Bioreactors

Bioreactors can be defined as a vessel, deployed to utilize the activity of a biological catalyst to achieve a desired chemical transformation.

Bioreactor generally provides a biomechanical and a biochemical environment that controls nutrient and oxygen transfer to the cells and metabolic products from the cells. It could also be defined as an engineered device designed for optimal growth and metabolic activity of the organism through the action of biocatalyst, enzyme or microorganisms and cells of animal or plants.

Bioprocess or fermentation technology is an important component of most 'old' and 'new' biotechnology processes and will normally involve complete living cells (microbe, mammalian or plant), organelles or enzymes as the biocatalyst, and will aim to bring about specific chemical and/or physical changes in biochemical materials derived from the medium.
In practice, many bioprocessing techniques will be used industrially because they are the only practical way in which a specific product can be made (e.g. vaccines, antibiotics).

Bioprocessing in its many forms involves a multitude of complex enzyme-catalyzed reactions within specific cellular systems, and these reactions are dependent on the physical and chemical conditions that exist in their immediate environment. Successful bioprocessing will only occur when all the essential factors are brought together.

The reasons of using bioreactor to produce new products are increasingly being derived from microbial, mammalian and plant cell fermentations, **namely the ability:**

(1) To overproduce essential primary metabolites such as acetic and lactic acids, glycerol, acetone, butyl alcohol, organic acids, amino acids, vitamins and polysaccharides.

(2) To produce secondary metabolites (metabolites that do not appear to have an obvious role in the metabolism of the producer organism) such as penicillin, streptomycin, cephalosporin, giberellins, etc.

(3) To produce many forms of industrially useful enzymes, e.g. exocellular enzymes such as amylases, pectinases and proteases, and intracellular enzymes such as invertase, asparaginase, restriction endonucleases, etc.

(4) To produce monoclonal antibodies, vaccines and novel recombinant products, e.g. therapeutic proteins.

The term bioreactor is often used synonymously with fermenter, which is a type of bioreactor using a living cell as the biocatalyst. Fermentation is referred to the growth of microorganisms on food, under either aerobic or anaerobic conditions. Fermenters are made up of glass, glass exotic alloys, stainless steel, glass-lined steel, plastic tanks equipped with gauges. These are used for the growth of specialized pure cultures of bacteria, fungi and yeast, production of enzymes and a wide spectrum of fermented products.

The sizes of the bioreactor can vary widely from the microbial cell (few mm3) to shake flask (100-1000 ml) to laboratory scale fermenter (1 – 50 L) to pilot level (0.3 – 10 m3) to plant scale (2 – 500 m3) for large volume industrial applications. There are several aspects of biotechnological processes, which require special attention in designing a bioreactor. The reaction rate, cell growth, and process stability depend on the environmental conditions in the bioreactor. The bioreactor's conditions like gas (i.e. air, oxygen, nitrogen, carbon dioxide) flow rates, temperature, pH and dissolved oxygen levels and agitation speed/circulation rate, foam production, etc. need to be closely monitored and controlled.

Bioreactor Design and Operations

A good bioreactor design should address improved productivity, validation of desired parameters towards obtaining consistent and higher quality products in a cost effective manner. The design and mode of operation of a bioreactor depends on the production of organism, optimum conditions required for desired product formation, product value and its scale of production. The effective bioreactor is to control and positively influence the biological reaction and must prevent foreign contamination.

The basic features of a bioreactor include headspace volume, agitator system, oxygen delivery system, foam control, temperature & pH control system, sampling ports, cleaning and sterilization system and lines for charging & emptying the reactor. These are briefly described as follows:

Headspace volume: The working volume of a bioreactor is the fraction of its total volume taken up by the medium, microbes, and gas bubbles and remaining volume is called the headspace. Generally, the working volume will be ~70-80% of the total reactor volume. This, however, depends on the rate of foam formation during the reaction.

Agitator system consists of an external power drive, impeller and the baffles for intense mixing and increased mass transfer rates through the bulk liquid and bubble boundary layers. It provides enough shear conditions required for breaking up of bubbles. Most microbial fermentations use a Rushton turbine type impeller.

Air delivery system consists of a compressor, inlet air, sterilization system and exit air sterilization system to avoid contamination.
Foam control system is an essential element of bioreactor as excessive foam formation leads to blocked air exit filters and builds up pressure in the reactor.

Temperature control **system** involves temperature probes, heat transfer system (jacket, coil). Heating is provided by electric heaters and steam generated in boilers and cooling is provided by cooling water produced by cooling towers or refrigerants such as ammonia.

pH control system uses neutralizing agents to control pH; these should be non-corrosive, non-toxic to cells when diluted in the medium. Sodium carbonate is commonly used in small scale bioreactor.

Sampling ports are used to inject nutrients, water, salts etc. in bioreactors and also for collecting samples.

Cleaning and sterilization system is important to avoid contamination. Thermal sterilization by steam is preferred option for economical and large-scale sterilizations of equipment. Sterilization by chemical substances is generally preferred for heat-sensitive equipment. Sterilization is carried out by radiation by UV for surfaces and x-rays for liquids and also by membrane filters having uniform microspores and depth filters with glass wool.

Charging & emptying lines are used for input of reactants and withdrawal of products in the bioreactor.

To achieve optimization of the bioreactor system, the following operating guidelines must be closely adhered to:

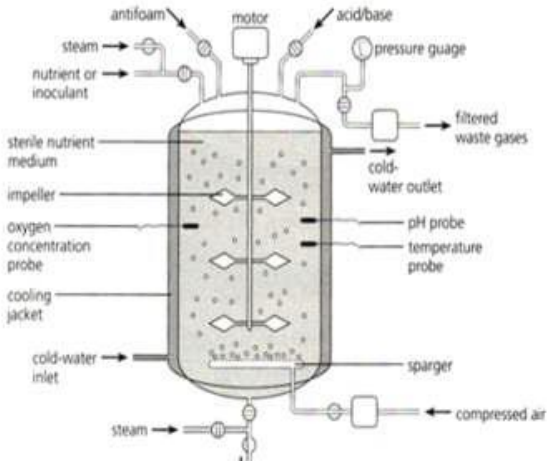
1. The bioreactor should be designed to exclude entrance of contaminating organisms as well as containing the desired organisms
2. The culture volume should remain constant, i.e. no leakage or evaporation
3. The dissolved oxygen level must be maintained above critical levels of aeration and culture agitation for aerobic organisms
4. Environmental parameters such as temperature, pH, etc., must be controlled and the culture volume must be well mixed.

Types of Bioreactor

There are mainly three types of reactions involved in fermentation process i.e. batch, continuous and semi-continuous or fed-batch depending on the feeding strategy of the culture and the medium into the bioreactor. Traditional batch stirred tank reactors (STRs) and continuously stirred tank reactors (CSTRs) have existed for centuries and are still widely adopted in the chemical and bioprocessing industry for production due to their simplicity. Other bioreactors, which have special design and operational attributes are photo-bioreactors, rotary drum reactors, mist bioreactor, membrane bioreactor, packed & fluidized bed bioreactors, bubble column & air lift bioreactors etc. These have been developed to application specific processes.

Batch Process

In the batch process, after sterilization, the sterile culture medium is inoculated with microorganisms. During this reaction period, cells, substrates including the nutrient salts, vitamins and concentrations of the products vary with time. The fermentation is allowed to run for a predetermined time and the product is harvested at the end. To promote aerobic cultivation, the medium is aerated to provide a continuous flow of oxygen. Gaseous by-products such as CO2 are removed.



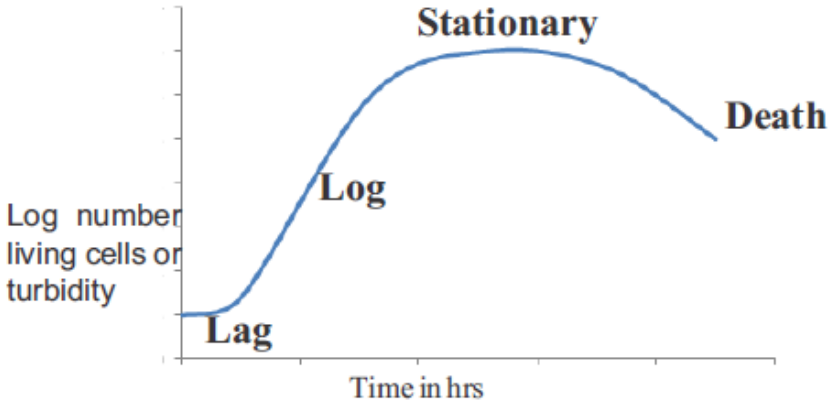
Batch Bioreactor

Lag phase: The growth of microbial population when it is inoculated with a fresh medium starts after a certain period of time called lag phase.

Log or Exponential phase: In this phase, the microbial cell numbers double per unit time period. When the cell number from such a reaction is plotted on logarithmic scale as function of elapsed time, a curve is obtained with a constantly increasing slope.

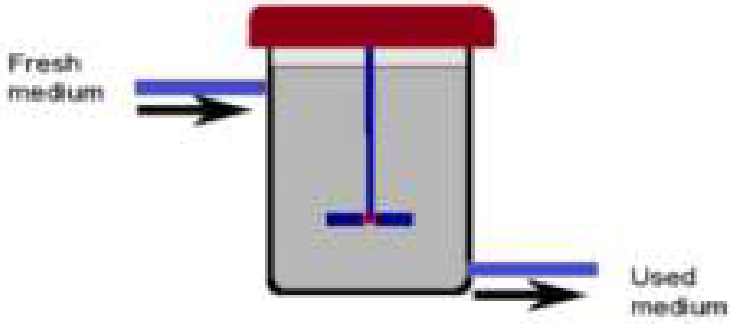
Stationary phase: In stationary phase there is no increase or reduction in cell number. The cell functions such as energy metabolism and some biosynthetic processes go on.

Death phase: The cells may start dying if the incubation is continued after the bacterial population arrive the stationary phase. Cells may die due to cell lysis, which is a much slower process than the growth phase.



Continuous Process

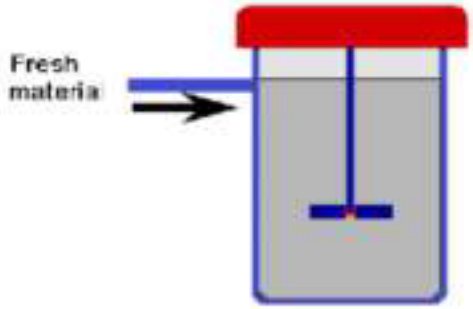
For a bioreactor on continuous mode operations, fresh medium is continuously added and the products, along with the culture are removed at the same rate, thus maintaining constant concentrations of nutrients and cells throughout the process. Continuous process is frequently used for high-volume production; for reactions using gas, liquid or soluble solid substrates; and for processes involving microorganisms with high mutation-stability. Typical end products include vinegar, baker's yeast and treated wastewater. Chemostat is a common example of continuous process reactor.



Continuous reactor

Semi-continuous or Fed-batch Process

The process uses a combination of batch and continuous reactions. In this process additional nutrients are added progressively to the reactor as the bioreactions are underway so as to obtain better yields and higher selectivity along with controlling the reaction temperature. The products are harvested at the end of the production cycle as in a batch bioreactor. Semi-batch reactors are stabler and perform safer operations than in a batch reactor.



Fed-batch bioreactor

Mode of Operation	Advantages	Disadvantages
Batch	Simple equipment; suitable for small production volumes along with multi-product flexibility	Downtime for loading and cleaning; reaction conditions change with time
Continuous	High productivity; better product quality due to constant conditions; good for kinetic Studies	Requires flow control, longevity of catalyst necessary, stability of organisms
Semi-batch or Fed-batch operation	Control of environmental conditions e.g. substrate concentration (inhibition), induction of product formation; most flexible for selecting optimal conditions; most frequently used in biotechnological processes and in fine chemical industry	Requires feeding strategy e.g. to keep constant temperature or substrate concentration