MICROBIOLOGY COURSE MATERIAL

Semester - V

DR. PRIYADARSHINI MALLICK

CC12: UNIT 3: PART B: BIOREACTORS

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
SEMESTER – V
CC12: UNIT - 3
Part – B: BIOREACTORS

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

❖ History of Fermentors:

De Becze and Liebmann (1944) used the first large scale (above 20 litre capacity) fermentor for the production of yeast. But it was during the First World War, a British scientist named Chain Weizmann (1914-1918) developed a fermentor for the production of acetone. Since importance of aseptic conditions was recognized, hence steps were taken to design and construct piping, joints and valves in which sterile conditions could be achieved and manufactured when required.

For the first time, large scale aerobic fermentors were used in central Europe in the year 1930's for the production of compressed yeast (de Becze and Leibmann, 1944). The fermentor consisted of a large cylindrical tank with air introduced at the base via network of perforated pipes. In later modifications, mechanical impellers were used to increase the rate of mixing and to break up and disperse the air bubbles. This process led to the compressed air requirements. Baffles on the walls of the vessels prevented forming a vortex in

the liquid. In the year 1934, Strauch and Schmidt patented a system in which the aeration tubes were introduced with water and steam for cleaning and sterilization. The decision to use submerged culture technique for Penicillin production, where aseptic conditions, good aeration and agitation were essential, was probably a very important factor in forcing the development of carefully designed and purpose-built fermentation vessels. In 1943, when the British Government decided that surface culture was inadequate, none of the fermentation plants were immediately suitable for deep fermentation. The first pilot fermentor was erected in India at Hindustan Antibiotic Ltd., Pimpri, Pune in the year 1950.

❖ Design of Fermentors:

All bioreactors deal with heterogeneous systems dealing with two or more phases, e.g., liquid, gas, solid. Therefore, optimal conditions for fermentation necessitate efficient transfer of mass, heat and momentum from one phase to the other. Chemical engineering principles are employed for design and operation of bioreactors. A bioreactor should provide for the following:

(i) Agitation (for mixing of cells and medium),
(ii) Aeration (aerobic fermentors); for O_2 supply,
(iii) Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, liquid level, etc.,
(iv) Sterilization and maintenance of sterility, and
(v) Withdrawal of cells/medium (for continuous fermentors).

Modern fermentors are usually integrated with computers for efficient process monitoring, data acquisition, etc. Generally, 20-25% of fermentor volume is left unfilled with medium as “head space” to allow for splashing, foaming and aeration. The fermentor design varies greatly depending on the type and the

fermentation for which it is used. Bioreactors are so designed that they provide the best possible growth and biosynthesis for industrially important cultures and allow ease of manipulation for all operations.

❖ Size of Fermentors:

The size of fermentors ranges from 1-2 litre laboratory fementors to 5,00,000 litre or, occasionally, even more, fermentors of upto 1.2 million litres have been used. The size of the fermentor used depends on the process and how it is operated.

TABLE 39.6. Fermentor sizes for various microbial fermentations

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

❖ Construction of Fermentors:

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

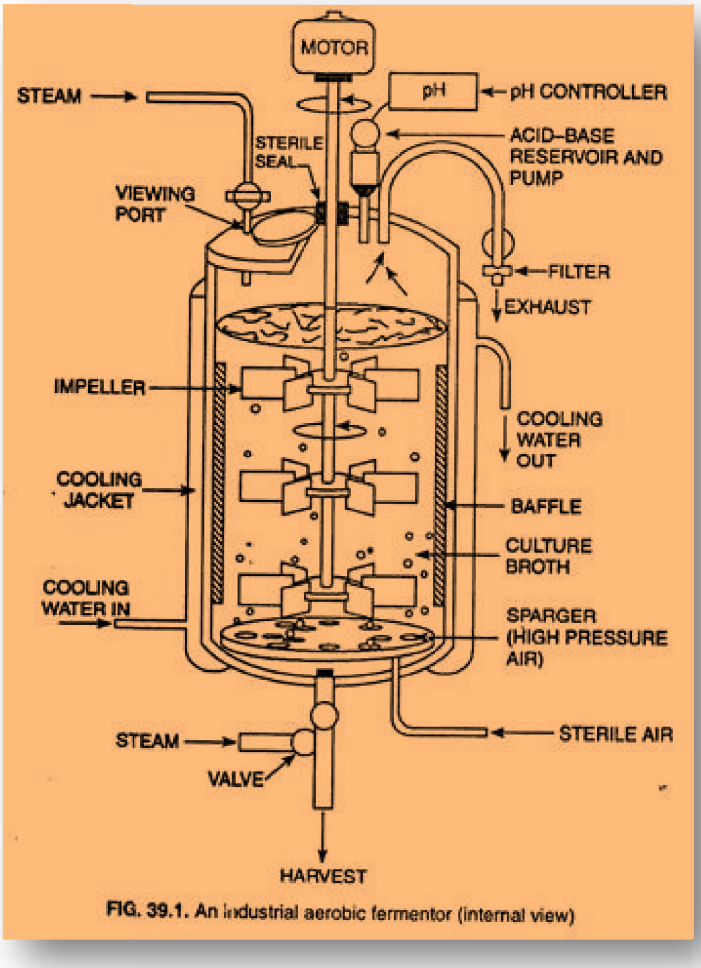


FIG. 39.1. An industrial aerobic fermentor (internal view)

1. Cooling Jacket:

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

therefore, internal coils are provided through which either steam or cooling water is runned.

2. Aeration System:

Aeration system is one of the most critical parts of a fermentor. In a fermentor with a high microbial population density, there is a tremendous oxygen demand by the culture, but oxygen being poorly soluble in water hardly transfers rapidly throughout the growth medium. It is necessary, therefore, that elaborate precautions are taken using a good aeration system to ensure proper aeration and oxygen availability throughout the culture. However, two separate aeration devices are used to ensure proper aeration in fermentor. These devices are sparger and impeller. The sparger is typically just a series of holes in a metal ring or a nozzle through which filter-sterilized air (or oxygen-enriched air) passes into the fermentor under high pressure. The air enters the fermentor as a series of tiny bubbles from which the oxygen passes by diffusion into the liquid culture medium. The impeller (also called agitator) is an agitating device necessary for stirring of the fermenter. The stirring accomplishes two things:

(i) It mixes the gas bubbles through the liquid culture medium, and
(ii) It mixes the microbial cells through the liquid culture medium.

In this way, the stirring ensures uniform access of microbial cells to the nutrients. The size and position of the impeller in the fermentor depends upon the size of the fermentor. In tall fermentors, more than one impeller is needed if adequate aeration and agitation is to be obtained. Ideally, the impeller should be 1/3 of the fermentor's diameter fitted above the base of the fermentor. The number of impeller may vary according to the size of the fermentor.

3. Baffles:

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

4. Controlling Devices for Environmental Factors:

In any microbial fermentation, it is necessary not only to measure growth and product formation but also to control the process by altering environmental parameters as the process proceeds. For this purpose, various devices are used in a fermentor. Environmental factors that are frequently controlled includes temperature, oxygen concentration, pH, cell mass, levels of key nutrients, and product concentration.

❖ Use of Computer in Fermentor:

Computer technology has produced a remarkable impact in fermentation work in recent years and the computers are used to model fermentation processes in industrial fermentors. Integration of computers into fermentation systems is based on the computers capacity for process monitoring, data acquisition, data storage, and error-detection. Some typical, on-line data analysis functions include the acquisition measurements, verification of data, filtering, unit conversion, calculations of indirect measurements, differential integration calculations of estimated variables, data reduction, tabulation of results, graphical presentation of results, process stimulation and storage of data.

❖ Types of Fermentor:

There are six types of bioreactors used in bioprocess technology. The six types are:

(1) Continuous Stirred Tank Bioreactors

(2) Bubble Column Bioreactors

(3) Airlift Bioreactors

(4) Fluidized Bed Bioreactors

(5) Packed Bed Bioreactors, and

(6) Photo-Bioreactors.

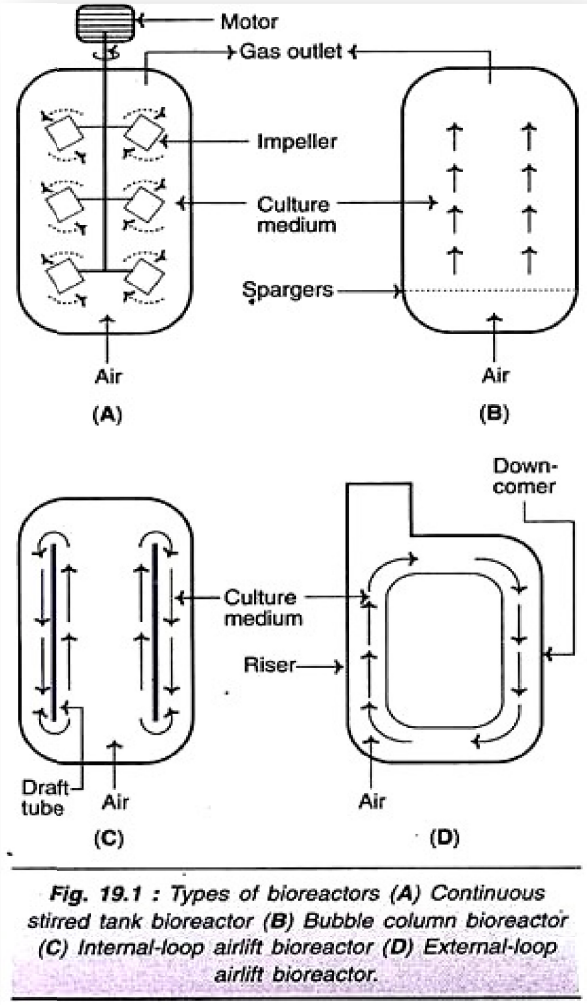
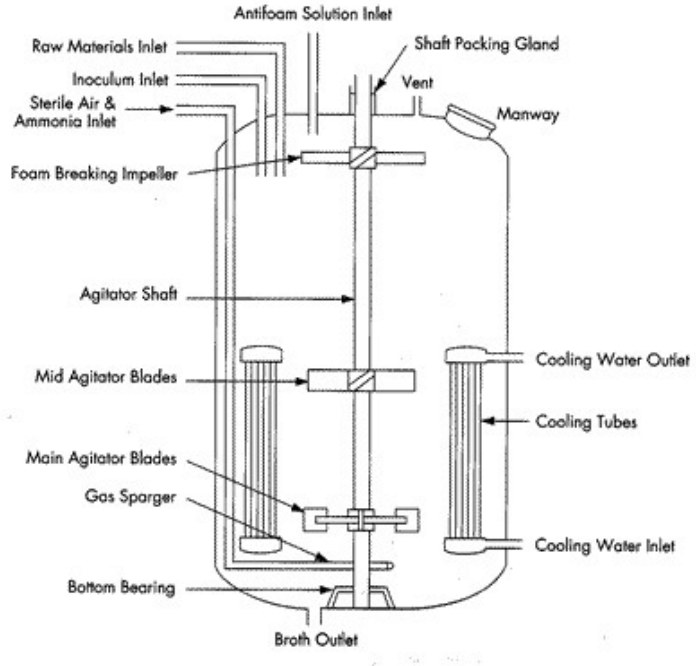


Fig. 19.1 : Types of bioreactors (A) Continuous stirred tank bioreactor (B) Bubble column bioreactor (C) Internal-loop airlift bioreactor (D) External-loop airlift bioreactor.

Type # 1: Continuous Stirred Tank Bioreactors:

Stirred tank fermentor consists of a cylindrical vessel with a motor driven central shaft that supports one or more impellers. 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. The aspect ratio of a stirred tank bioreactor lies usually between a range of 3-5. However, for animal cell culture applications, the aspect ratio is less than 2. The diameter of the impeller is usually 1/3 rd of the vessel diameter. The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use. In stirred tank bioreactors or in short stirred tank reactors (STRs), 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.



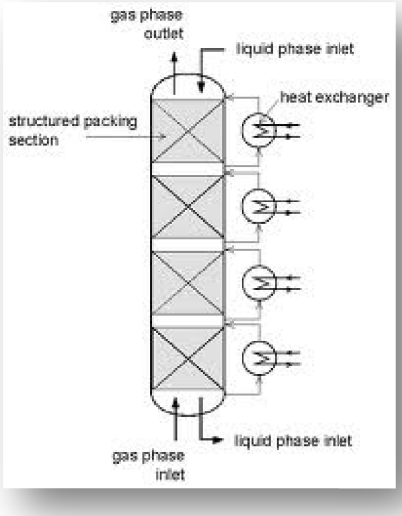
Schematic Diagram of a Stirred Tank Bioreactor

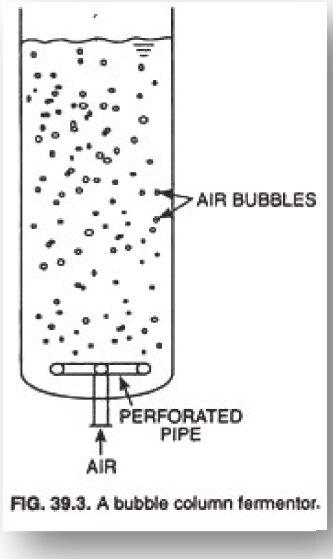
✓ **Advantages of STRs:**

There are many advantages of STRs over other types. These include the efficient gas transfer to growing cells, good mixing of the contents and flexible operating conditions, besides the commercial availability of the bioreactors.

Type # 2: Bubble Column Bioreactors:

In the bubble column bioreactor, the air or gas is introduced at the base of the column through perforated pipes or plates, or metal micro-porous spargers. The bubble column bioreactors may be fitted with perforated plates to improve performance. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio of 4-6 (i.e., height to diameter ratio). O_2transfer, mixing and other performance factors are influenced mainly by the gas flow





Diagrammatic views of a Bubble Column Fermentor

rate and the rheological 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. The column diameter does not affect its behavior so long as the diameter exceeds 0.1 m. One exception is the axial mixing performance. For a given gas flow rate, the mixing improves with increasing vessel diameter. Mass and heat transfer and the prevailing shear rate increase as gas flow rate is increased.

Type # 3: Airlift Bioreactors:

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.

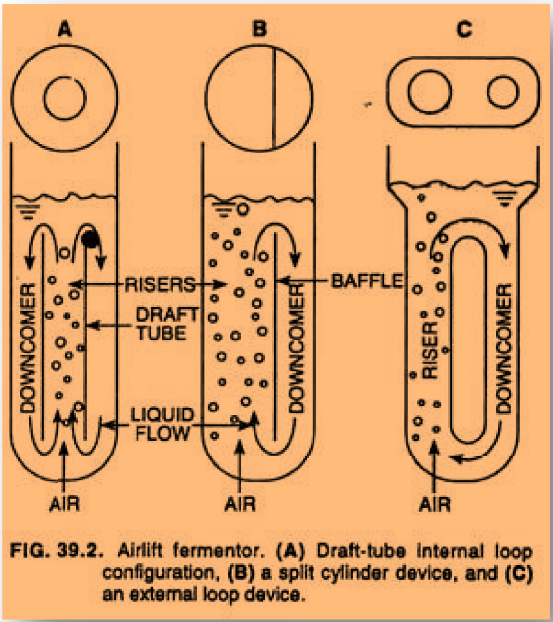


FIG. 39.2. Airlift fermentor. (A) Draft-tube internal loop configuration, (B) a split cylinder device, and (C) an external loop device.

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 the bubble column fermentors. All performance characteristics of airlift fermentor are related ultimately to the gas injection rate and the resulting rate of liquid circulation. Usually, 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.

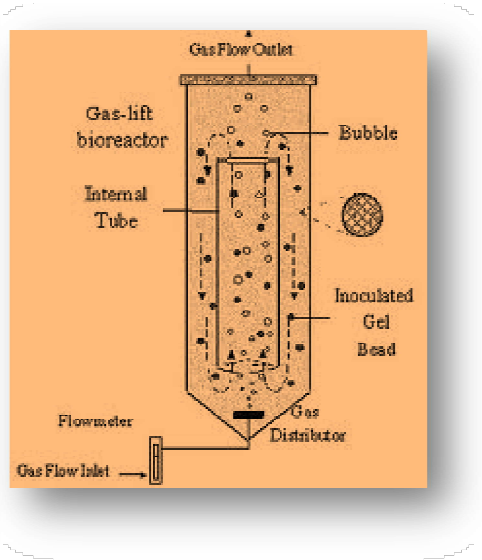


Diagram showing the Mechanism of an Airlift Fermentor

There are two types of airlift bioreactors:

- Internal-loop airlift bioreactor has a single container with a central draft tube that creates interior liquid circulation channels. These bioreactors are simple in design, with volume and circulation at a fixed rate for fermentation.

- External loop airlift bioreactor possesses an external loop so that the liquid circulates through separate independent channels. These reactors

can be suitably modified to suit the requirements of different fermentations. In general, the airlift bioreactors are more efficient than bubble columns, particularly for denser suspensions of microorganisms. This is mainly because in these bioreactors, the mixing of the contents is better as compared to bubble columns.

Airlift bioreactors are commonly employed for aerobic bioprocess technology. They ensure a controlled liquid flow in a recycle system by pumping. Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

❖ Two-stage airlift bioreactors:

Two-stage airlift bioreactors are used for the temperature dependent formation of products. Growing cells from one bioreactor (maintained at temperature 30°C) are pumped into another bioreactor (at temperature 42°C). There is a necessity for the two-stage airlift bioreactor, since it is very difficult to raise the temperature quickly from 30°C to 42°C in the same vessel. Each one of the bioreactors is fitted with valves and they are connected by a transfer tube and pump (Fig. 19.2A). The cells are grown in the first bioreactor and the bioprocess proper takes place in the second reactor.

Tower bioreactors:

A pressure-cycle fermenter with large dimensions constitutes a tower bioreactor (Fig. 19.2B). A high hydrostatic pressure generated at the bottom of the reactor increases the solubility of O_2 in the medium. At the top of the riser, (with expanded top) reduces pressure and facilitates expulsion of CO_2. The medium flows back in the down comer and completes the cycle. The advantage

with tower bioreactor is that it has high aeration capacities without having moving parts.

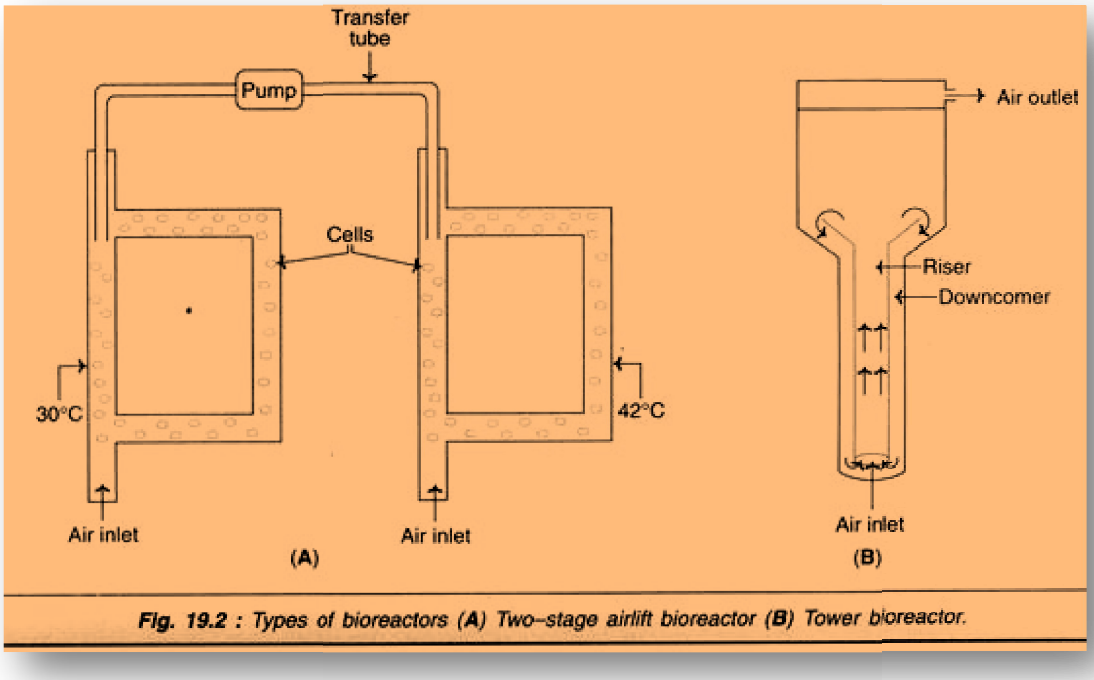


Fig. 19.2 : Types of bioreactors (A) Two–stage airlift bioreactor (B) Tower bioreactor.

Type # 4. Fluidized Bed Bioreactors:

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.

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 too 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 enables good efficiency of bioprocess.

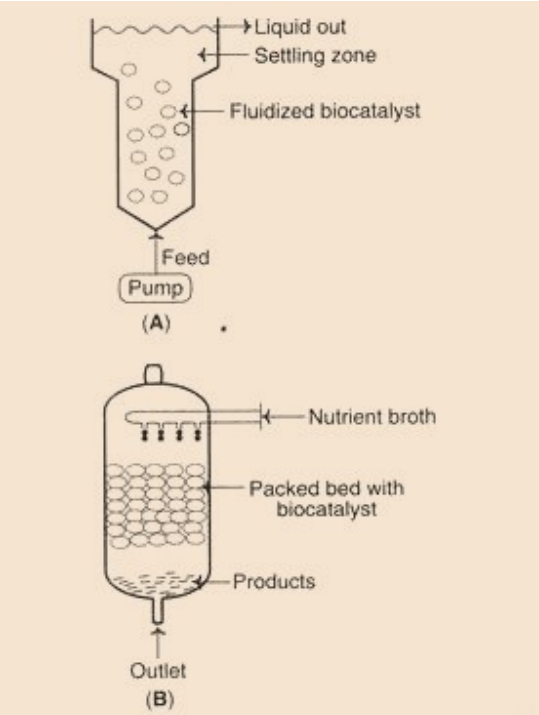
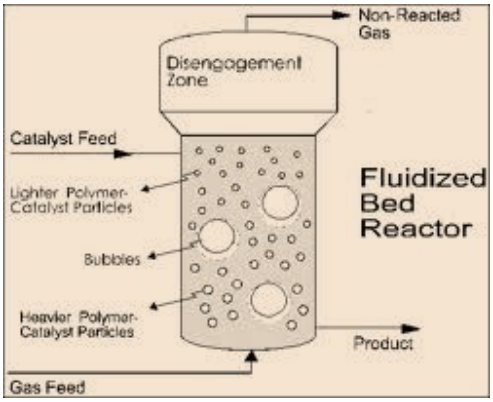


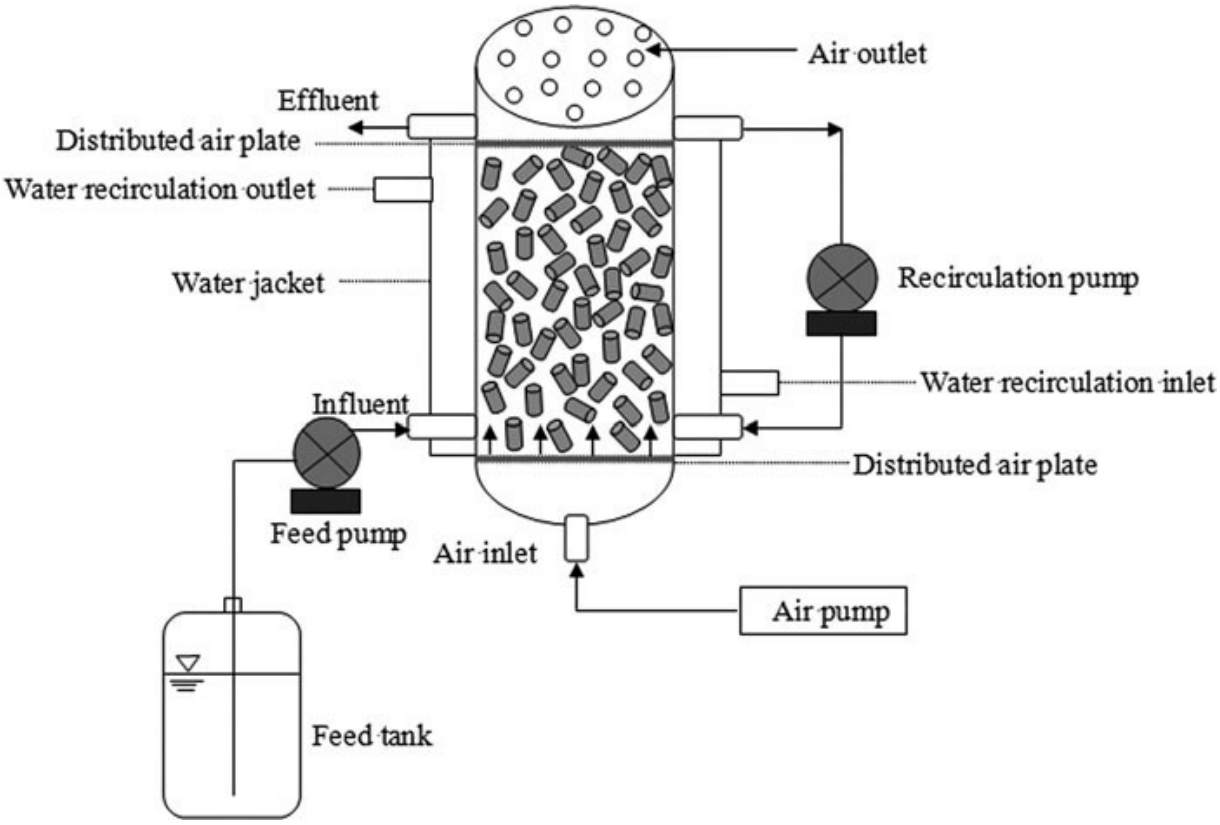
Fig. 19.3 : Types of bioreactors (A) Fluidized bed bioreactor (B) Packed bed bioreactor.



Type # 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 (Fig. 19.3B). 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 bioprocess technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

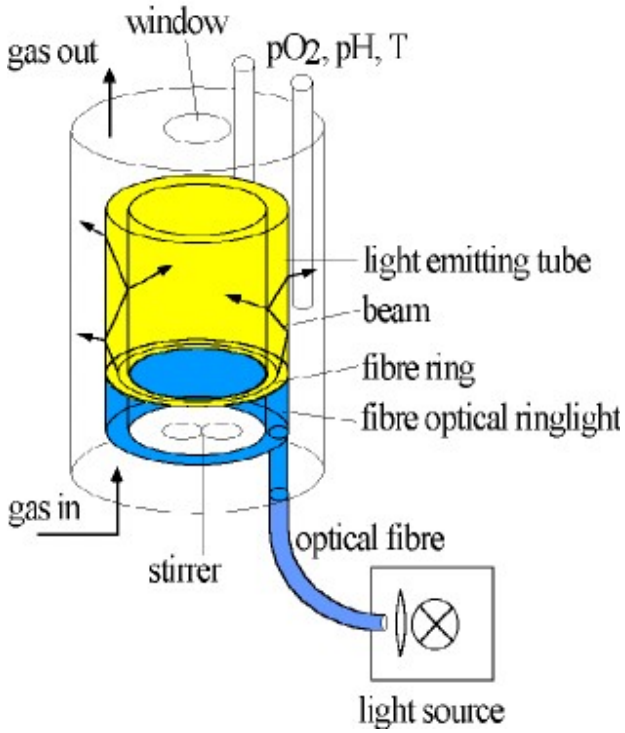


SCHEMATIC REPRESENTATION OF A PACKED BED BIOREACTOR

Type # 6. 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. The different types of photo-bioreactors are depicted in Fig. 19.4. They are made up of glass or more commonly transparent

plastic. The array of tubes or flat panels constitutes 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.



DIAGRAMMATIC VIEW OF A PHOTO BIOREACTOR