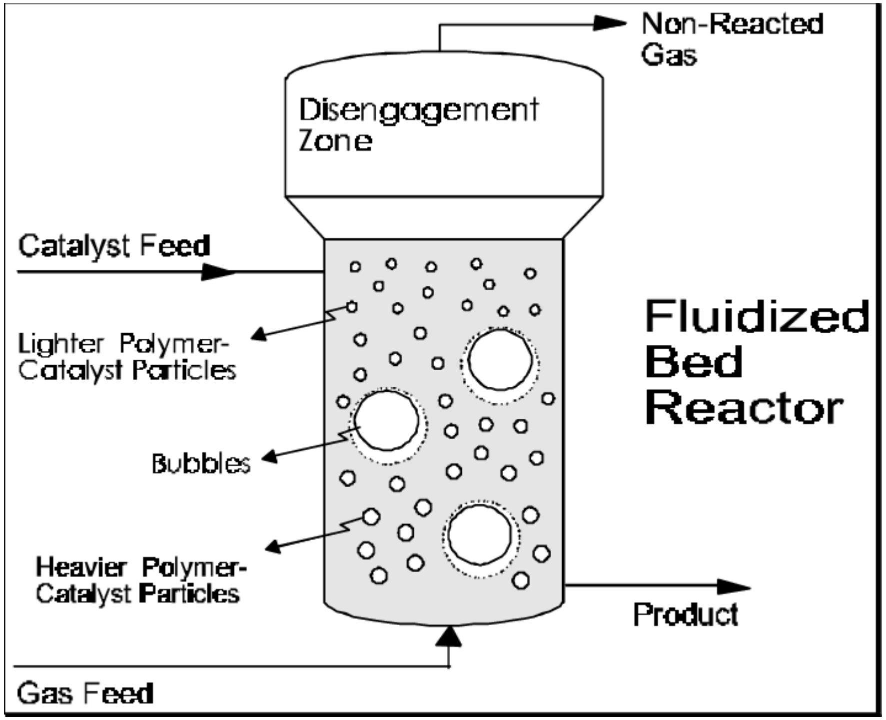


Fluidized Bed Bioreactor

- A fluidized bed bioreactor is an immobilized cell reactor which is a combination of stirred tank and packed bed continuous flow reactors.
- It can be explained as beds of regular molecules that are suspended in a flowing liquid stream.
- This can be used for particles such as immobilized enzymes, immobilized cells, and microbial flocs.
- It involves cell as biocatalysts including 3 phases – gas-liquid-solid.
- Usually fluidization is obtained either by external liquid recirculation or by gas fed to the reactor.
- In the case of immobilized enzymes the usual situation is of two-phase systems involving solid and liquid but the use of aerobic biocatalyst necessitate introduction of gas (air) as the third phase.
- Basically the particles used in FBBs can be of three different types: (i) inert core on which the biomass is created by cell attachment. (ii) Porous particles in which the biocatalyst is entrapped.(iii) Cell aggregates/ flocs (self-immobilization).

... Fluidized Bed Bioreactor

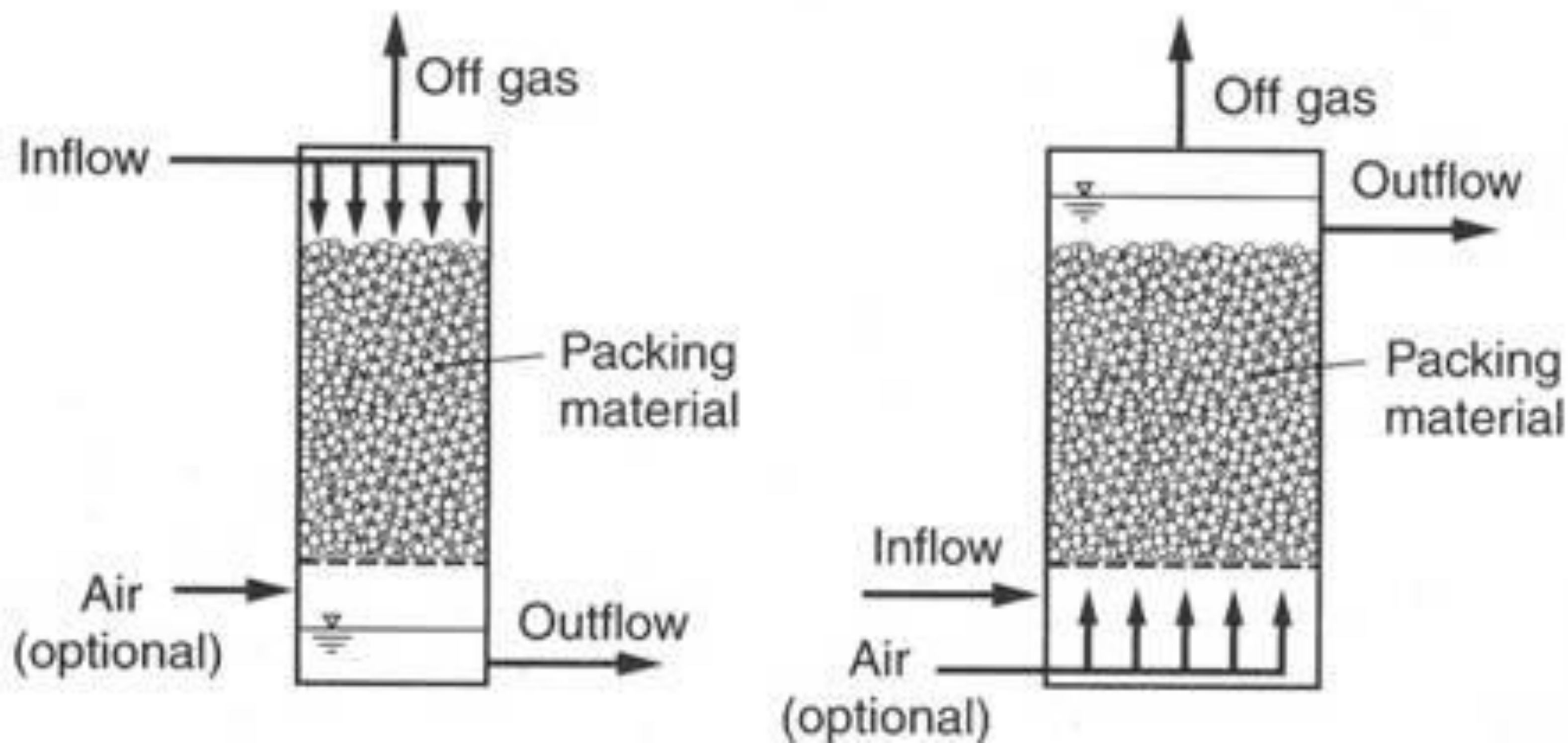
- **Advantages of Fluidized Bed Bioreactor**
- It is used for wastewater treatment and hydrogen production.
- **Disadvantages of Fluidized-Bed Bioreactor**
- It requires more energy in order to achieve fluidization in the bioreactor.



Packed Bed Bioreactor

- A bed of solid particles, with biocatalysts or cells 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 immobilised biocatalyst/ cells.
- 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.
- 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.
- Packed beds arc generally used where substrate inhibition governs the rate of reaction.
- 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.

Packed Bed Bioreactor



Photobioreactor

- These are 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.
- 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.
- Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25-40°C.
- Microalgae and cyanobacteria are normally used.
- The organisms grow during day light while the products are produced during night.

Photobioreactor

Advantages

- Contamination is lower.
- It can be space-saving as it can be placed vertically, horizontally or at an angle, indoors or outdoors.

Disadvantages

- The control of PH and temperature is quite difficult.
- It is somewhere susceptible to contamination.

Membrane Bioreactor

- It consists of a biological reactor with suspended biomass and solids removal by ultra- and microfiltration membranes.
- 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.
- Product is forced out through the membrane.
- Membrane holds back the enzyme.
- Good mixing in the reactor can be achieved by using a stirrer.
- The most widely used membrane materials includes polysulfonte, polyamide and cellulose acetate.

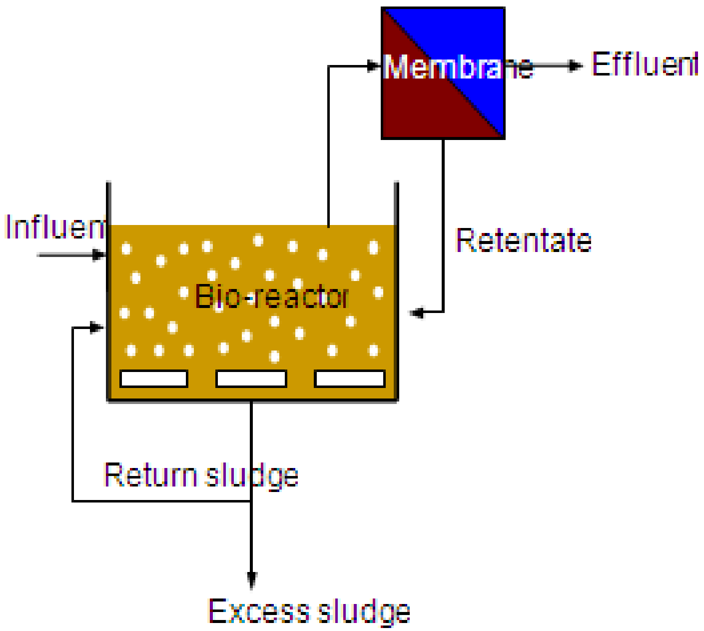
Advantages of Membrane Bioreactor

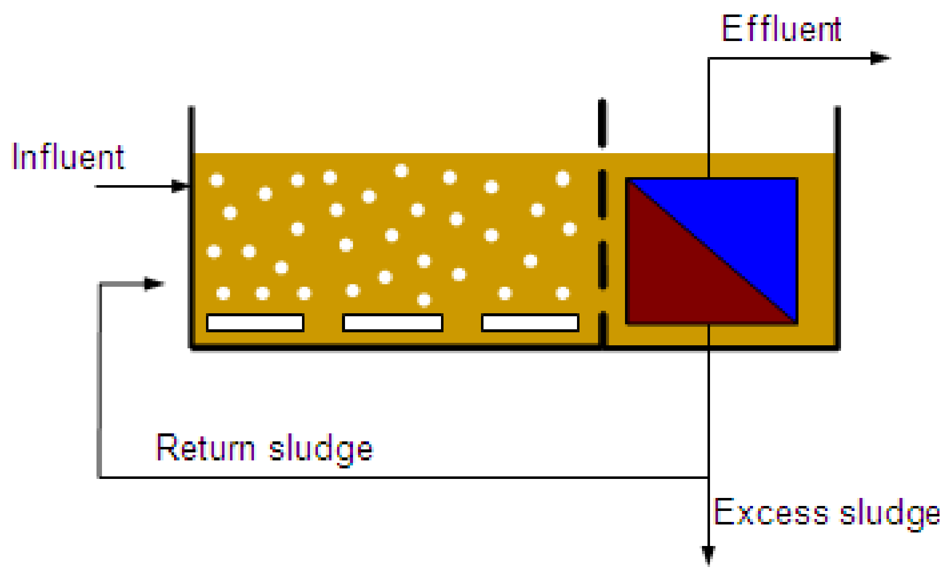
- The loss of the enzyme is minimized.
- Effluent quality is high
- The effluent is properly disinfected from all the pathogenic microbes.

Membrane Bioreactor

Disadvantages of Membrane Bioreactor

- Quite costly and energy-consuming
- The aeration is limited
- Membrane pollution is also a drawback





Questions

- Diagrammatically represent the design of basic fermenter/ stirred tank bioreactor.
- Discuss in brief about the basic features of fermenter design.
- Write a short note on continuous stirred tank bioreactors.
- Write short note on
  - Photobioreacters
  - bubble column fermenter
  - air-lift fermenter.
  - Fludized bed reactor
  - Packed bed reactor
  - Impiller
  - Spargers
  - Baffels
- How many types of fermenters used in industries? Discuss their structure and applications.
- What are the different components or parts of a typical fermenter and their function.
- Write short note on antifoam agents, types and their function.