

BIO REACTORS AND FERMENTORS

I.BIOREACTOR

A bioreactor, or a fermentation vessel, is a device or system used to cultivate and produce biological cells, tissues, or organisms in a controlled environment.

It functions as a vessel-like equipment that offers a stable environment for microorganisms to thrive while also maintaining a steady equilibrium in the biochemical processes carried out by these bacteria to produce the necessary chemicals.

A bioreactor is a device or system that uses controlled conditions to cultivate and produce biological cells, tissues, or organisms.

Bioreactor Principle

The bioreactor's basic premise is to offer an optimum environment for the growth of microorganisms and the generation of metabolites via biotransformation and bioconversion processes. The bioreactor is the central element of any biological process.

A bioreactor's main function is to facilitate the conversion of biological materials into desired products, which includes the production of various enzymes and the performance of biocatalysis processes. Bioreactors create an environment conducive to the growth and metabolic activity of microorganisms by carefully controlling factors such as temperature, pH, aeration, agitation, nutrient feeding, and sterility.

An ideal Bioreactor should have the following properties

1. Aseptic operation: The bioreactor vessel must enable aseptic operation for several days. This keeps microbial growth in the bioreactor clean, allowing desirable products to be generated without interference from undesired organisms.
2. Proper agitation and aeration: The bioreactor must have effective and efficient agitation and aeration mechanisms. Agitation ensures proper mixing of cells and culture medium, while aeration provides oxygen for aerobic fermentation. These processes are critical for ensuring optimal growth and metabolic activity of microorganisms.
3. Minimum energy consumption: The bioreactor should be built to consume the least amount of energy while still performing optimally. This lowers energy costs and improves the economic viability of bioreactor operations.
4. Temperature and pH regulation: The bioreactor must offer precise temperature and pH

regulation. Microorganisms require precise temperatures and pH levels to flourish and produce products. The bioreactor offers an optimum environment for the growth of microorganisms by keeping these parameters within acceptable limits.

5. Sampling facilities: The bioreactor should be provided with conditions for easy and convenient sampling. Regular sampling allows microbial growth and productivity to be monitored and analyzed, allowing for process optimization and quality control.

6. Low Evaporation Loss: During the fermentation process, the bioreactor must limit evaporation loss. Excessive evaporation can result in the loss of important products and have a negative impact on the process's efficiency and output.

7. Minimal labor: Industrial cleaning, harvesting, and maintenance of the bioreactor should be labor-intensive. This lowers personnel expenses while improving the overall performance of the bioreactor system.

8. Smooth interior surfaces: The bioreactor's internal surfaces should be smooth to prevent the collection of bacteria or dirt and to facilitate cleaning and maintenance.

9. Containment policy: The bioreactor must be built to avoid viable cell leakage from the fermenter or downstream equipment. This protects the production process's integrity and prevents contamination.

10. Aseptic protection: Contamination prevention is required for aseptic activities in a bioreactor. To preserve the purity and quality of cultivated cells or organisms, appropriate steps must be taken to prevent the introduction of unwanted microbes.

Bioreactor Design

A well-designed bioreactor may boost productivity, enhance product quality, and lower manufacturing costs. To do this, the bioreactor is often composed of several parts and systems. An agitator system for correct mixing, an oxygen supply system for aerobic operations, a foam control system to regulate foam formation, and temperature and Ph control systems are examples of these. In addition, sampling ports for monitoring and analysis, as well as cleaning and sterilizing systems, may be included in the bioreactor. Loading and emptying lines are also used to add and remove materials from the reactor.

- Several key qualities must be considered when selecting materials for the building of a bioreactor. To preserve the bioreactor's integrity and endurance, the material must be noncorrosive.

It must not introduce any hazardous compounds into the fermentation medium that might damage microorganisms or compromise product quality. The material must be able to survive the steam

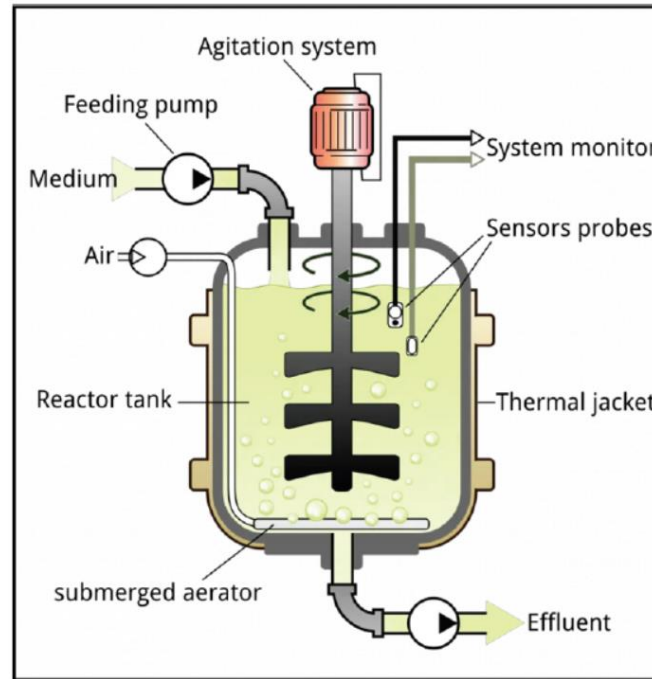
sterilizing procedure used in bioreactors. Furthermore, it must tolerate extreme pressure and pH variations.

An excellent fermenter should possess the following qualities:

Heat and oxygen transport parameters; Sterilization procedures and foam control, as well as a quick and thorough cleaning method; A reliable monitoring and control system.

Bioreactor Parts and Their Function

Fermenter Design



General structure of a continuous stirred-tank type bioreactor

Traditional designs use open circular or rectangular stone or wood jars.

- To avoid contamination, most fermentations are performed in closed systems.
- It must be constructed of non-toxic, corrosion-resistant materials.
- Small fermenters with a few liters of capacity are constructed of glass or stainless steel.
- The stainless-steel pilot scales and other manufacturing containers have a polished inner surface.
- Large fermenters are frequently made of mild steel and covered with plastic or glass to save money. If an aseptic procedure is necessary, the piping that conveys the inoculum, air, and fermentation materials must be sterilized, which is commonly done using steam.
- The majority of vessel cleaning methods are now automated using nozzles and are known as cleaning in situ (CIP). It is contained within the vessel.

- Piping must be built to reduce the likelihood of microbiological contamination. There should be

no horizontal junctions, extraneous pipes, or stagnant places where contaminants might gather; otherwise, poor sterilization may occur.

- Typically, 1000 liter fermenters have an outer jacket. Internal coils are standard in bigger containers.
- Pressure gauges and safety valves must be utilized (needed during sterilization and operation).
- The medium is transferred via pumps. Centrifugal pumps (which generate strong shear forces and establish paths for contamination), magnetically connected jet pumps, and peristaltic pumps are all examples of pumps.

Components of continuous stirred type bioreactor

1. Fermenter Vessel/Vessel

- A fermenter vessel, also known as a fermenter, is a large cylindrical container with top and bottom closures and connections to numerous pipelines and valves. The vessel's design is intended to function under regulated circumstances in bioprocesses.
- There are two types of fermenter vessels: glass vessels and stainless-steel vessels. Glass containers are commonly utilized in small businesses. They are non-toxic and resistant to corrosion. The use of glass containers has the benefit of providing transparency, making it simple to watch and investigate the internal processes going place inside the vessel. Autoclaves are typically used to sterilize glass glassware. These vessels are typically around 60 centimetres in length.

2. Apparatus for heating and cooling

- Heating and cooling equipment are critical in maintaining ideal temperature conditions in the fermenter vessel during fermentation. Here are some details regarding these devices:
- Cooling jacket: A cooling jacket is installed on the exterior of the fermenter tank, which closes the tank and circulates the cooling water. The cooling jacket is required to sanitize the nutrient medium and remove heat produced during fermentation. It aids in the regulation and control of the temperature within the vessel.
- Heat supply: To supply heat within the fermenter vessel, thermostatically controlled baths or internal coils are typically employed. By immersing the vessel in a temperature-controlled bath, thermostatic baths allow accurate temperature control. Internal coils, on the other hand, transport heat directly to the fermenter by cycling hot water or steam through the coils.

- **Heat Removal:** Excess heat generated during fermentation must be removed to maintain the desired temperature. Silicone shirts are often used for heat dissipation.

3. Sealing Assembly

The seal assembly in the fermenter is critical for optimum mixing and preventing leaking from the agitator shaft. Here are some details concerning the seal assembly:

3.1 Purpose of the seal assembly: To ensure appropriate mixing in the fermenter, the seal assembly is employed to seal the agitator shaft. This reduces shaft leaking and aids in maintaining aseptic conditions throughout operation.

4. Partitions

Baffles are crucial components used in fermenters for a variety of reasons, including reducing vortex formation and enhancing aeration within the fermenter. They are typically constructed of metal strips and are mounted radially to the fermenter's wall. Here is some further information on partitions:

1. Swirl Prevention: The baffles' primary role is to prevent the creation of a vortex or swirling movement of the liquid inside the fermenter. Whirlwind might inhibit the fermentation broth from properly mixing and agitating.

2. Improve aeration: Baffles also help to improve aeration in the fermenter. They aid in the uniform distribution of entering air or oxygen throughout the liquid medium, ensuring that bacteria obtain sufficient oxygen for growth and metabolism

3. Baffle design: Baffles are typically flat metal strips affixed to the fermenter's internal wall. The number, size, and position of the baffles are determined by the fermentation process's unique needs.

4. Influence on mixing and mass transfer: The addition of baffles to the fermenter optimizes mixing and mass transfer processes. They serve to break up any stagnant spots in the fermentation broth, ensuring that nutrients, gases, and metabolites are distributed evenly throughout the liquid media.

5. Working wheel

Impellers are essential in the functioning of fermenters because they ensure a homogenous suspension of microbial cells in diverse nutritional mediums.

1. Suspension of microbial cells: The impellers' primary job is to induce agitation in the fermentation tank, ensuring that the microbial cells are equally suspended in the nutritional media. This encourages effective communication between cells and nutrients, resulting in optimum development and metabolic activity.

2. Design and Construction: Impellers are typically made up of blades connected to a central

hub. The impeller assembly is linked to a motor positioned on the fermenter's top. The number, shape, and size of the paddles may vary based on the fermentation process's unique requirements.

3. Bubble Reduction and Distribution: The impeller blades are also important in lowering the size of the air bubbles supplied during aeration and spreading them uniformly throughout.

Types of Impellers: In fermenters, several types of impellers are employed, and their selection is influenced by criteria such as the characteristics of the microbe, the qualities of the culture medium, and the intended process outputs

Disc turbines, Variable Pitch Open Turbine are the types.

6. Sparger

By delivering sterile air, the bubbler ensures optimum aeration in the fermentation tank. It is a mechanism that stimulates microbial growth and metabolism during fermentation. Here are some facts regarding sprinklers:

1. Aeration and oxygen supply: The bubbler's primary role is to give a regulated and sterile flow of air to the fermentation tank. Small holes, generally 5-10 mm in diameter, are supplied in the bubbler tubes via which compressed air is discharged into the vessel

2. Types of atomizers: In fermentation operations, three types of bubblers are used:

a. Porous Bubbler: This type of diffuser is made of a porous material that allows air to travel through equally dispersed pores, such as sintered metal or ceramic.

The porous structure aids in the formation of tiny air bubbles

7. Feed ports

Feed ports are important in the operation of the fermenter because they allow the input of nutrients, acids, alkalis, or other chemicals required for the fermentation process.

1. Nutrient Supplement: Feed ports are used to deliver nutrients to the fermenter. These solutions include the primary elements required for microbial growth and metabolism

2. Acid-base addition: The supply ports allow you to manage the input of acid or alkali solutions to the fermenter in addition to nutrients. This helps to manage the pH of the fermentation medium, which is important for microbial growth and activity.

3. Construction: Feed ports are often tubes made of silicone or other compatible materials with the fermentation processes.

8. Foam Control

Foam management is an important part of fermenter operation because it prevents excessive foaming, which can cause fouling and hinder the fermentation process from working correctly.

9. Valves

The flow and movement of liquids in the fermenter vessel is controlled by valves. They are utilized to control the flow of liquid, allowing for exact control of the fermentation process. Here are some crucial points concerning fermenter valves:

a. Purpose of valves: The flow rate, direction, and pressure of liquids inside the fermenter are controlled via valves. They enable you to manage and isolate distinct process streams, which contributes to the fermentation system's effective operation and maintenance.

10. Aeration System

The aeration system in the fermenter is crucial, especially in aerobic fermentation processes.

Some details on the aeration system:

1. The Importance of Aeration: Microorganisms in aerobic fermentation require oxygen for development and metabolic functions. Bacteria and yeast cells cannot grow or digest the substrate adequately without enough aeration.

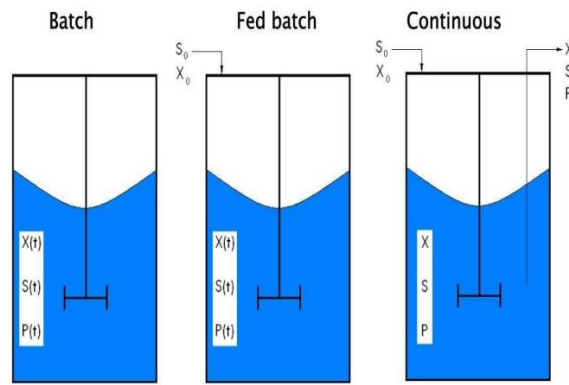
11. Environmental monitoring devise

Control devices have long been a source of contention in the biotech business. Many characteristics, including as temperature, pH, dissolved oxygen and carbon dioxide content, must be considered while developing a bioreactor. Throughout the process, all of this must be managed at various levels. This will minimize pollution, enhance production, and improve product quality while controlling growth.

Bioreactor types

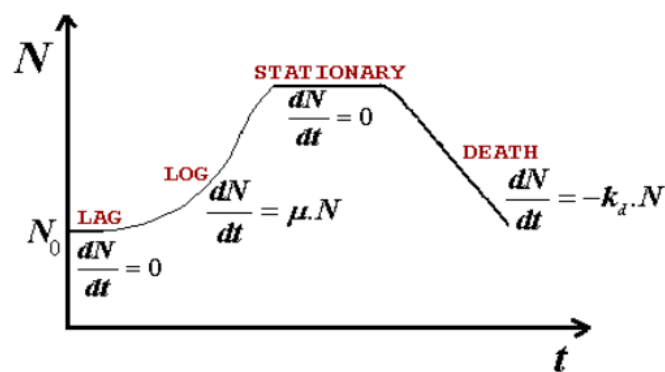
1	Continuous bioreactors with a stirrer
2	Bubble column bioreactors
3	Airlift bioreactors
4	Packed bed reactors
5	Fluidized bed bioreactor
6	Photobioreactor
7	Membrane bioreactor
8	Rotating drum bioreactor

II.Types Of Fermentation



1. Batch fermentation: $\rightarrow$ Nutrients are added in the fermentation for the single time only and growth continues until the particular nutrients are exhausted $\rightarrow$ In the batch process when the microorganism is added into a medium which supports its growth, the culture passes through number of stages known as „growth curve“

Growth kinetics of batch culture: The number of living cells (population of growth rate dN/dt) varies with time in a batch system as shown below:



where;

N = number of bacteria at any time t in the reactor

t = time

N_0 = initial number of bacteria in the reactor after inoculation

2. Fed-batch fermentation:

In this type of fermentation, freshly prepared culture media is added at regular intervals without removing the culture fluid. This increases the volume of the fermentation culture. This type of fermentation is used for production of proteins from recombinant microorganisms. The total amount of the biomass in the vessel increases but biomass concentration is maintained constant.

3. Continuous fermentation:

The growth rate and physiological conditions of microorganisms can be maintained by using a process of continuous culture (chemostat). In this the products are removed continuously along with the cells and the same is replenished with the cell growth and addition of fresh culture media. This results in a steady or constant volume of the contents of the fermenter. This type of fermentation is used for the production of single cell protein (SCP), antibiotics and organic solvents.

Continuous Bioreactor with stirrer (agitator)

A Continuous Stirred Tank Reactor (CSTR) is an agitated vessel used in flow chemistry, that has a continuous feed of reactants and a continuous discharge of the reaction mixture (product). The

feed and discharge rates are controlled to maintain constant reaction conditions (concentration, temperature and reaction rate) ensuring a consistent product stream is produced.

A continuous stirred tank reactor (CSTR) is a reaction vessel in which reagents, reactants, and solvents flow into the reactor while the products of the reaction concurrently exit the vessel. In this manner, the tank reactor is considered to be a valuable tool for continuous chemical processing.

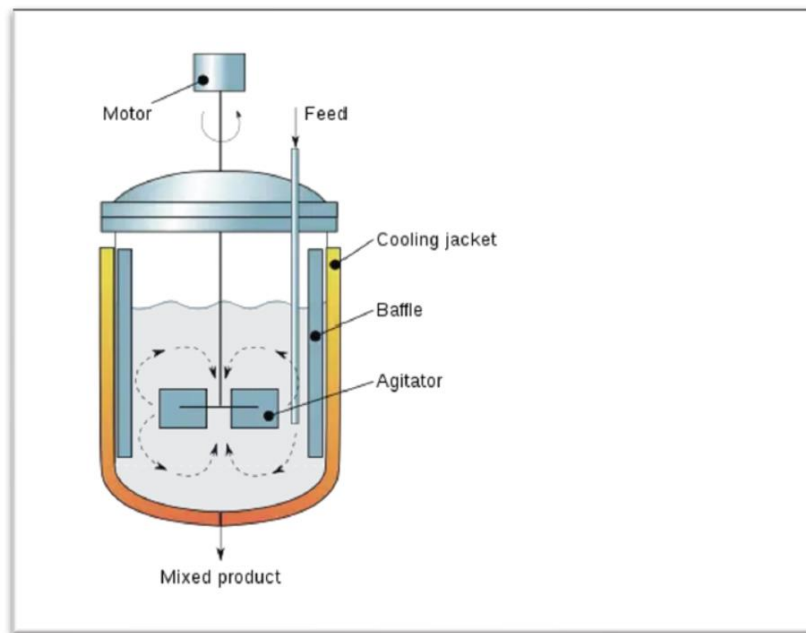
CSTR reactors are known for their efficient mixing and stable, uniform performance under steady-state conditions. Typically, the output composition is the same as the material inside the reactor, which depends on the residence time and reaction rate

It is made consisting of a cylindrical tank with a central shaft powered by a motor that supports one or more agitators or impellers. The combination of sparger and impellers provides for effective gas distribution throughout the vessel, resulting in ideal conditions for microbial growth and fermentation.

- In bioprocessing, CSTF has various advantages. It may operate constantly to provide consistent fertilizer delivery and effective product removal. Temperature control is simple in the fermenter, creating ideal conditions for microbial activity. The CSTF is inexpensive to produce, making it a viable option for large-scale production. It is simple to run, which lowers labour expenses, and the vessel is simple to clean, which simplifies maintenance and prevents contamination.
- A cylindrical CSTF vessel has a height-to-diameter ratio (size ratio) in the 3 to 5 range. Inside the vessel, baffles are frequently fitted to avoid eddies and swirls. These baffles protrude from the vessel's walls and are typically one-tenth or one-twelfth the reservoir's breadth. When employing animal cell cultures, which are more susceptible to turbulence, the vessels are typically not blocked to prevent cell injury.

The number and configuration of impellers in the CSTF are determined by the vessel's aspect ratio. The bottom impeller is typically situated around one-third of the tank's diameter above the bottom. Additional impellers are spaced one to two diameters apart from each other. A single, large-diameter, low-shear impeller, such as a marine propeller, is frequently used in animal cell culture tanks. The gas enters the liquid via a bubbler, which can be either a ring of perforated tubing with a slightly smaller diameter than the impeller or a single-hole bubbler.

The surface aeration rate (gas flow rate divided by vessel cross-sectional area) must remain below the impeller fill point to enable optimal gas dispersion. Flooding happens when the impeller receives more gas than it can adequately disperse, resulting in poor mixing. Surface aeration typically does not exceed 0.05 m/s.



Features of bioreactors with a stirrer

Agitated bioreactors, also known as agitated fermenters, are types of bioreactors that are used to hold and mix a liquid culture of microorganisms or cells. A cylindrical tank with a stirrer or impeller to mix the contents and give oxygen for breathing is typical. The following are some important characteristics of agitated bioreactors

Agitation: To agitate the culture and provide oxygen for respiration, a stirrer or impeller is utilized. To maximize growing conditions, the kind and speed of agitation may be modified.

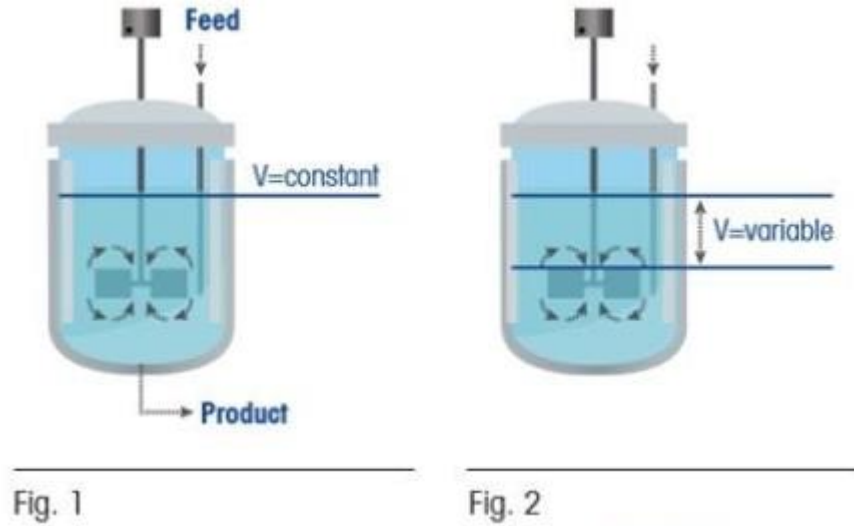
- Temperature control: The culture temperature is normally kept constant by heating or chilling the tank.
- pH Control: Culture pH is normally kept constant by adding acid or base as needed.
- Aeration: Oxygen is supplied into the culture through a stirrer or by bubbling air or oxygen through it.
- Sterilization: To avoid contamination of the culture, the bioreactor and accompanying equipment can be sterilized.

CSTR vs Batch Reactor

In general, reactors can be classified as either continuous (Fig. 1) or batch reactors (Fig. 2). CSTRs are typically smaller in size and enable the seamless addition of reactants and reagents while the product can flow out continuously without interruption.

In contrast, a batch reactor is a chemical reactor that involves the addition of a fixed amount of reactants to the reactor vessel, followed by the reaction process until the desired product is obtained. Unlike a continuous reactor, reactants are not added continuously, and products are not removed continuously. Furthermore, batch reactors are not as uniformly mixed, and the temperature and pressure conditions may vary during the reaction.

CSTRs have the unique ability to handle higher reactant concentrations, as well as more energetic reactions due to their superior heat transfer properties in comparison to batch reactors. In this manner, a CSTR is considered a tool supporting flow chemistry.



Design and Operation of CSTRs

Continuous stirred tank reactors (CSTRs) consist of:

- A tank reactor
- Stirring system to mix reactants (impeller or fast-flowing introduction of reactants)
- Feed and exit pipes to introduce reactants and remove products

CSTRs are most commonly used in industrial processing, primarily in homogeneous liquid-phase flow reactions where constant agitation is required. However, they are also used in the pharmaceutical industry and for biological processes, such as cell cultures and fermenters.

Batch Fermentation	Continuous Fermentation
Type of Fermentation	
It is a closed batch.	It is an open batch.
Nutrient addition	
All the nutrients are added at once in the beginning.	Nutrients are added throughout the batch.
Product extraction	
The product is extracted at the end of the batch.	The product is continuously removed while the batch is operating.
Turnover rate	
The turnover rate (formation of product) is low.	The turnover rate is high.

Type of product	
It is used to extract secondary metabolites such as antibiotics.	It is used to extract primary metabolites such as amino acids and proteins.
Growth conditions inside the fermenter	
The growth conditions can be changed by altering pH and dissolved oxygen.	The growth conditions have to be maintained throughout the operation of the batch.
Size of the fermenter	
Large fermenters are used.	Small-size fermenters are used.
Chances of contamination	
It has less chance of contamination.	It has a high chance of contamination.
Microbial phases	
The microbes go through three phases – lag, exponential and stationary.	The microbes go through a very short lag phase and then maintain themselves at the exponential phase.
Nutrient consumption	
Nutrients are consumed at a slower rate.	Nutrients are consumed rapidly.

Batch cultivation is closed system where there is no interaction between the system and the surrounding during the process. Except air during the aerobic cultivation.

n In Batch cultivation we prepare medium, sterilize it and inoculate the culture into the bioreactor.

n Allow the cells to grow and produce the product.

n Once the product formation reaches to maximum harvest the fermentation broth.

In a batch process, all nutrients are provided at the beginning of the cultivation, without adding any more in the subsequent bioprocess. During the entire bioprocess, no additional nutrients are added – just control elements such as gases, acids and bases; it is a closed system. The bioprocess then lasts until the nutrients are consumed. This strategy is suitable for rapid experiments such as strain characterization or the optimization of nutrient medium. The disadvantage of this convenient method is that the biomass and product yields are limited. Since the carbon source and/or oxygen transfer are usually the limiting factor, the microorganisms are not in the exponential growth phase for a long time.

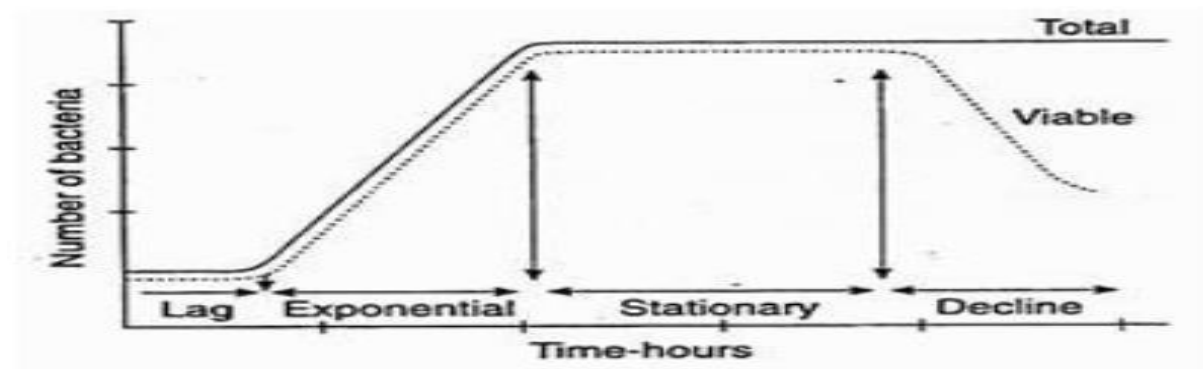
How cells grow during Batch cultivation

After inoculating the medium and start measuring the biomass at different time intervals, you may find six different phases intervals. They are as follows--

Lag phase

Accelerated growth phase

Exponential growth phase
Decelerated growth phase
Stationary phase
Death phase



Lag Phase

- ✓ Lag Phase is an initial period of cultivation during which the change of cell number is zero or negligible.
- ✓ Even though the cell number does not increase, the cells may grow in size during this period.
- ✓ The Lag phase results from several factors.
- ✓ When cells are placed in the fresh medium, the intracellular concentrations of cofactors, amino acids, ions will decrease and these have to be synthesized / transported first before cell division to occur.
- ✓ When the cells are inoculated into medium containing different carbon source then the enzymes for its metabolism have to be transported.

When cell are placed in medium containing several carbon sources then several lag phases may result. This is known as diauxic growth. This is known as diauxic growth. When glucose and lactose are present then glucose will be utilized first then lactose. Presence of glucose will have catabolite repression on galactosidase enzyme which is required for lactose utilization

How to avoid lag phase --- Lag phase is non productive period in the industrial fermentations. Hence industrial fermentations minimizing it is essential.

The stage of culture from where the stage of culture from where the inoculum is drawn is important.

Exponentially growing cells will have adequate concentrations of intermediates and intracellular pool of compounds. Hence if the inoculum is drawn from this stage they will not suffer dilution effect.

Size of the inoculum – If the size of the inoculum is large then the lag phase can be minimized. Generally 10% are used for yeast and mold and 5% for bacteria. Medium of inoculum should be same of that production medium.

Accelerated growth phase ---- At the end of Lag when growth begins the division rate increase gradually and reaches a maximum value. The growth rate increases to maximum during this phase

Exponential growth phase --- Cell division occurs in this phase. Often cell dry weight is used for cell concentration.

Stationary Phase --- In this phase, the growth i.e., cell division, almost ceases due to exhaustion of nutrients and also the accumulation of toxic products. At this stage the cell death starts at a slow rate and is compensated by the formation of new cell through cell division. The total cell number increases at a slow rate, but the viable count remains almost constant. The duration of this phase

is variable which ranges from few days to few hours. Secondary metabolites like antibiotics, toxins etc. are produced in this phase.

Decline Phase ---- In the phase of decline, the total number of cells remains constant, but the number of viable cells gradually decreases due to exhaustion of nutrients and also the accumulation of toxic products. In some cases a few- cells remain viable for long time, even after death of most of the cells. These viable cells probably grow by utilizing nutrients released from dead cells. The cells attain maximum size at the end of lag phase and become smaller in log phase (exponential phase). In spore forming species, the sporulation occurs at the end of log phase (exponential phase) or in the early part of stationary phase.

Micro-organisms used for fermentation process grow on or in growth medium which satisfies the nutritional needs of microbes.

‘ Complete analysis is needed to be done to establish the most favourable medium for the growth of the microbe used for fermentation.

‘ Formulating medium at lab scale can be done by adding main ingredients like water, carbon source, nitrogen source, minerals and other supplements in pure form and in required quantities is very easy which supports the growth of the microbe whereas, the same may not support the satisfactory growth of the same organism at industrial level.

Following criteria need to be satisfied for the material to be treated as medium at industrial level --

‘ It should give maximum yield of product.

‘ It should give minimum yield of undesired product.

‘ It should be consistently available throughout the year.

‘ It should be cheap.

NUTRIENTS

‘ Most fermentations require liquid media, often referred to as broth; although some solid substrate fermentations (SSF) are operated.

‘ Fermentation media must satisfy all the nutritional requirements of the microorganism and fulfil the technical objectives of the process.

‘ All microorganisms require water, sources of energy, carbon, nitrogen, mineral elements and possibly vitamins plus oxygen if aerobic.

‘ The nutrients should be formulated to promote the synthesis of the target product, either cell biomass or a specific metabolite.

‘ In most industrial fermentation processes there are several stages where media are required. They may include several inoculum (starter culture) propagation steps, pilot scale fermentations and the main production fermentation. The technical objectives of inoculum propagation and the main fermentation are often very different, which may be reflected in differences in their media formulations.

Batch culture technique

- Batch culture technique is also called as closed system of cultivation.
- In this technique at first nutrient solution is prepared and it is inoculated with inoculum (culture organism) and then nothing is added in the fermentation tank except aeration.
- In batch culture, neither fresh medium is added nor used up media is removed from the cultivation vessel. Therefore volume of culture remains same.
- Since fresh media is not added during the course of incubation, concentration of nutrition decreases continuously. Furthermore various toxic metabolites also accumulates in the culture vessel. Therefore batch culture technique gives characteristics growth curve with lag phase, log phase, stationary phase and decline phase.

Advantage:

- Chance of contamination of culture is minimum in batch culture technique because it is closed system of cultivation.

Disadvantage:

- It gives low product yield and it is not economic procedure.

Fed-batch culture technique:

- Fed-batch culture is also called as semi-closed system of cultivation.
- In this technique, at first nutrient media is prepared and it is inoculated with culture organism and then incubated for particulate time.
- During the course of incubation a particular nutrient is added at intervals without removing the used up media. so the volume of culture increases continuously.
- Fed batch culture technique is applied in many types of fermentation process.
- In fermentation some nutrient is very essential for the process but when these nutrients are provided in higher concentration in the culture they inhibit the growth of bacteria ultimately ceasing the fermentation. Therefore such nutrients are kept in lower concentration initially and it is added slowly and continuously during the course of fermentation.

Advantage:

- Fed batch culture gives greater product yields than batch culture technique.

Disadvantage:

- Chance of contamination of culture is higher in fed-batch than batch culture technique.

III. Biological treatment of wastewater

The objective of the biological treatment of wastewater is to remove organic matter from the wastewater which is present in soluble and colloidal form or to remove nutrients such as nitrogen and phosphorous from the wastewater. The microorganisms (principally bacteria) are used to convert the colloidal and dissolved carbonaceous organic matter into various gases and into cell tissue. Cell tissue having high specific gravity than water can be removed in settling tank. Hence, complete treatment of the wastewater will not be achieved unless the cell tissues are removed.

Biological treatment usually is divided into aerobic and anaerobic processes. “Aerobic” refers to a process in which oxygen is present, while “anaerobic” describes a biological process in which oxygen is absent.

Aerobic Wastewater Treatment

Aerobic wastewater treatment processes include simple septic or aerobic tanks, and oxidation ditches; surface and spray aeration; activated sludge; oxidation ditches, trickling filters; pond and lagoon-based treatments; and aerobic digestion. Constructed wetlands and various types of filtration are also considered biological treatment processes. Diffused aeration systems may be used to maximize oxygen transfer and minimize odors as the wastewater is treated. Aeration provides oxygen to the helpful bacteria and other organisms as they decompose organic substances in the wastewater.

Anaerobic Treatment

In contrast, anaerobic treatment uses bacteria to help organic material deteriorate in an oxygen-free environment. Lagoons and septic tanks may use anaerobic processes, but the best-known anaerobic treatment is anaerobic digestion, which is used for treating effluent from food and beverage manufacturing, as well as municipal wastewater, chemical effluent, and agricultural waste.

oxidation ditches

Oxidation ditch is a particular type of extended aeration process, where aeration tank is constructed in the ditch shape (oval shape). It is a modified activated sludge biological treatment process that makes use of long solid retention times to remove biodegradable organic matter.

Typical oxidation ditch treatment system consists of-

Basin: The aeration tank consists of a single or multi-channel configuration within a ring shaped basin 1.0 to 1.5 m deep through which the wastewater flows and of suitable width forming a trapezoidal or rectangular channel cross-section.

Bar screens: Preliminary treatment such as bar screens normally precedes the oxidation ditch.

Surface aerators: An aeration rotor, such as brush rotors, disc rotors or fine bubble diffusers is placed across the ditch to provide aeration and wastewater circulation at velocity of about 0.3 to 0.6 m/s. The aeration increases the dissolved oxygen concentration. The horizontal or vertically mounted mechanical aerators are used to provide circulation, oxygen supply, aeration and at the same time, to provide sufficient horizontal velocity for not allowing the cells to settle at the bottom of the oxidation ditch.

The oxidation ditch can be operated as an intermittent type with *fill and draw* cycles consisting of:

- (a) Inlet valve and outlet valve;
- (b) Stopping aeration and circulation device;
- (c) Sedimentation tank to settle the sludge. This settled sludge then gets re-circulated to maintain necessary MLVSS in the oxidation ditch. The sludge generated in oxidation ditch is less and can be directly applied to the sand-bed for drying.

Working

The oxidation ditch is a circular basin through which the wastewater flows. Activated sludge is added to the oxidation ditch so that the microorganisms will digest the B.O.D. in the water. This mixture of raw wastewater and returned sludge is known as mixed liquor.

Oxygen is added to the mixed liquor in the oxidation ditch using rotating biological contactors (RBC's.)

Once the B.O.D. has been removed from the wastewater, the mixed liquor flows out of the oxidation ditch. Sludge is removed in the clarifier. This sludge is pumped to an aerobic digester where the sludge is thickened with the help of aerator pumps. This method greatly reduces the amount of sludge produced. Some of the sludge is returned to the oxidation ditch while the rest of the sludge is sent to waste.

Advantages of Oxidation Ditch

The main advantages of the oxidation ditch is that it can commonly be installed as 'fit and forget' technology with low operational requirements and maintenance costs. Some of the advantages of oxidation ditches include:

- i) This process is reliable over other biological treatment processes owing to a constant water level and continuous discharge;
- ii) Resilient to the impact of shock load or hydraulic surge due to long hydraulic retention time and complete mixing;
- iii) Production of less sludge than other biological treatment processes. Only 15% of original BOD ends up as sludge;
- iv) Energy efficient operations results in reduced energy costs;

Disadvantages of Oxidation Ditch

- i) Suspended solids concentrations of wastewater are relatively higher;
- ii) Requires a larger land area, limiting the feasibility of oxidation ditches in urban, suburban, or other areas where land acquisition costs are relatively high.

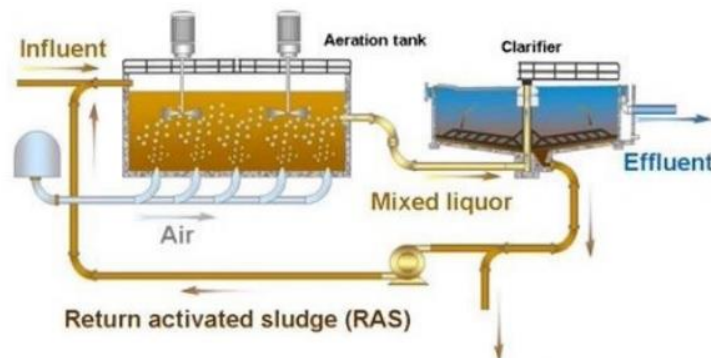
Activated sludge process

The activated sludge process is the most commonly used method of reducing the organic load of the wastewater stream after primary treatment. When the concentration of organic pollutants is

reduced, the oxygen demand placed on the receiving waters is also reduced. This process is also able to oxidize ammonia, reduce the Carbonaceous Biochemical Oxygen Demand (CBOD) and remove nitrogen and phosphorus from the waste stream.

BOD stands for Biochemical Oxygen Demand and it represents the amount of oxygen required by microbes to biochemically oxidize organic and inorganic contaminants in the waste stream. The BOD must be reduced before the wastewater is released into the receiving water. This ensures that an excessive oxygen demand will not be placed on the receiving water body, which could cause fish kills or algal blooms. Total BOD represents the total oxygen demand caused by both carbonaceous BOD (CBOD) and nitrogenous BOD (NBOD), which all play a role in the treatment of wastewater.

Carbonaceous BOD (CBOD) reflects the oxygen demand created by the oxidation of organic carbon substances (sugars, fats and proteins). Nitrogenous BOD measures the oxygen demand created by the oxidation of inorganic nitrogen compounds, like ammonia and nitrite. Using the activated sludge process to remove nutrients like nitrogen or phosphorus is referred to as tertiary treatment. The main goal is to remove CBOD to the following EPA established limits (based on 30 day average):



Biological Reactor (Aeration Tank):

This is where the microbes do their work in the aeration basin. The contents of this basin are referred to as Mixed Liquor (MLSS), since it is comprised of several components, such as soluble, colloidal and suspended material both biodegradable and non-biodegradable in nature. The organic portion of the MLSS is called Mixed Liquor

Volatile Suspended Solids (MLVSS) and makes up approximately 70-80% of the MLSS. These reactors must be sized to provide sufficient hydraulic retention time for treatment to occur, which is in the range of 6-8 hours.

Oxygen Source:

The microbes in the reactor are aerobic, which means they need oxygen to thrive and treat waste. This is supplied in different ways, including mechanical aeration, blowers and diffusers.

Mixing Mechanism:

Each basin must have a way to keep the microbes in suspension so they are able to come into contact with as much waste as possible. This is achieved through mixing mechanisms, which also ensures sufficient dissolved oxygen (DO) is distributed throughout the basin.

Clarifier:

After spending adequate contact time in the biological reactor (aeration basin), the mixed liquor flows to a clarifier where the solids are separated from the liquid. The clarified liquid flows over the effluent weir to the next process, while the solids collect on the bottom of the clarifier for removal.

Sludge Collection System (Return Activated Sludge (RAS))

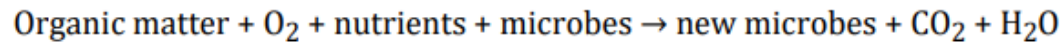
The sludge collection system moves the settled sludge to a common point where it can be pumped back to the aeration tank for further treatment. The volumetric flow of the returned sludge must be accurately measured in order to control the microbial population in the reactor.

Sludge Wasting System (Waste Activated Sludge (WAS))

While the microbes are in the aeration tank they reproduce very quickly, becoming overpopulated (increasing MLVSS concentration), removing the excess microbial population from the system. It is important to maintain a desired microbial population for process control, which is done through sludge wasting. This is linked with the Mean Cell Residence Time (MCRT), which is the amount of time microbes are in the process. The sludge can simply be dewatered if the sludge has already been stabilized through an extended aeration process.

Biochemical Reactions

In the activated sludge process, the BOD and suspended solids are reduced through a variety of biochemical reactions. In the first step, carbonaceous BOD (CBOD) is reduced through a chemoheterotrophic aerobic reaction:

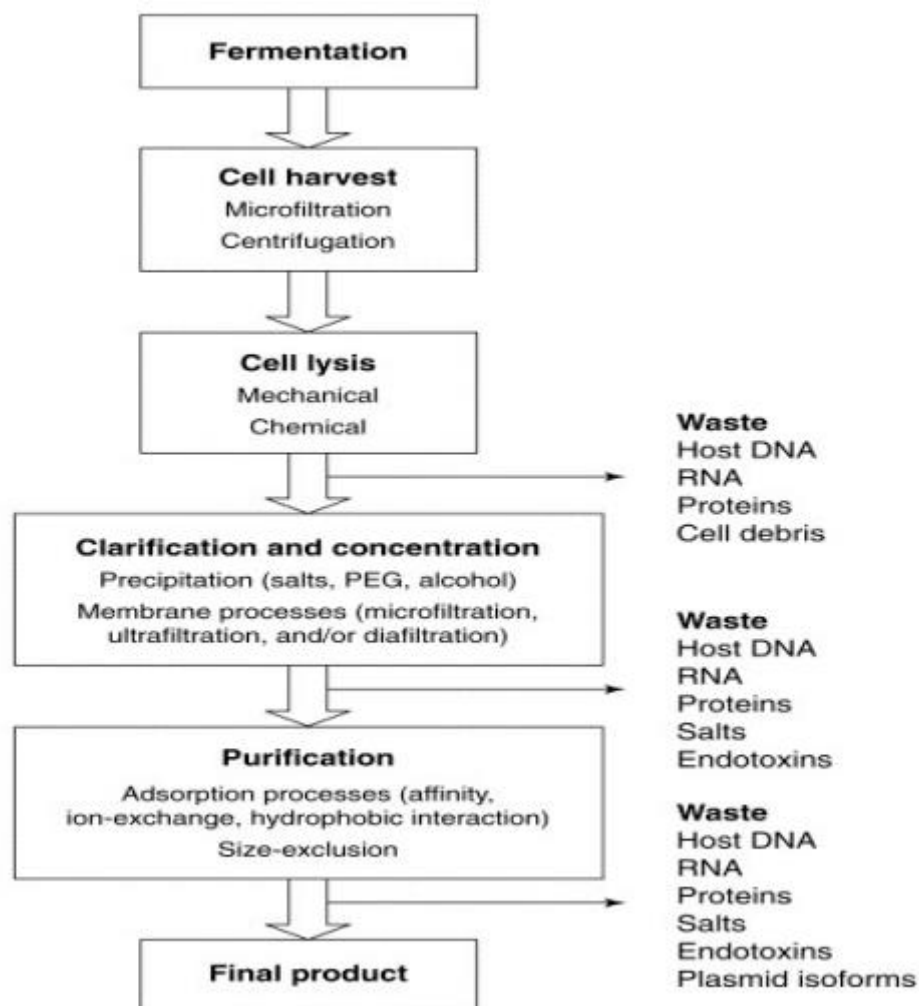


When the organic matter, nutrients and microbes in the aeration basin are given oxygen, it produces new microbes, water and carbon dioxide as a byproduct. These new microbes are either wasted (WAS) or returned (RAS) to do more work in the aeration basin. The second reaction that occurs helps to reduce the nitrogenous BOD (NBOD) in the waste stream. This occurs through a chemoautotrophic aerobic reaction known as nitrification. Nitrification is an aerobic process in which ammonia-oxidizing bacteria, known as Nitrosomonas, change the ammonia (NH_4) and organic nitrogen in the wastewater into oxidized nitrogen, usually nitrate (NO_2).

IV. Separation of bioproducts (Downstream processing)

The extraction and purification of a biological product from the fermentation broth is called as downstream processing (DSP) or product recovery. DSP is quite complex and variable depending upon the type of the product.

Broadly it can be divided into five stages: 1) Cell harvesting 2) Lyses/breakage of cells 3) Concentration 4) Purification 5) Formulation



Definition of Downstream

The downstream stage in the production process involves processing the materials collected during the upstream stage into a finished product. The downstream stage further includes the actual sale of that product to other businesses, governments or private individuals. The type of end user will vary depending on the finished product. Regardless of the industry involved, the downstream process has direct contact with customers through the finished product.

Steps of Downstream Processing

Downstream processing is generally considered to be a specialized field in biochemical/chemical engineering. It encompasses harvesting, purification and packaging of a specific industrial product synthesized in marketable quantities. Analytical bio separation refers to the separation or purification of biological products at different scales of operation, done at analytical scale, the purpose of which is to maintain the desired quality of the product. Downstream processing involves four important steps that result in the following progressive improvements in purity and concentration of the desired product:-

1.Cell harvesting or Solid-Liquid Separation: