UNIT- I

INDUSTRIAL MICROBIOLOGY

Definition:

Industrial microbiology is basically associated with the commercial use of microbes for the benefit of mankind. These microbial products have direct or indirect impact on the economics, environment and social parameters of the society. The use of microbes for the production of industrially important products is not a recent event. Mankind has been producing alcoholic beverages and dairy products since the beginning of the civilization but they were not known about the role of microbes in the production of these products.

Industrial microbiology deals with the production of microbial biomass or microbial products by a process called fermentation.

Fermentation:

Any process in which a product of economic value is obtained by using microorganisms is called fermentation.

Industrial microbiology is an important branch of microbiology dealing with those areas of microbiology involving economic aspects, where valuable products are prepared from cheaper and waste material by using microorganisms. The cost of production of antibiotics or other chemicals by fermentation is less than chemical process.

Scope of Industrial Microbiology:

Many different branches of microbiology and non-microbiological fields are directly or indirectly involved in the study of industrial microbiology. Which include: soil and Agricultural Microbiology, Medical Microbiology, Microbial Physiology, Cytology and Morphology, Virology, Genetics, Marine Microbiology, Food and Dairy Microbiology and Immunology.

Disciplines important to industrial microbiology include organic, inorganic and physical chemistry, biochemistry, engineering, medicine, economics, sales and law, particularly patent law and labor law, governmental regulations on the use of certain substrates and the sale of certain products also are relevant to industrial microbiology.

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- Historical Events of Industrial Microbiology

Sr. No.	Events	Time /Era	Scientists
1	Fermentation to produce alcoholic beverages	6000-2000 BC	
2	Vinegar formation	Pre-3000 BC	
3	Production of beer	4000 BC	
4	Visualization of microbes	mid 17th century	Anton Van Leeuwenhoek
5	Discovery of alcohol fermentation by yeasts	1818	Louis Pasteur
6	Involvement of microbes in the production of lactic acid	1881	
7	Discovery of fermentation enzymes from yeast	1897	
8	Microbial process for the production of butanol and acetone	1915	
9	Discovery of penicillin	1930	Alexander Fleming
10	Microbial transformations	1937	
11	Commercial production of penicillin	1941-44	
12	Discovery of other antibiotics	1950	
13	Production of single–cell protein	1960	
14	Use of immobilized enzymes	1960	
15	Commercial use of genetically engineered microbes	1982	
16	Cloning of secondary metabolite operons	1990	
17	High throughput screening of industrially significant metabolites	2000	

Phase	Period	Main products	Scientists
I	Period before 1900	Alcohol, Vinegar, Bakers yeast, glycerol, citric acid, lactic acid and acetone /butanol	Louis Pasteur, ***Hansen***
II	Period between 1900-1940	Penicillin, streptomycin other antibiotics	Alexander Fleming, Waksman *et al.*
III	Period between 1940-1964	Gibberellins, amino acids, nucleotides, enzymes, transformations	
IV	Period between 1964-1979	Single cell protein using hydrocarbons and other feed stocks	
V	Period 1979-onward	Production of heterogenous proteins by microbial and animal cells; Monoclonal antibodies produced by animal cells	

A vast range of industrial products which were earlier made by chemical processes are now being made with the help of microbes.

Microbial products of industrial importance

Category	Products
Primary metabolites	Enzymes, amino acids, nucleotides, organic acids, ethanol, butanediol
Secondary metabolites	Antibiotics, gibberellins, hormones, pigments, alkaloids
Microbial biomass	Baker's yeast, single cell protein (SCP), probiotics, vaccines
Recombinant products	Insulin, streptokinase, interferons, Interleukins, growth hormones, vaccines

Category	Name of Product	Producing organism	Use
Alcohol	Ethyl alcohol	*Saccharomyces cerevisae*	Alcoholic beverages, Industrial solvent
Antibiotics	Penicillin	*Penicillium notatum*	Treatment of bacterial infection
	Cephalosporin	*Cephalosporium acremonium*	
	Streptomycin	*Streptomyces griseus*	
Acids	Citric acid	*Aspergillus niger*	Soft drink, preservative, detergents
Amino acids	Glutamic acid, Lysine	*Corynebacterium glutamicum*	Food supplement
Enzymes	amylases	*Bacillus, Aspergillus*	starch hydrolysis
	Protease	*Aspergillus niger*	Prevention of haze formation
Foods	Sauerkraut	*Lactobacilli, Leuconostoc*	Lactic acid production
	Bread, Idli, Dosa,	Saccharomyces, *Lactobacilli*,	Fermented food
Hormones	Insulin	*E. coli*, *Pichia pastoris*	Control of diabetes
	Human growth hormone	*E. coli*	Increase in height
Milk products	Butter, Curd, Cheese, Youghrt, Probiotics,	*Lactobacilli, Leuconostoc*	Lactic acid, flavor and vitamins production
Vaccines	BCG	*Mycobacterium tuberculosis*	Protection against tuberculosis
	Typhoid	*Salmonella typhi*	Protection against typhoid
Vitamins	Vit. A	*Blakeslea trispora*	Eye and skin care
	Vit. B complex	*Ashbya gossypii*	Improve metabolism

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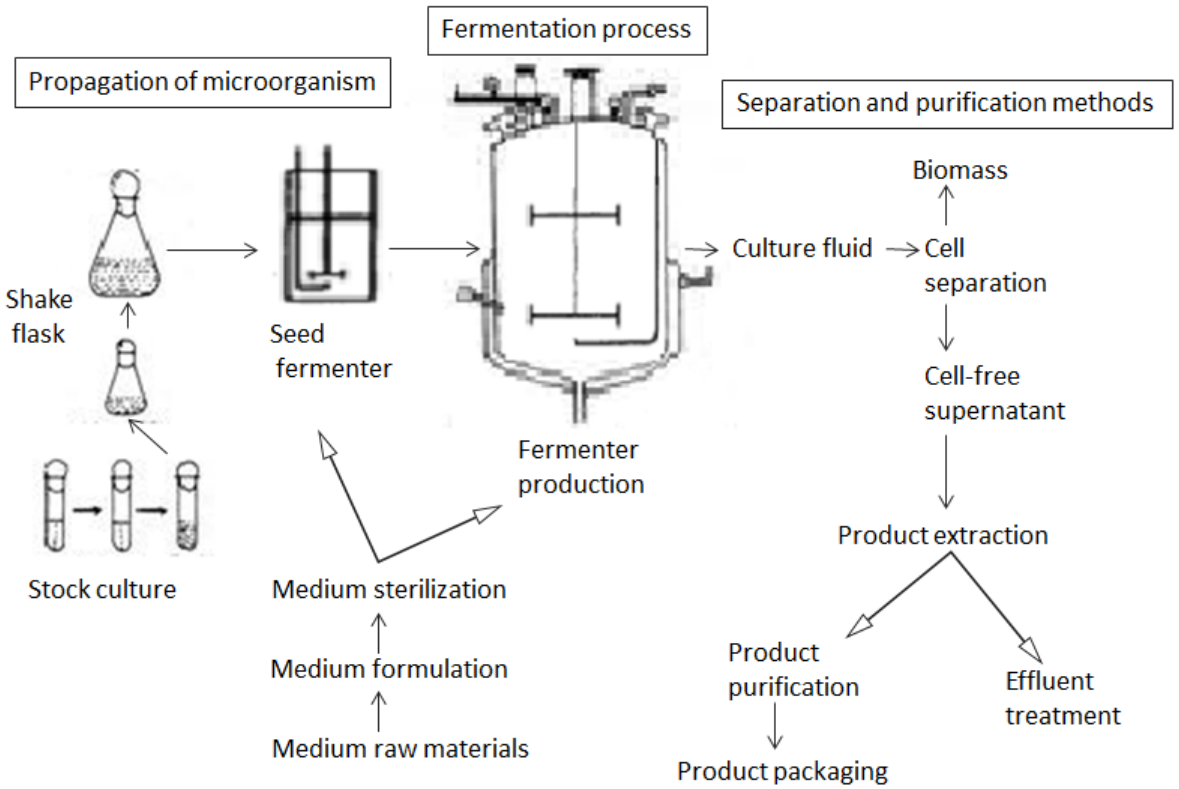
- **Lay out of fermentation process in industry**



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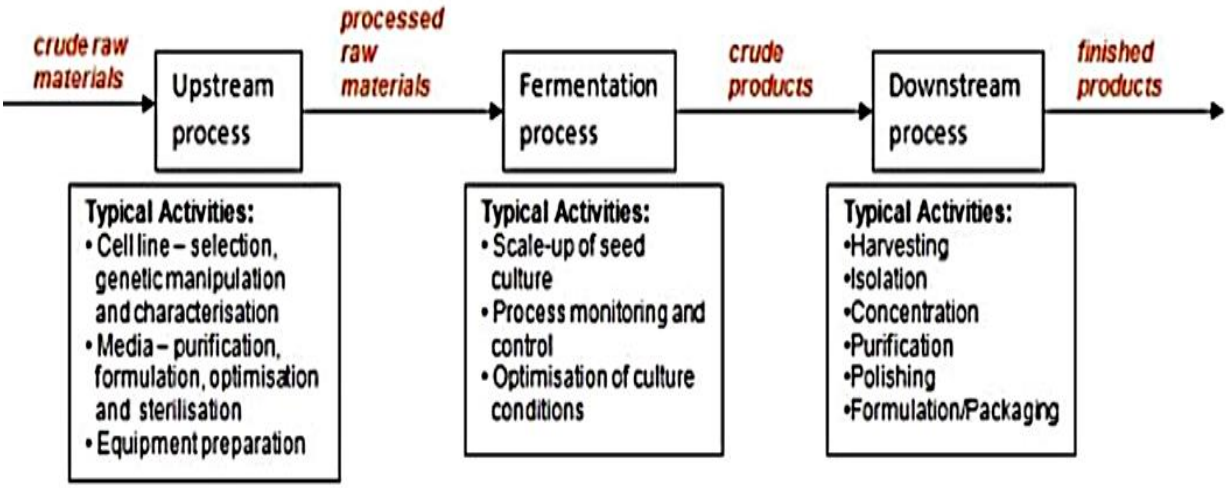
- **Different units / departments in the fermentation industry and their functions**

Following are the different units / departments in the fermentation industry

A) Upstream processing unit
B) Fermentation process unit
C) Downstream processing unit
D) Quality Control and Quality Assurance unit
E) Research and Development unit
F) Packaging Unit



A) Upstream processing unit: includes

1. Formulation of the fermentation medium unit
2. Sterilization of air, fermentation medium and the fermentor unit
3. Stock culture maintenance and Inoculum preparation unit

B) Fermentation process unit: includes

1. Fermentation and Production unit

C) Downstream processing unit: includes

1. Recovery of the products in a pure state

2. Effluent treatment

D) Quality Control and Quality Assurance unit

E) Research and Development unit

F) Packaging Unit

A) Upstream processing unit: includes

1. Formulation of the fermentation medium unit
2. Sterilization of air, fermentation medium and the fermentor unit
3. Stock culture maintenance and Inoculum preparation unit

1. Formulation of the fermentation medium unit

The *fermentation medium* should contain an energy source, a carbon source, a nitrogen source and micronutrients required for the growth of the microorganism along with water and oxygen, if necessary. A medium which is used for large scale fermentation should have the following characteristics;

a) It should be cheap and easily available.

b) It should maximize the growth of the microorganism, productivity and the rate of formation of the desired product.

c) It should minimize the formation of undesired products.

Usually, waste products from other industrial processes, such as molasses, lignocellulosic wastes, cheese whey and corn steep liquor, after modifying with the incorporation of additional nutrients, are used as the substrate for many industrial fermentations.

2. Sterilization of air, fermentation medium and the fermentor unit

Sterilization is essential for preventing the contamination with any undesired microorganisms. Air is sterilized by membrane filtration while the medium is usually heat sterilized. Any nutrient component which is heat labile is filter-sterilized and later added to the sterilized medium. The fermentor may be sterilized together with the medium or separately.

3. Stock culture maintenance and Inoculum preparation unit

Inoculum build up is the preparation of the seed culture in amounts sufficient to be used in the large fermentor vessel. This involves growing the microorganisms obtained from the pure stock culture in several consecutive fermentors. This process cuts down the time required for the growth of microorganisms in the fermentor, thereby increasing the rate of productivity. Then the seed culture obtained through this process is used to inoculate the fermentation medium.

B) Fermentation process unit

1. Fermentation and Production unit

The fermentation process involves the growth of the microorganism and production of the desired product. The fermentation process can be categorized depending on various parameters. It can be either aerobic fermentation, carried out in the presence of oxygen or anaerobic fermentation, carried out in the absence of oxygen. Most of the industrial fermentations are carried out under aerobic conditions where a few processes such as ethanol production by yeast require strictly anaerobic environments.

The fermentation process can also be divided into three basic systems, namely **batch, continuous or fed-batch**, depending on the feeding strategy of the culture and the medium into the fermentor. Each of these processes has their own advantages and disadvantages.

In a batch operation, the medium and the culture are initially fed into the vessel and it is then closed. After that, no components are added apart from oxygen (in an aerobic process) and acid or alkali for the pH adjustment. The fermentation is allowed to run for a predetermined period of time and the product is harvested at the end.

In a **continuous process**, fresh medium is continuously added and the products, along with the culture is removed at the same rate, thus maintaining constant concentrations of nutrients and cells are maintained throughout the process.

A **fed-batch system** is a combination of these two systems where additional nutrients are added to the fermentor as the fermentation is in progress. This extends the time of operation but the products are harvested at the end of the production cycle as in a batch fermentor.

The process can also be categorized as solid state fermentation (SSF) or submerged fermentation (SmF), depending on the amount of free water in the medium.

Solid state fermentation: In this process, the medium contains no free flowing water. The organisms are grown in a solid substrate which is moistened. This is used in certain industrial process such as ‘koji’ fermentation from soybeans, production of amylase and protease by *Aspergillus oryzae* on roasted soybeans and wheat, bioremediation, detoxification of agro-industrial wastes, etc.

Submerged fermentation: In this process, microorganisms grow submerged in a liquid medium where free water is abundant. This is the method of choice for many industrial operations over SSF although SSF is also rapidly gaining interest in the present.

C) Downstream processing unit: includes

1. Recovery of the products in a pure state
2. Effluent treatment

1. Recovery of the products in a pure state:-

Product recovery is carried out through a series of operations including *cell separation* by settling, centrifugation or filtration; *product recovery* by disruption of cells (if the product is produced intracellularly); *extraction* and *purification* of the product.

2. Effluent treatment: -

The effluents are treated by chemical, physical or biological methods.

D) Quality Control and Quality Assurance unit

Quality Control

The term quality control may generally be defined as a system that maintains a desired level of quality. This is done by comparing a specific quality characteristic of some product or service with a reference. Process analysis is the complete analysis of the industrial process including every single activity involved in the manufacturing of the product. Thereby, all material and virtual flows are considered. Process analysis is performed on an instrumental basis.

Quality control is accomplished by

i) Off-line quality control procedures,
ii) Statistical process control

i) **Off-line quality control procedures: -** involves selecting and defining controllable product and process parameters in such a way that deviations between process output and a standard will be minimized. A typical tool for

such a product or process design is the statistical experimental design approach or design of experiment (DoE). Quality is here basically defined 'off-line' before the process has actually been implemented or started.

ii) **Statistical process control (SPC): -** It compares the results or output of a process with the selected reference states and measures are taken when deviations from a desired condition of a process are statistically significant. When the process is poorly designed (by inappropriate off-line quality control measures, that is, unsuitable or sub-optimal processes) these deviations may be large and cannot be compensated for by statistical process control. Hence, it is obvious that off-line quality control by well-designed processes which are based on a thorough understanding of the effects of the involved process factors on the critical quality features of the product will govern the achievable product performance, or in other words: quality cannot be tested into products afterwards.

Quality Assurance

Quality assurance ensures that all procedures that have been designed and planned to produce quality of a certain level are appropriately followed. Hence, quality assurance acts on a meta-level and continually surveys the effectiveness of the quality philosophy of a company. Internal and external audits, standardized procedures and comprehensive documentation systems (traceability) are important tools to achieve this 'watchdog' function within the company.

Strict process descriptions determining every single step required during manufacturing a product, including the required evaluation procedures, may be defined, and deviations from these fixed procedures may be indicative of potential deteriorations in quality.

Good Manufacturing Practice (GMP) approach or ISO certifications are typical for quality assurance on a highly sophisticated level. However, defined procedures and certification alone do not necessarily lead to improved performance or functionality of a product. Specified procedures must be followed for quality assurance of the product.

Pre-defined and fixed processes that are certified and commissioned by the regulatory authorities like Food and Drug Administration (FDA) may even prove to be hard, sub-optimal and difficult to develop further. But every small

deviation from the standard routine processing is considered a potential quality risk and, especially in the case of pharmaceuticals or biologicals, may comprise a potential health hazard. All such deviations are required to be communicated to the authorities. FDA has promoted the PAT (Process Analytical Technology) initiative which, in a similar form, is also supported by the European Medicine Agency (EMA).

E) Research and Development unit

A company's research and development department plays an important role in the life cycle of a product. While the department usually is separate from sales, production and other divisions, the functions of these areas are related and often require collaboration. A thorough understanding of the functions of the research and development department allows you to maximize those duties at your small business, even if you don't have a big department.

New Product Research

Before a new product is developed, a research and development department conducts a thorough study to support the project. The research phase includes determining product specifications, production costs and a production time line. The research also is likely to include an evaluation of the need for the product before the design begins to ensure it is a functional product that customers want to use.

New Product Development

The research covers the way for the development phase. This is the time when the new product is actually developed based on the requirements and ideas created during the research phase. The developed product must meet the product guidelines and any regulatory specifications.

Existing Product Updates

Existing products of the company also fall under the scope of research and development. The department regularly evaluates the products offered by the company to ensure they are still functional. Potential changes or upgrades are considered. In some cases, the research and development department is asked to determine a problem with an existing product that works insufficiently or to find a new solution if the manufacturing process must change.

Quality Checks

In many companies, the research and development team handles the quality checks on products created by the company. The department must have knowledge of the requirements and specifications of a particular project. This allows team members to ensure the products meet those standards so the company puts out quality products. If the company also has a quality assurance team, it may collaborate with research and development on quality checks.

Innovation

The research and development team helps the company in staying competitive with others in the industry. The department is able to research and analyze the products other businesses are creating, as well as the new trends within the industry. This research helps the department in developing and updating the products created by the company. The team helps the good future of the company based on the information it provides and products it creates.

F) Packaging unit

The various functions of packaging are divided into primary, secondary and tertiary functions. The primary functions concern the technical nature of the packaging and secondary functions relate to communications. Primary, secondary and tertiary functions are divided into the following sub-functions:

1. **Protective function**
2. **Storage function**
3. **Loading and transport function**
4. **Sales function**
5. **Promotional function**
6. **Service function**
7. **Guarantee function**
8. **Additional function**

1. Protective function

It involves protecting the contents from the environment and vice versa. The protective function ensures full retention of the utility value of the

packaged products. The packaging helps to protect the products from loss, damage and theft.

In addition, packaging must be able to withstand from forces to which it is subjected during transport, handling and storage operations. The products frequently also require protection from climatic conditions, such as temperature, humidity, precipitation and solar radiation, which may require "inward packaging measures" in addition to any "outward packaging measures".

2. Storage function

The packaging materials and packaging containers required for producing packages must be stored in many different locations both before packaging of the products and once the package contents have been used. Packaging must thus also fulfill a storage function.

3. Loading and transport function

Convenient products handling requires designing transport packaging in such a manner that it may be held, lifted, moved, set down and stowed easily, efficiently and safely. Packaging thus has a crucial impact on the efficiency of transport, handling and storage of products. Packaging should therefore be designed to be easily handled and to permit space-saving storage. The shape and strength of packages should be such that they may not only be put side by side leaving virtually no empty space but may also be put safely one above the other.

4. Sales function

The purpose of the sales function of a package is to enable or promote the sales process and to make it more efficient.

5. Promotional function

It is intended to attract the potential purchaser's attention and to have a positive impact upon the purchasing decision. Promotional material on packaging plays a particularly important role on sales packaging as it is directly addressed to the consumer.

6. Service function

The various items of information printed on packaging provide the consumer with details about the contents and use of the particular product.

Examples are the nutritional details on yogurt pots or dosage information on medicines.

7. Guarantee function

By supplying an undamaged and perfect package, the manufacturer guarantees that the details on the packaging correspond to the contents. The packaging is therefore the basis for branded goods, consumer protection and product liability. There are legislative requirements which demand that goods be clearly marked with details indicating their nature, composition, weight, and quantity and storage life.

8. Additional function

The additional function in particular relates to the extent to which the packaging materials or packaging containers may be reused once the package contents have been used. The most significant example is the recycling of paper, paperboard and cardboard packaging as waste paper.

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❖ Importance of sterility maintenance and checking

Fermentation product is produced by the culture of certain organism, or organisms, in a nutrient medium. If any foreign microorganism enters the fermentation process, then product will not be produced thus it is very necessary to sterilize the medium and other materials so that only required organisms which we inoculate will grow and give high yield of fermentation product. Thus process of sterilization plays very important role in the fermentation processes.

Fermentor has various openings or points through which contaminants many enter inside the vessel

- Improperly sterilized media
- Partially sterilized air
- Water used for cooling system
- Through different openings like outlets, inlets or other openings
- Defect in inoculum procedure
- Due to defective process of pretreatment of crude ingredients

- Due to leakage in fermentor vessel

Why sterilization is required?

If foreign microorganisms enter in the fermentation, then the following problems may occur

- The medium would have to support the growth of both the production organism and the contaminant, resulting in a loss of productivity.
- foreign microorganism destroys or decrease product.
- If the fermentation is a continuous one then the contaminant may 'outgrow' the production organism and displace it from the fermentation.
- The foreign organism may contaminate the final product, e.g. single-cell protein where the cells, separated from the broth, constitute the product.
- The contaminant may produce compounds which make subsequent extraction of the final product difficult.
- The contaminant may degrade the desired product; this is common in bacterial contamination of antibiotic fermentations where the contaminant would have to be resistant to the normal inhibitory effects of the antibiotic and degradation of the antibiotic is a common resistance mechanism, e.g. the degradation of β-lactam antibiotics by β-lactamase-producing bacteria.
- Contamination of a bacterial fermentation with phage (virus) could result in the lysis of the culture.

Avoidance of contamination may be achieved by

- Using a pure inoculum to start the fermentation.
- Sterilizing the medium to be used.
- Sterilizing the fermentor vessel.
- Sterilizing everything that is used during the process.
- Maintaining aseptic conditions during the fermentation.

Principles of Sterilization

Microorganisms will be removed by sterilization process which otherwise will create problems in fermentation process.

There are two main methods for the sterilization

1. Destruction of microorganism
2. Removal of microorganism

There are many methods for sterilization

Methods of Sterilization

Sterilization is carried out by many methods like

1. Filtration
2. Radiations
3. Ultra sonic treatment
4. Heat treatment
5. Chemical treatment

The extent to which these procedures are used is determined by the likely probability of contamination and the nature of its complications.

Microbes that may contaminate the fermentor vessel include bacteria, fungi, protozoa, spores and phages (viruses).

Destruction of microbes may be achieved by either chemical of physical methods.

The common physical agent is Moist Heat.

- Moist heat is the satisfactory sterilization process in industry
- Removal of microorganisms can be done by filtration
- Various kinds of filters are available for this purpose

Filtration for the removal of microbes depends on

1. Size of microorganism

2. Effectiveness of filter

The strictness of sterilization condition depends on

1. Types of fermentation process
2. Time of fermentation process
3. Type and magnitude of contamination of the other article

Some fermentations are described as '***protected***' - that is, the medium may be utilized by only a very limited range of microorganisms, or the growth of the process organism may result in the development of selective growth conditions, such as a reduction in pH.

The brewing of beer falls into this category; hop resins tend to inhibit the growth of many micro-organisms and the growth of brewing yeasts tends to decrease the pH of the medium. Thus, brewing worts are boiled, but not necessarily sterilized, and the fermentors are thoroughly cleaned with disinfectant solution but are not necessarily sterile. Also, the precautions used in the development of inoculum for brewing are less complicated than in an antibiotic fermentation.

However, the vast majorities of fermentations are ***not 'protected'*** and, if contaminated, would suffer some of the complications previously listed

Which article to be sterilized?

- Sterilization is required for Media, Air, Fermentor, Feed and additives.
- Different articles are sterilized by different methods
- Heat labile (Serum, blood or animal tissue) medium is sterilized by filter
- Heat stable media is sterilized by steam under pressure
- Air is mainly sterilized by filter

Depending on the volume and process sterilization of media may be

1. continuous sterilization process or
2. batch sterilization process

- **Indian Pharmacopoea and World Health Organization guidelines for Sterility testing**

A **sterility test** may be defined as — **'a test that critically assesses whether a sterilized pharmaceutical product is free from contaminating microorganisms'.**

According to **Indian Pharmacopoea (1996)** the **sterility testings** are used for detecting the presence of viable forms of microorganisms in or on the pharmacopoeal preparations. In actual practice important guidelines and precautionary measures that must be followed strictly to accomplish the utmost accuracy and entire concept of sterility testing for life-saving secondary pharmaceutical products (drugs). A few such **fundamental factors, guidelines, and necessary details** are as listed under:

(*a*) Sterility testing is associated with a statistical process wherein the portion of a batch is sampled almost randomly. So the chance of the particular batch (lot) duly passed for actual usage (consumption) depends upon the 'sample' having passed the stringent sterility test.
(*b*) Sterility tests should be performed under conditions designed to avoid accidental contamination of the product (under investigation) during the test. Such particular precautions exactly taken for this purpose must not adversely affect any microbes that should be exposed in the test ultimately.
(*c*) Working environment wherein the sterility tests are carefully carried out must be adequately monitored at regular intervals by sampling the air and the surface of the working area by performing necessary control tests.
(*d*) Sterility tests are exclusively based upon the principle that in case the bacteria are purposefully placed in a specific medium that provides for the necessary nutritive material and water, and maintained duly at a favourable temperature (37 ± 2°C), the microbes have a tendency to grow, and their presence may be clearly indicated by the appearance of a turbidity in the originally clear medium.
(*e*) Extent of probability in the detection of viable microorganisms for the tests for sterility usually increases with the actual number apparently present in a given quantity of the preparation under examination, and is found to vary according to the species of microorganisms present. However, extremely low

levels of contamination cannot be detected conveniently on the basis of random sampling of a batch.

(*f*) In case, observed contamination is not quite uniform throughout the batch, random sampling cannot detect contamination with absolute certainty. Therefore, compliance with the tests for sterility individually cannot certify absolute assurance of freedom from microbial contamination. Nevertheless, greater assurance of sterility should invariably originate from reliable strict manufacturing procedures *with reference to* strict compliance with Good Manufacturing Practices (GMPs).

(*g*) Tests for sterility are sufficiently designed to make known the presence of microorganisms in the 'samples' used in the tests. However, the interpretation of results is solely based upon the assumption that the contents of each and every container in the batch, had they been tested actually, would have complied with the tests. As it is not practically possible to test every container, a sufficient number of containers must be examined to give a suitable degree of confidence in the ultimate results obtained of the tests.

(*h*) It has been duly observed that there exists no definite sampling plan for applying the tests to a specified proportion of separate units selected carefully from a batch is capable of demonstrating that almost all of the untested units are in fact sterile absolutely. Therefore, it is indeed quite related that while determining the number of units to be tested, the manufacturer must have adequate regard to the environment parameters of manufacture, the volume of preparation per container together with other special considerations specific to the preparation under investigation. For this Table records the guidance on the exact number of items recommended to be tested with regard to the number of items in the batch on the assumption that the preparation has been duly manufactured under specified stringent parameters designed carefully to exclude any untoward contamination.

Table: Profile of Guidance: Number of Items in a Batch *Vs* Minimum Number of Items Recommended to be Tested

S.No.	Product Variants	Number of Items in a Batch	Minimum Number of Items Recommended to be Tested
I	Injectable Preparations	(*a*) Not more than 100 containers	Either 10% or 4 containers whichever is greater.
		(*b*) More than 100, but not more than 500 containers.	10 containers.
		(*c*) More than 500 containers.	Either 2% or 20 containers whichever is less.
II	Ophthalmic and Other Non-Injectable Preparations	(*a*) Not more than 200 containers.	Either 5% or 2 containers whichever is greater.
		(*b*) More than 200 containers.	10 containers
III	Surgical Dressings	(*a*) Not more than 100 packages.	Either 10% or 4 packages whichever is greater.
		(*b*) More than 100, but not more than 500 packages.	10 packages.
		(*c*) More than 500 packages.	Either 2% or 20 packages whichever is less.
IV	Bulk Solids	(*a*) Less than 4 containers	Each container.
		(*b*) 4 containers, but not more than 50 containers.	Either 20% or 4 containers whichever is greater.
		(*c*) More than 50 containers.	Either 2% or 10 containers whichever is greater.

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- **FERMENTOR: DESIGN AND ROLE OF DIFFERENT PARTS OF FERMENTOR / BIOREACTOR**

In fermentation industries, microbes are to be grown in specially designed vessels loaded with particular type of nutritive media. These vessels are called as **Fermentor or Bioreactors.**

For production of a desired microbial product, it is of extreme importance to optimize physical (pH, temperature, aeration etc) and chemical (carbon, nitrogen, mineral sources etc) composition of the fermentation medium. To maintain these stringent conditions, microbes are grown in containers called as **fermentors or bioreactors.**

Bioreactors or fermentors are complicated in design, because they must provide for the control and observation of many facts of microbial growth and biosynthesis. The design of fermentor depends upon the purpose for which it is to be utilized. Industrial fermentors are designed to provide the best possible growth and biosynthesis conditions for industrially important microorganisms and allow no difficulty of manipulation for all operations associated with the use of the fermentors.

(Fermentor is the proper term for an apparatus, i.e. a bioreactor, while fermenter is the proper term for an organism that uses fermentation as a metabolic process, i.e. the thing that goes in the fermentor.)

Characteristics of an Ideal Fermentor or bioreactor:

There cannot be a fermentor ideal for almost all fermentation processes. But fermentors should have following characteristics:

1. Material used in the manufacture of fermentor should be strong enough to resist the interior pressure due to the fermentation media, it should be resistant to corrosion and free form any toxic effect for the microbial culture and the product formed by the microbial culture.
2. A fermentor should permit easy control of contaminating microbes.
3. It should be provided with the inoculation point for aseptic transfer of inoculum.
4. Should be equipped with the aerating device (Spargers).

5. Should be equipped with a stirring device for uniform distribution of air, nutrients and microbes (Impellers).
6. There should be provision of baffles to avoid vortex formation.
7. Fermentor should be provided with a sampling valve for aseptic withdrawing of sample for different laboratory tests.
8. Fermentor should possess a device for controlling temperature (Temperature sensor and water jacket internally fitted with heating coil).
9. Fermentor should be provided with pH controlling device for monitoring and maintaining pH of media during fermentation process (pH probe and Acid base reservoir).
10. Should be provided with a facility for intermittent addition of antifoam agents for controlling foam formation (Reservoir of sterile Antifoming agents or mechanical foam breakers).
11. There should be provision for feeding certain media components during the progress of fermentation (Precursors).
12. A drain at the bottom is essential for the removal of the completed fermentation broth for further processing.
13. A man hole should be provided at the top of fermentor for entry inside the fermentor for different purposes like repairing and thorough cleaning of fermentors between runs.
14. An exit valve should be provided at the top for the exit of metabolic gases produced during fermentation processes.

- **DESIGN OF TYPICAL FERMENTOR *(AERATED STIRRED TANK BATCH FERMENTOR)***

1) Fermentor Vessel:

A suitable vessel is used to carry out the whole fermentation process. The vessel is capable of being operated aseptically and meets the control requirements. The vessel is designed in such a way that it requires minimal labour operation and maintenance. It has smooth internal surfaces and a similar geometry. Depending upon the fermentation process two types of vessels are used. For small scale fermentation, glass vessels are used and for industrial scale stainless steel vessels are used.

Glass vessel:

Glass vessels are smooth which makes it non toxic, corrosion proof. It also makes it easy to examine the interior of the vessel. The large vessels are usually borosilicate battery jars with a round or flat bottom and a top flanged carrying plate. These vessels require autoclaved sterilization. The diameter of glass fermentor is nearly 60cm.

Stainless steel vessel:

Stainless steel is the most satisfactory material for large scale fermentations. These vessels can be sterilized in situ and hence have the capability to withstand pressure and corrosion. The corrosion resistance of stainless steel is dependent upon the thin hydrous film on the surface of the metal. This film is stabilized by chromium and is continuous, non porous, insoluble and self heating. Corrosion resistance can be improved by tungsten, silicone and other elements.

Capacity:

The capacity of the fermentor may range from a few hundred to several thousand gallons. The capacity of the fermentor is usually stated on the basis of the total volume capacity of the same. Thus, based on total volume capacity the fermentors are of following types:

i) Small Laboratory fermentors (range from 1-2 liters with a maximum up to of 12 –15 liters.)

ii) Pilot plant fermentors (range from 25 –100 gallons up to 2000 gallons) (100 to 400 liter)

iii) Large industrial fermentors (range form 5,000-10,000 gallons up
   a. to 1, 00,000 gallons.) (20000 to 40000 liter)

iv) Horton spheres fermentors (range from 2, 50,000 to 5, 00,000 gallons) (10 lakh to 25 lakh liter)

Actually the working volume in a fermentor is always less than that of the total volume. In other words, a 'head space' is left at the top of the fermentor above the level of fermentation media. The reason for keeping a head space is to allow aeration, splashing and foaming of the aqueous medium. This head space usually occupies a fifth to a quarter or more of the volume of the fermentor.

2) Heating and cooling apparatus (Temperature control):

Temperature control is achieved by a water jacket around the vessel. This is often supplemented by the use of internal coils, in order to provide sufficient heat-transfer surface. Heat is provided by passing hot water through these coils. During fermentation process, heat is generated due to microbial activity and mechanical agitation which is removed by passing cold water through these coils. Thus optimum temperature is maintained by observing readings on thermometer fitted to fermentor.

3) pH Control:

pH control is achieved by acid or alkali addition, which is controlled by an auto- titrator. The auto-titrator in turn is connected to a pH probe.

4) Impellers (Agitation):

Impeller is used for agitation. Agitation is required to ensure that a uniform suspension of microbial cells is achieved in an homogeneous nutrient medium. The agitating device consists of a strong and straight shaft to which impellors are fitted. An impeller, in turn consists of a circular disc to which blades are fitted with bolts. Different types of blades are available and are used according to the requirements. The shaft passes through a bearing in the lid of the fermentation tank. It is rotated with the help of an electric motor mounted externally at the top of the tank. The liquid medium is thrown up towards the walls of the fermentor while rotating the impeller blades at a high speed.

Impellers are classified as:

Disc turbine: It consists of a disc with a series of rectangular vanes. The vane disc again has a series of rectangular vanes attached vertically on the underside.

Air from the sparger hits underside of the disc and is displaced towards the vanes where the air bubbles are broken up into smaller bubbles.

Variable pitch open turbine: It also contains vane disc, the vanes of which are attached to the blades of a marine propeller on the agitator shaft. The air bubbles in this turbine do not hit any surface before dispersion by vanes or blades.

5) **Baffles (to prevent vortex and to improve aeration capacity):**

The liquid medium is thrown up towards the walls of the fermentor while rotating the impeller blades at a high speed. This results in the formation of a vortex, which is eliminated, usually by four equally spaced baffles attached to the walls of the fermentor. Baffles are metal strips nearly one tenth of the vessel diameter and attached radially to the wall. They are incorporated into agitated vessels of all sizes to prevent vortex and to improve aeration capacity. Baffles maintain a gap between them and the vessel wall to enable scouring action thus minimizing microbial growth on the walls of the fermentor.

6) Sparger (Aeration):

Sparger is used for aeration. The purpose of aeration is to provide sufficient oxygen to the aerobic microorganisms for metabolic requirements. Sparger introduces air into liquid in the fermentor.

Usually, the aerating device consists of a pipe with minute holes, through which pressurized air escapes into the aqueous medium in the form of tiny air bubbles. The size of the holes in a sparger ranges from 1/64 to 1/32 of an inch or larger, holes smaller than this requires too high air pressure for economical bubble formation. One should always remember that the smaller the air bubbles, the greater is the bubble surface area. It is desirable to adjust the size of the air bubbles to give the greatest possible aeration without greatly increasing the overall cost of the fermentation process. The reason for this is that sterile air is a costly item for large-scale fermentation.

The cheapest means of sterilization of air is to pass it through a sterile filter composed of glass wool, carbon particles or some other finely divided material that will trap microorganisms present in the air. Spargers in fermentors for growth of mycelium forming organisms often utilize 1/4 inch holes to prevent plugging of the holes by hyphal growth. Pipes crimped at the end or

with a single small hole to produce a stream of air bubbles also are employed in some instances.

The air bubbles from the sparger are picked up and dispersed through the medium by the action of the impeller blade mounted above the sparger. In some very large fermentation tanks, an impeller is not utilized. The medium is stirred by the directed rush of air bubbles from a sparger at the bottom of the tank. These tanks are specially designed and usually do not contain baffles.

7) Feed ports:

Feed ports are silicone tubes connected to the nutrient reservoir. They are used to add nutrients and acid/alkali in the fermentor. They are sterilized in situ with stem after connection has been completed and before any additions are made.

8) Foam Control (Antifoam agents):

Foams are dispersions of gas in liquid. Aeration and agitation of a liquid medium can cause the production of foam. This is particularly true for the media containing high levels of proteins or peptides. If the foam is not controlled, it will rise in the head space of the tank and be forced from the tank along with the exit valve. This condition often causes contamination of the fermentation from organisms picked up by breaking of some of the foam which then drains back into the tank. Excessive foaming also causes other problems for fermentation.

Foams in industrial fermentations are controlled either by chemical or mechanical means. *Chemicals controlling foams* have been classified into **antifoams**, which are added in the medium to prevent foam formation, and **defoamers** which are added to knock down foams once these are formed. Some may not see much in the distinction and in this discussion the term antifoam will refer to both. The usual procedure for controlling foam is to add an antifoaming agent, although a supplementary impeller blade mounted high in the tank may at times be effective.

When antifoam is required in a tank, it is added either manually or electrically. Obviously, manually addition requires that someone continuously observe the tank so that the antifoam can be added as required. Electrical

addition of antifoam is usually preferred. To accomplish this automatic addition, a sensing mechanism is employed to determine when the foam has risen into the head space of bioreactor. Such a device is provided with two electrodes mounted in the top of the fermentor. These electrodes are connected to a pump associated with a reservoir of sterile antifoam and as the foam rises in the reactor it touches the two electrodes in the process allowing current to flow between them so as to activate the pump for addition of antifoam. The foam then collapses away from the electrode thus breaking the electrical connection between them and stopping further addition of antifoam agent.

9) Valves:

Valves are required to control the flow of gases and liquids in a variety of way. Five types of valves are used.

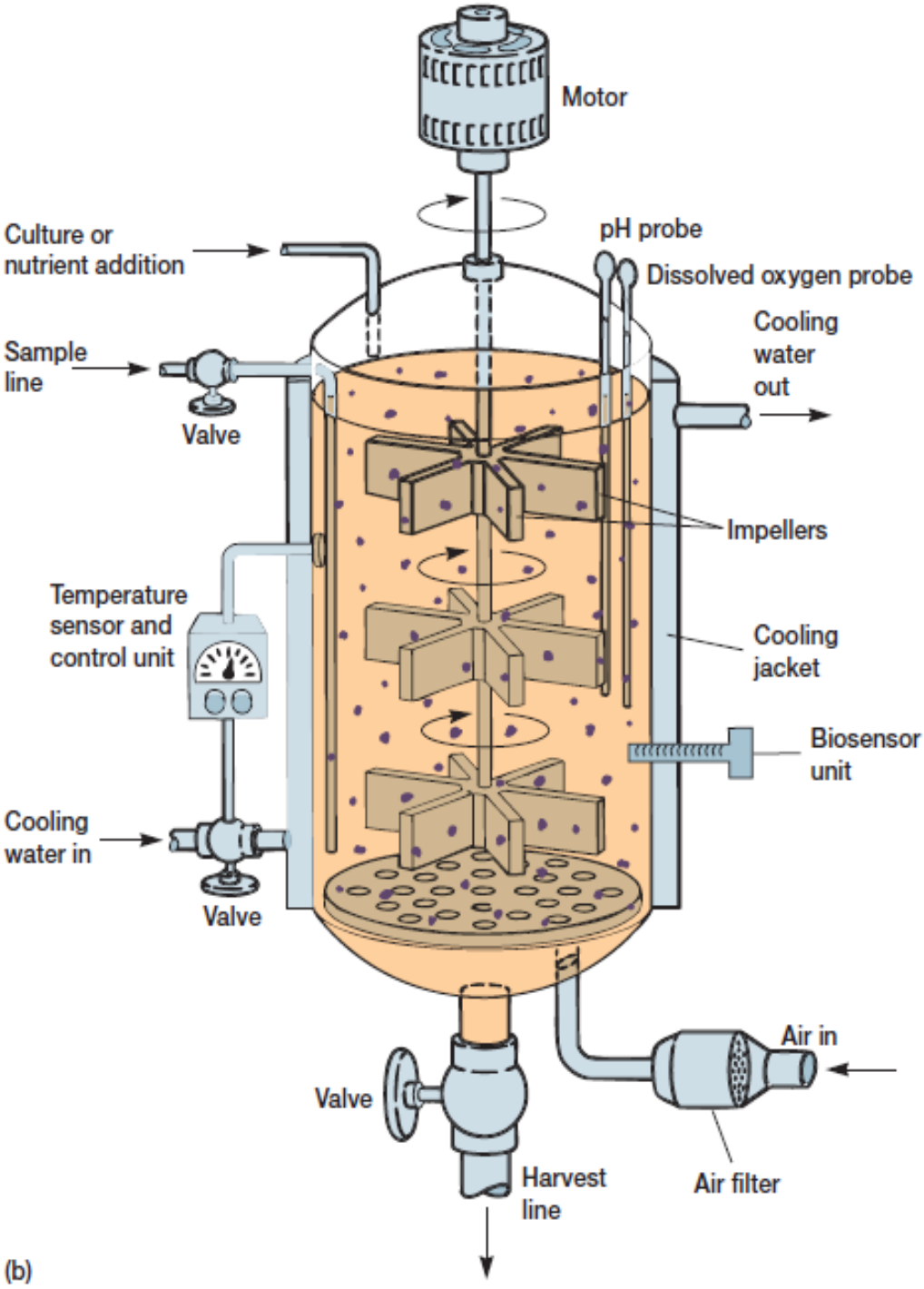
Globe valves: They are suitable for general purposes on stem or a water line for use when fully opened or fully closed. They do not regulate flow.

Butterfly valves: They are used in large diameter pipes operating under low pressure. They are not suitable for aseptic operation.

Ball valves: They are suitable for aseptic condition. They can handle mycelial broths and can be operated under high temperature.

Diaphragm valves: They are used for flow regulation and for stem services within pressure limits.

Safety valves: Safety valve is incorporated in every air or stem vessel and pipe layout which is subjected to work under pressure. These valves ensure that the pressure never exceeds the safe upper limit of the specified value. In simple safety valves, a spindle is lifted from its seating against the pressure of gas. Once the pressure falls below the value set by the spring, the spindle returns to its fermentor.


Motor
Culture or nutrient addition
pH probe
Dissolved oxygen probe
Cooling water out
Sample line
Valve
Impellers
Temperature sensor and control unit
Cooling jacket
Biosensor unit
Cooling water in
Valve
Air in
Valve
Harvest line
Air filter
(b)

Part of Bioreactor	Function
Motor	Provides energy to the impeller in order to generate it.
Impeller	Mix the media by stirring.
Sparger	Introduces air in the form of bubbles
Baffles	Prevent vortexing of culture.
Inlet Air Filter	Remove contaminants; adjust flow rate
Exhaust Ait Filter	Filter used air moving out to the environment
Rotameter	Measures the flow rate of the air.
Pressure Gauge	Measures the pressure.
Temperature Gauge	Measures temperature, giving the culture broth an appropriate warmness to maintain in.
Coolinng Jacket	Control Temperature
pH probe	Measures pH
Dissolved Oxygen Probe	Measures the amount of dissolved oxygen
Foam Probe	Detect the presence of foam
Acid	Added when pH too high (Alkaline)
Base	Added when pH too low (Acidic)
Antifoam pump	Adds anti-foam agent when foam is present in the fermentor with a peristaltic pump.
Sampling Tube	Inoculation, addition of acid or base, and sample removal.
Control Panel	Controls the fermentor that is working
Level Probe	Measures the level of probe

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- **Types of Bioreactors / Fermentors**

Depending on the design of the reactor, the bioreactors are of following types:

i) Batch (Stirred tank batch) bioreactors

ii) Continuous stirred tank bioreactors

a) Single-stage continuous bioreactors

b) Multiple-stage continuous bioreactors

c) Recycled single or multiple stage continuous bioreactors

d) Semi-continuous bioreactors

iii) Bubble column bioreactors

iv) Airlift bioreactors

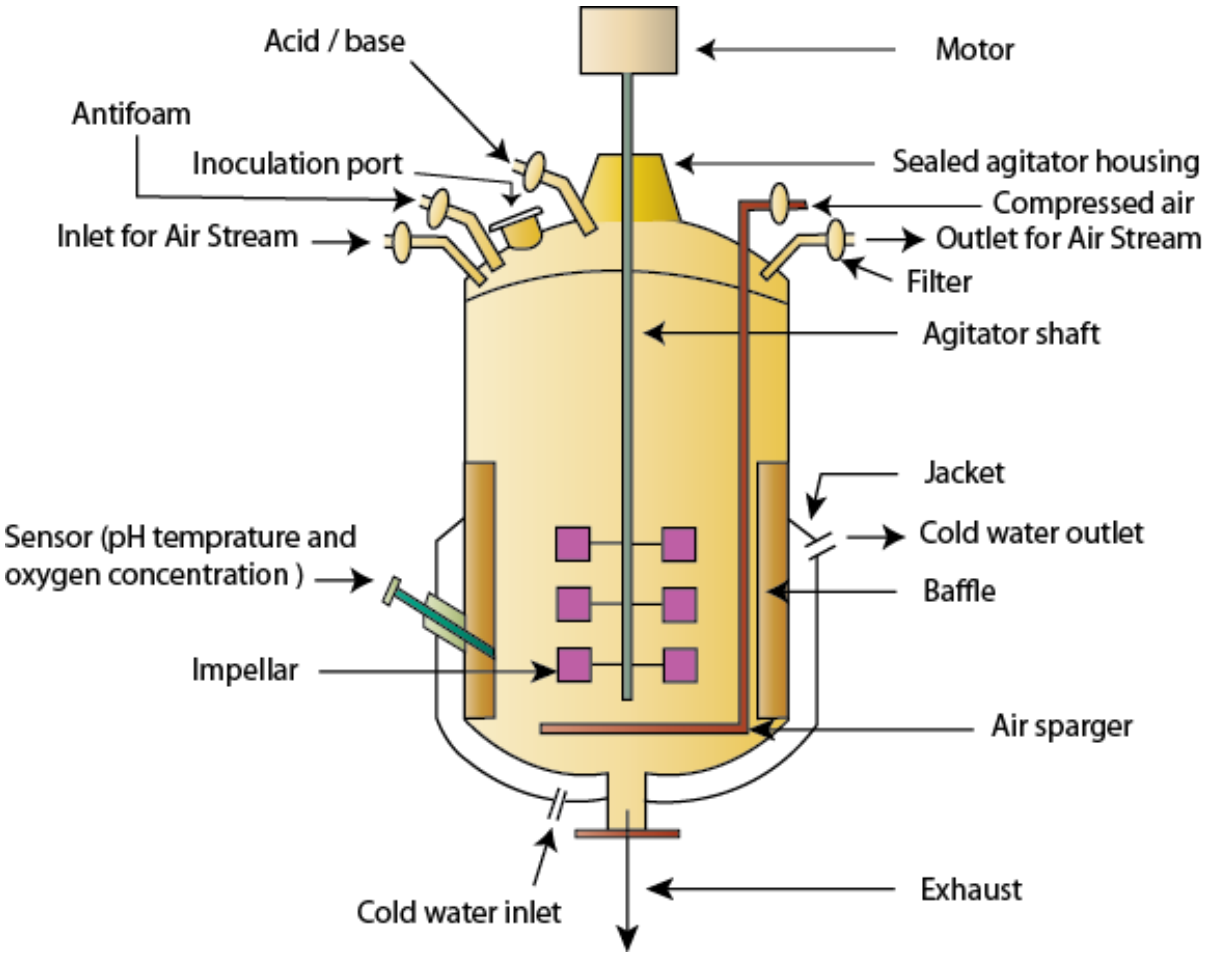
v) Fluidized bed bioreactors

vi) Packed bed bioreactors

vii) Photobioreactors

i) Batch (Stirred tank batch) bioreactors -

These bioreactors have a cylindrical vessel with motor driven central shaft which gives support to one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor. The diameter of the impeller is usually one third of the vessel diameter. The impellers are available in different designs like- Rustom disc, concave bladded, marine propeller etc. In stirred tank reactors, the air is added to the culture medium under pressure through a device called sparger. The sparger along with the impellers (agitators) enables better and efficient gas distribution throughout in the vessel. The advantages of using stirred tank reactors are: the efficient transfer of gas to growing cells which keeps the growth of cells in healthy limits, stirring ensures good mixing of the contents, the operating conditions are flexible and the bioreactors are easily available which makes them commercially viable products.

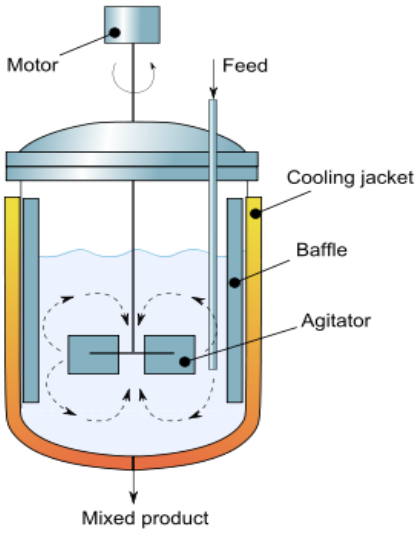


(ii) Continuous stirred tank bioreactors:

The tank used in this system is essentially similar to that of the batch fermentor. It differs only in so far as there is provision for the inlet of medium and the outlet of broth. In continuous fermentations nutrients are continuously added, and products are also continuously removed. There are four types of continuous stirred tank fermentors –

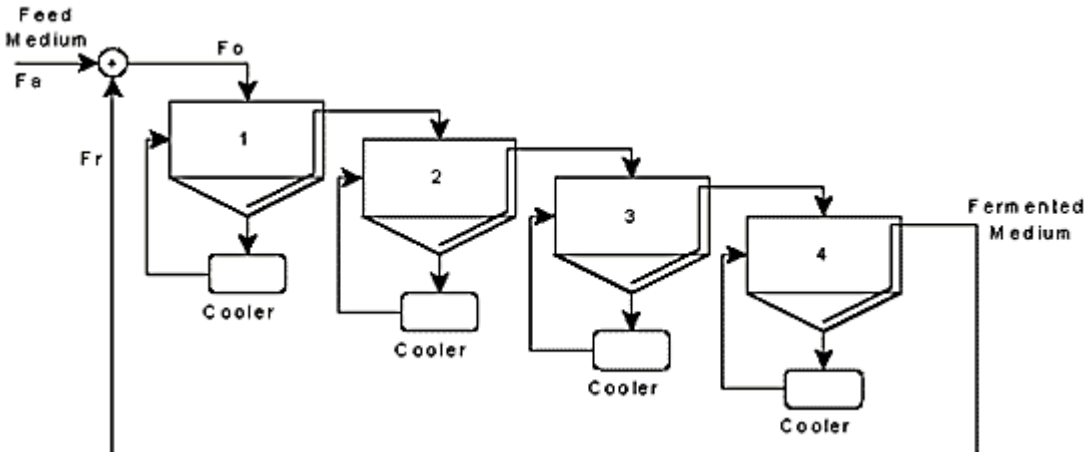
a) Single-stage continuous bioreactors

There are fermentations in which the entire operation is carried out in one vessel, the nutrient being added simultaneously with broth outflow. This system is suited for growth related fermentations such as yeast, alcohol, or organic acid production.



b) Multiple-stage continuous bioreactors

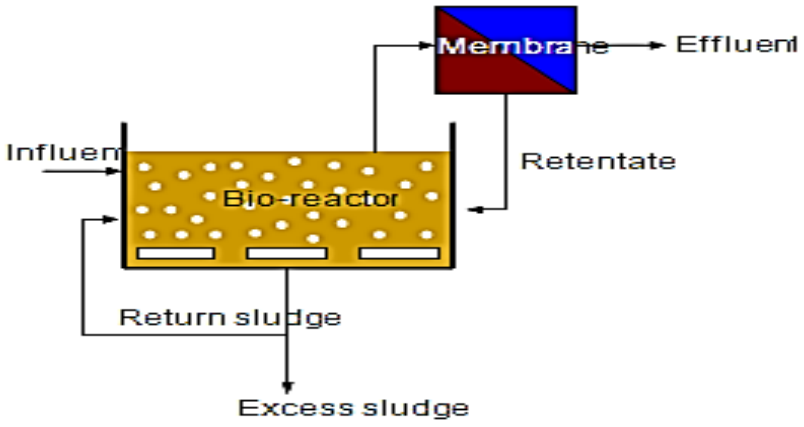
This consists of a series of fermentation tanks. The medium is led into the first and the out flow into the second, third, or fourth as the case may be. This is most frequently used for the fermentation involving metabolites. The first tank may be used for the growth phase and subsequent tanks for production.



c) Recycled single or multiple stage continuous bioreactors

The out flowing broth may be feed of the organisms by centrifugation and the supernatant returned to the system. This system is particularly useful where the substance is difficult to degrade or not easily miscible with water such as in hydrocarbons. Recycling can be applied in a single stage fermentor. In a

multiple stage fermentor, recycling may involve all or some of the fermentation vessels in the series depending on the need.

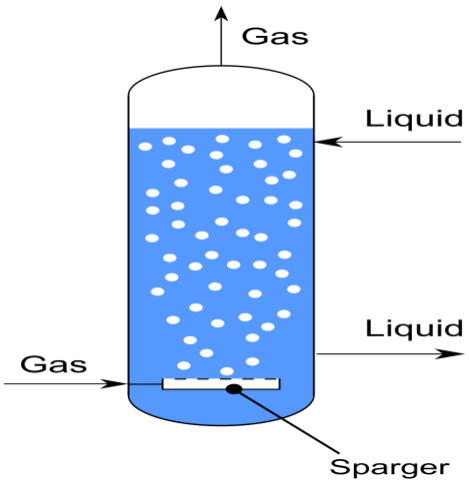


d) Semi-continuous bioreactors

In semi-continuous fermentations, simultaneous nutrient addition and outflow withdrawal are carried out intermittently, rather than continuously.

iii) Bubble column bioreactors–

In these bioreactors, the gas or air is introduced at the base of the column through perforated pipes or plates, or metal microporous spargers. The vessel used for bubble column bioreactors is usually cylindrical with an aspect ratio (height to diameter ratio) of 4-6. The rate of flow of gas affects the O_2 transfer and mixing.



iv) Airlift bioreactors -

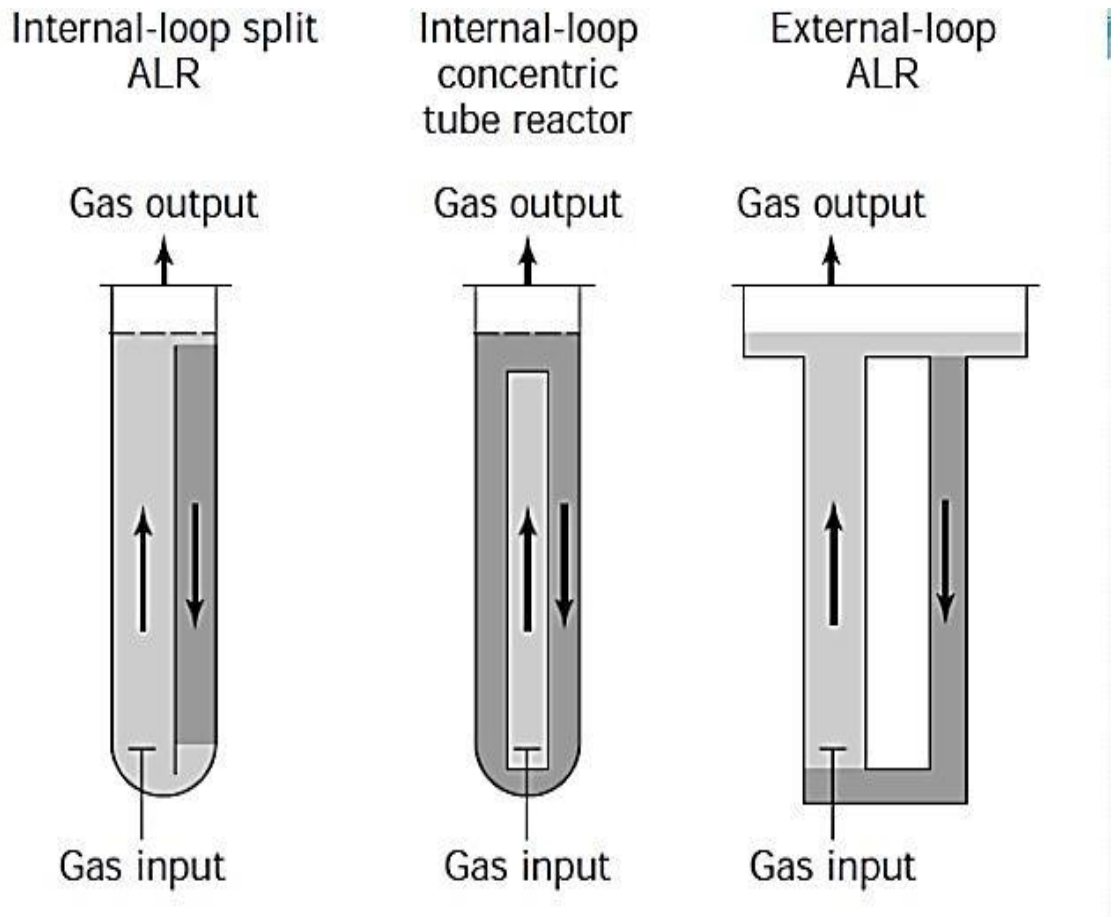
Airlift bioreactors are commonly used for aerobic bioprocessing technology. In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of baffle or draft tube. The air/gas is pumped into one of the two zones referred to as 'riser' and the other zone that receives no gas is known as 'downcomer'. The dispersion flows up the riser zone while the down flow occurs in the downcomer. Further there are two types of bioreactors:

1) **Internal loop bioreactor** - These bioreactors have a single container with a central draft tube that creates interior liquid circulation channels which keeps the volume and circulation at a fixed rate for fermentation.

(2) **External loop airlift bioreactor**-These have an external loop to keep the liquid in circulation through separate independent channels. The modifications can be made in these bioreactors depending on the requirements of different fermentation processes.

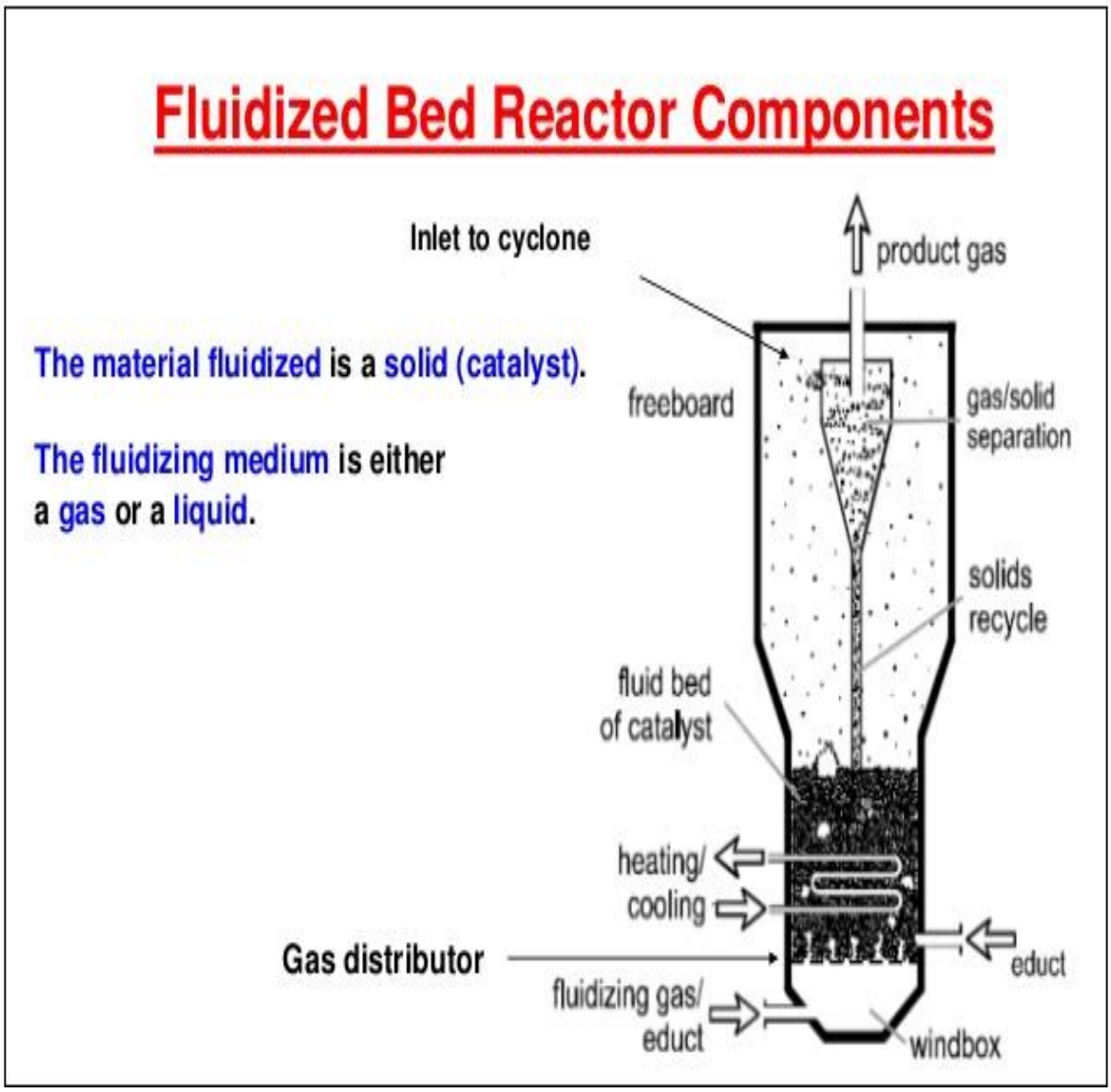
(3) **Two stage airlift bioreactors** -These bioreactors have two bioreactors which are basically used for the temperature dependent formation of products. The growing cells from one bioreactor (maintained at temperature 30^0C are pumped into another bioreactor (at temperature 42^0C). This is done because it is very difficult to increase the temperature quickly from 30^0C to 42^0C in the same vessel. The cells are grown in the first bioreactor and with the help of the fitted valves and a transfer tube and pump, they are transferred into the second bioreactor, where the actual bioprocessing takes place.

(4) **Tower bioreactors** - In this type of bioreactor, a high hydrostatic pressure is generated at the bottom of the reactor which increases the solubility of O_2 in the medium. Since the top is expanded, the pressure is reduced which helps in the expulsion of CO_2. The cycle completes with the medium flowing back into the downcomer. The advantage with Tower bioreactor is that it has high aeration capacities without having moving parts.



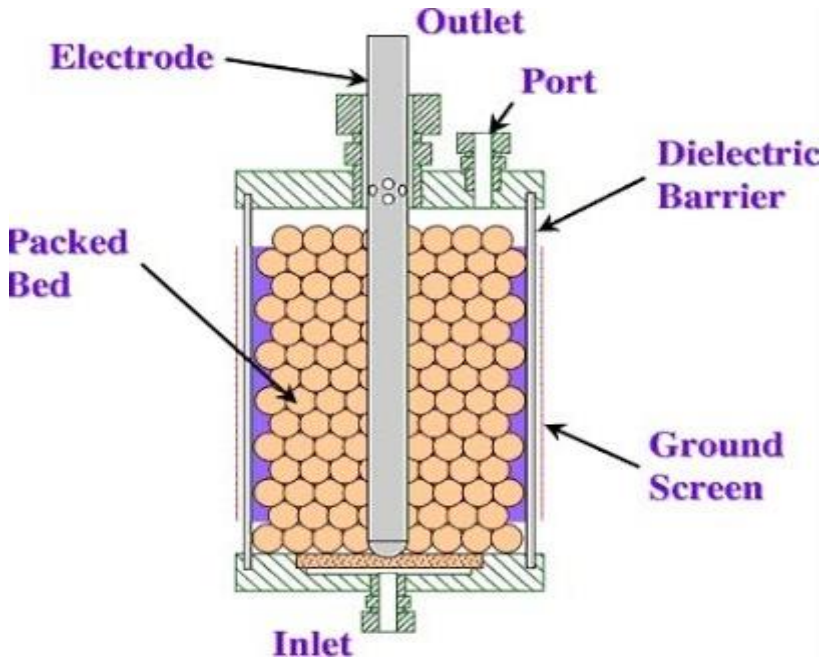
v) Fluidized bed bioreactors–

These bioreactors are mainly suitable to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, microbial flocs etc. The design of the bioreactors is such that the top is extended and the reaction column is narrow which retains the solids in the reactor and allows the liquids to flow out. To maintain an efficient operation of fluidized beds, gas is sparged to create a suitable gas-liquid-solid fluid bed. The recycling of the liquid ensures continuous contact between the reaction contents and biocatalysts which increases the efficiency of bioprocessing.



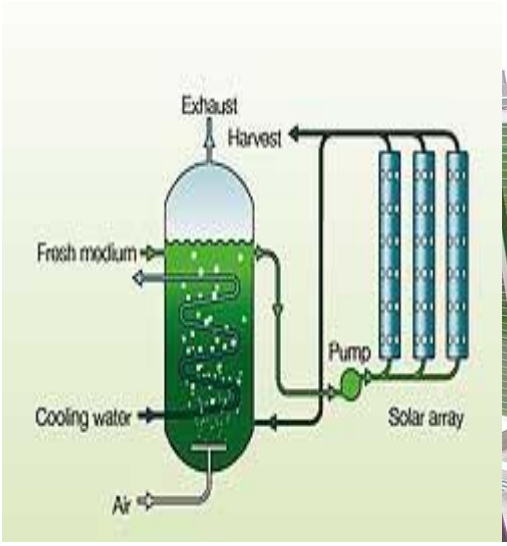
vi) Packed bed bioreactors-

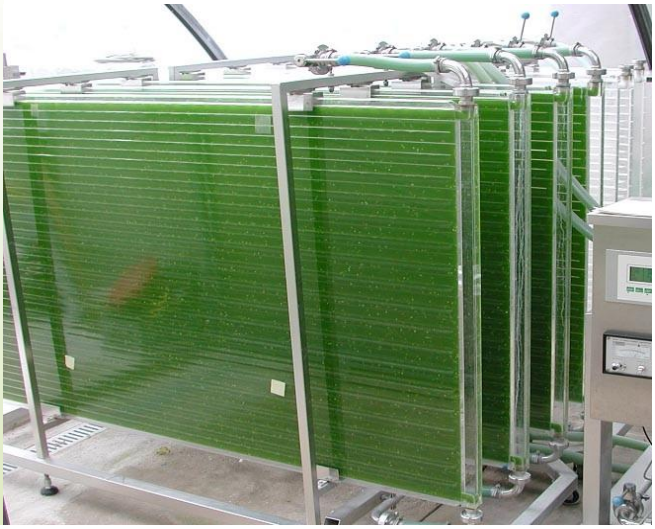
A packed bed bioreactor consists of a bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column. The solids are generally porous or non-porous gels which may be compressible or rigid in nature. The nutrient broth continuously flows over the immobilized biocatalyst and the products are released into the fluid from where they are removed. However, due to poor mixing, it is difficult to control the pH of packed bioreactors by the addition of acid or alkali.



vii) Photobioreactors–

These bioreactors are specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. The photobioreactors are made up of glass or transparent plastic which are the solar receivers. The cell cultures are circulated through the solar receivers by using centrifugal pumps or airlift pumps. These bioreactors work in the temperature which ranges from 25-40^{0}C. In these bioreactors, the microorganisms e.g. microalgae, cyanobacteria etc. grow during the day time while the products (e.g. beta-carotene, asthaxanthin) are produced during the night.





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- **Scale up and scale down of fermentation**

Two of the most common phrases one often met in fermentation technology research are 'Scaling up' and 'Scaling down' studies. However, the phrase 'scaling up' is more commonly understood and practiced during the designing of industrial scale fermentors. Whereas 'scaling down' studies are rarely heard that frequent.

A good example is when we intend to start with a fermentation process with the ultimate objective of producing the fermentation products on the level of industrial scale. Products need to be produced at large volume so that the process is economically viable. This requires scaling up. We do scaling up studies to ensure that the fermentation process is technically and economically viable to be produced in the end at a large scale.

SCALE UP STUDIES

Scale up studies are studies carried out at the laboratory or even pilot plant scal fermentors to yield data that could be used to extrapolate and build the large scale industrial fermentors with sufficient confidence it will function properly with all its behaviours anticipated. More important during scale up exercises you are trying to build industrial size fermentor capable or close of producing the fermentation products as efficient as those produced in small scale fermentors.

INITIAL SCALE UP STUDIES

Most scale up studies are usually carried at different phases involving different scales of fermentors. Preliminary work is carried out at the level of petri dishes and small scale laboratory fermentors to establish whether the process is:

1 Technically viable, meaning it is possible to produce such fermentation process and the products on the small scale. Additional parameters not provided by petri dishes studies and for more confidence are obtained by carrying further studies using submerged liquid fermentation using various sizes laboratory scale fermentors and even a pilot plant fermentor.

There are a few rules of the thumb followed when doing scale up studies such as:

a) Similarity in the geometry and configuration of fermentors used in scaling up
b) A minimum of three or four stages of increment in the scaling up of the volume of fermentation studies. Each jump in scale should be by a magnitude or power increase and not an increase of a few litres capacity. Slight increase in the working volume would not yield significant data for scale up operation.

It must be appreciated as the size of fermentation increases during scale up various parameters measured might not show a predictable linear co relationship. Certain parameters change some remained constant. Some parameters need to be modified and adjusted during scale up studies. The objective is to try to get the same fermentation efficiency as obtained in small scale fermentors at the most economical values.

The exercise in scaling up involved a number of programmed research or steps that has to be established so as to predict the final behaviour of the final large scale production fermentor. Studies carried out during scale up include:

1. Inoculum development.
2. Sterilization establishing the correct sterilization cycle at larger loads.
3. Environmental parameters such as nutrient availability, pH, temperature, dissolved oxygen, dissolved carbon dioxide,
4. Shear conditions, foam production.

SCALE DOWN STUDIES

In scale down studies the main objective is to carry out studies on smaller bioreactors in order to gain data and confidence and predict the behaviour how things actually will behave in large production fermentor. Scales down studies are also used while during the operation of large industrial scale fermentors in trouble shooting or trying to optimize the industrial scale fermentation. This method is called the fermentation monitoring experiment.

The goal when scaling down is to create a small-scale or lab-scale system that mimics the performance of its large-scale (pilot or manufacturing)

counterpart, when both the process parameters are varied within their operating ranges and also when a process parameter deviates outside its operating range.

The main type of studies in scale down such as:

1. Medium design
2. Medium sterilization
3. Inoculation procedures
4. Number of generations
5. Mixing
6. Oxygen transfer rate

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