UNIT 5

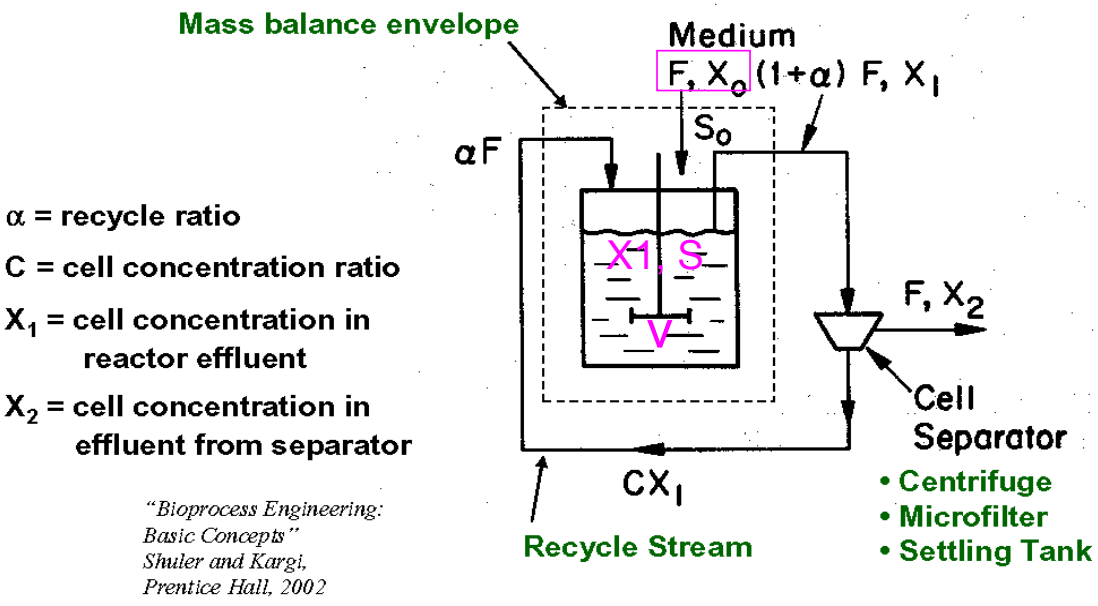
Bioreactor Design

Bioreactors are classified based on their mode of operation –as batch and continuous

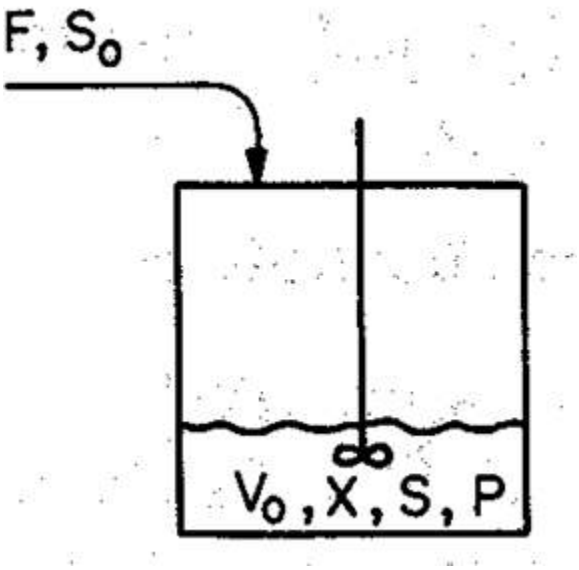
Modified types of the above modes are fed-batch, chemostat with recycle, multi-stage continuous reactors

Chemostat with Cell Recycle

- Designed to keep the cell concentration higher than the normal steady state level in a chemostat.
- To increase the cell and growth associated product yield
- For low product value processes. e.g.waste treatment ,ethanol production

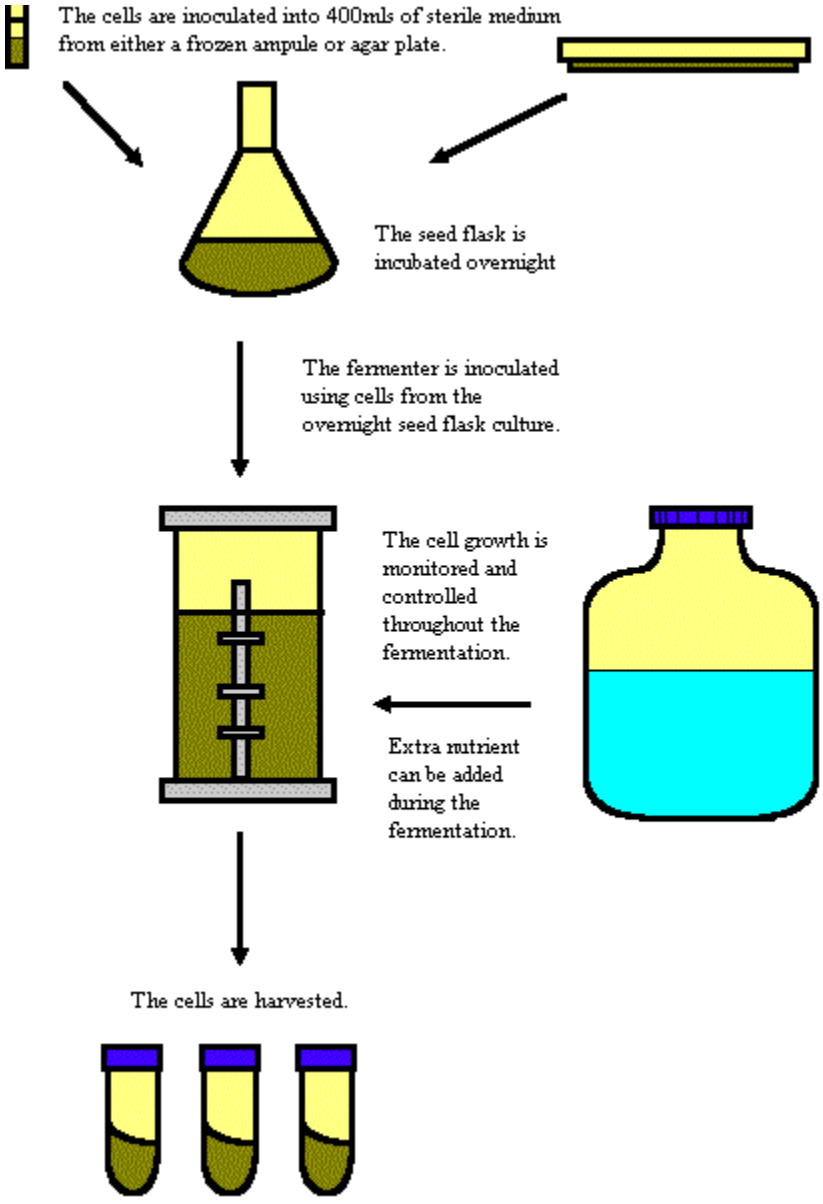


Fed-Batch Reactor



Nutrients are continuously or semi- continuously fed, while effluent is removed discontinuously. It overcomes substrate inhibition or catabolite repression by intermittent feeding of substrate by maintaining low substrate concentration. It is used for production of secondary metabolites e.g. antibiotics, lactic acid, *E.Coli* making proteins from recombinant DNA technology.

Batch Bioreactor



A typical batch bioreactor consists of a tank with agitator and integral heating/cooling system. These vessels may vary in size from less than 1 litre to more than 15,000 litres.They are usually fabricated in steel,glass or alloy.liquids and solids are charged via connections in the top cover of the reactor.vapors and gases also discharge through connections at the top.liquids are usaually dischsarged from the bottom.

Advantages:

1. Easy operation
2. A single vessel can carry out a sequence of different operations

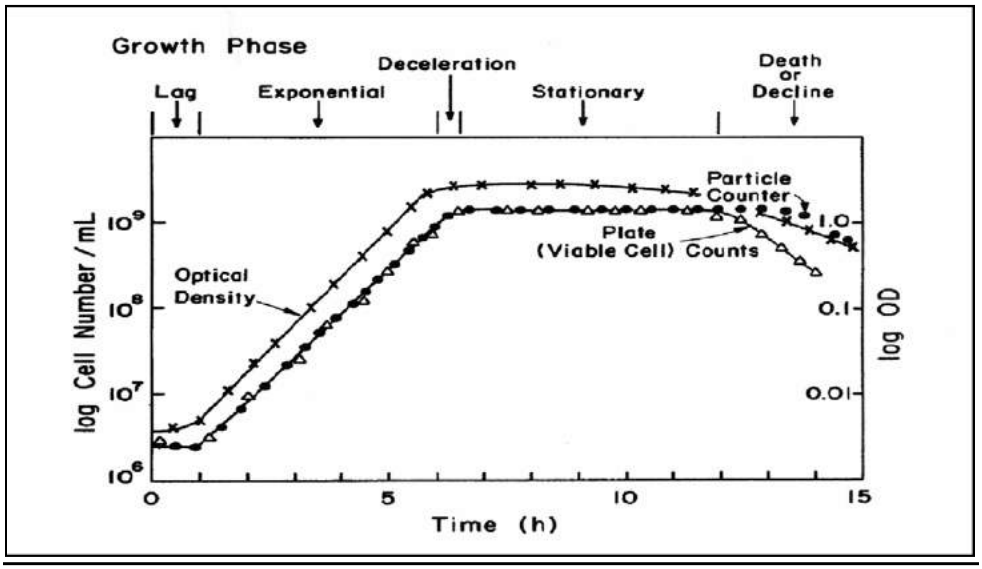
Disadvantages

- Where mixing is a critical parameter, they are not ideal solution.
- Can process small amounts for limited period.

The rate describing the conversion of cell mass into maintenance energy or the loss of cell mass due to cell lysis:

$$\frac{dX}{dt} = -k_d X$$

k_d is the rate constant for endogenous metabolism.



Log Phase

During log phase growth reaches maximum (max)

After depletion of substrate, growth rate decreases and finally ceases

μ= specific growth rate

$\mu = \mu_{max} s / (K_s + s)$

Exponential growth phase

In this phase, the cells have adjusted to their new environment and multiply rapidly (exponentially)

• Balanced growth

–all components of a cell grow at the same rate.

• Growth rate is independent of nutrient concentration, as nutrients are in excess.

The balance of cell mass in a batch culture gives:

$$\frac{dX}{dt} = \mu_{net} X, \quad X = X_0 \quad at \quad t = 0$$

Integration of the above equation yields:

$$\ln \frac{X}{X_0} = \mu_{net} t \text{ , or } X = X_0 e^{\mu_{net} t}$$

X and X_0 are cell concentrations at time t and $t = 0$

$\mu_{net} = \mu_R = \mu_m$

μ_m is the maximum specific growth rate (1/time)

Doubling time of cell mass: the time required to double the microbial mass:

$$\tau_d = \frac{\ln X / X_0}{\mu_{net}} = \frac{\ln 2}{\mu_{net}} = \frac{0.693}{\mu_{net}}$$

Deceleration growth phase

Very short phase, during which growth decelerates due to either:

• Depletion of one or more essential nutrients

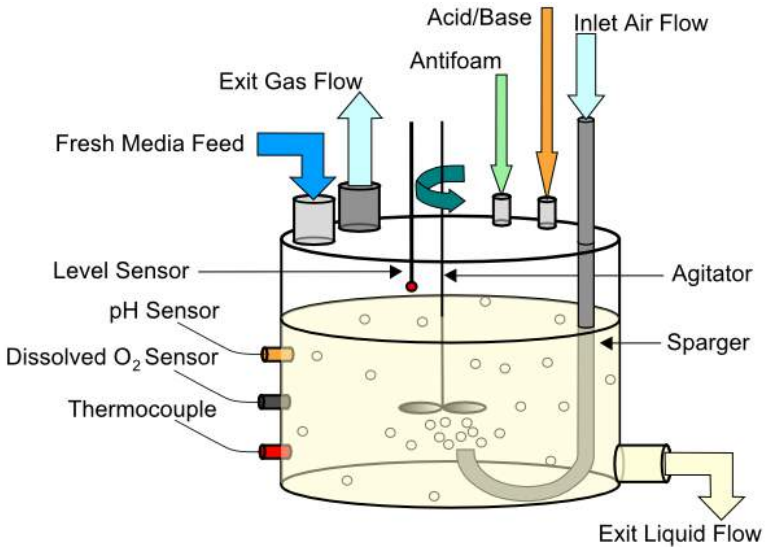
• The accumulation of toxic by-products of growth (e.g. Ethanol in yeast fermentations)

- Period of unbalanced growth: Cells undergo internal restructuring to increase their chances of survival

$$\frac{dN}{dt} = -k_d' N$$

k_d' is the first - order death rate constant.

Continuous Stirred Tank Reactor (CSTR)



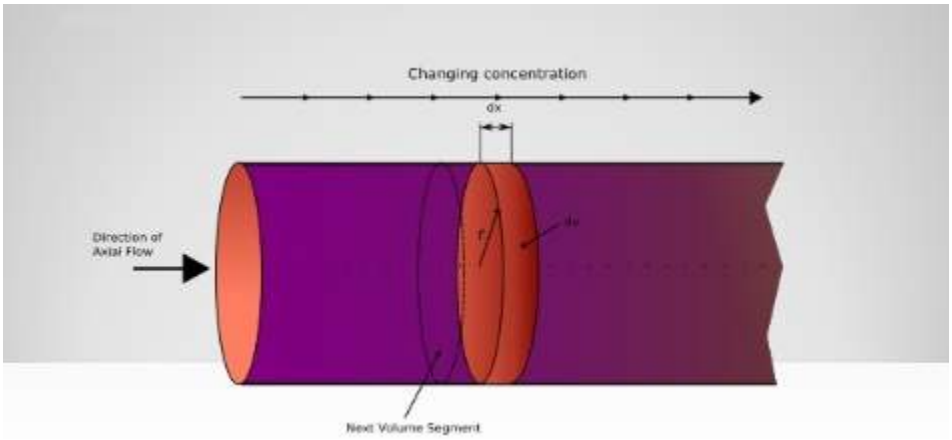
A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor. The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio. The aspect ratio of a stirred tank bioreactor is usually between 3-5. However, for animal cell culture applications, the aspect ratio is less than 2. The diameter of the impeller is usually 1/3 rd of the vessel diameter. The distance between two impellers is approximately 1.2 impeller diameter. Different types of impellers (Rustom disc, concave bladed, marine propeller etc.) are in use. In stirred tank bioreactors or in short stirred tank reactors (STRs), the air is added to the culture medium under pressure through a device called sparger. The sparger may be a ring with many holes or a tube with a single orifice. The sparger along with impellers (agitators) enables better gas distribution system throughout the vessel.The bubbles generated by sparger are broken down to smaller ones by impellers and

dispersed throughout the medium. This enables the creation of a uniform and homogeneous environment throughout the bioreactor.

Advantages of CSTRs:

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

Plug flow bioreactor



This is also referred to as tubular reactor or piston flow reactor. The liquid or slurry system continuously enters one end and leaves at the other end. In the ideal plug flow reactor we envision that flow moves through the reactor with no mixing with earlier or later entering flows. The concentration of substrates and microorganisms vary throughout the reactor. Concentrations of substrates are highest at the entrance of the reactor , which tends to make rates high.

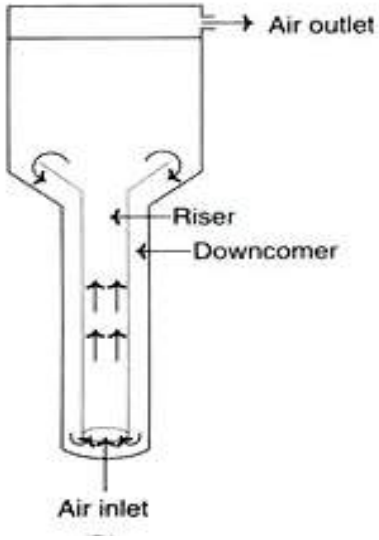
Advantages:

- Can run for long periods of time without any maintenance.
- The heat transfer rate can be optimized by using thinner tubes or few thicker tubes in parallel.

Disadvantages

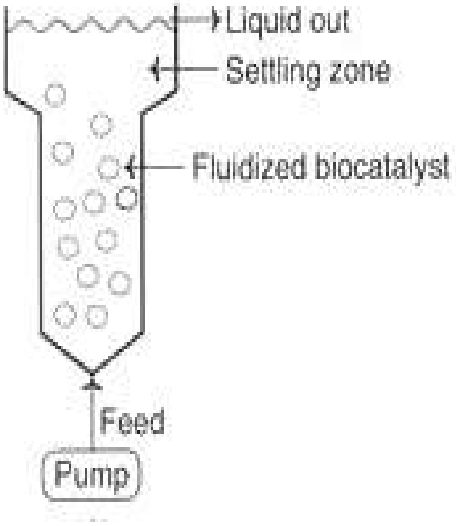
- Temperatures are hard to control and can result in undesirable temperature gradients
- High Installation cost.

Airlift Bioreactors



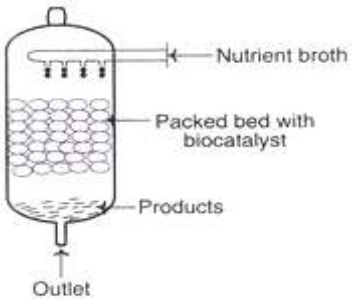
In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube. In one of the two zones referred to a riser, the air/gas is pumped. The other zone that receives no gas is the down comer. The dispersion flows up the riser zone while the down flow occurs in the down comer. There are two types of airlift bioreactors. External loop airlift bioreactor possesses an external loop so that the liquid circulates through separate independent channels. These reactors can be suitably modified to suit the requirements of different fermentations. In general, the airlift bioreactors are more efficient than bubble columns, particularly for more denser suspensions of microorganisms. This is mainly because in these bioreactors, the mixing of the contents is better compared to bubble columns. Airlift bioreactors are commonly employed for aerobic bioprocessing technology. They ensure a controlled liquid flow in a recycle system by pumping. Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

Fluidized Bed Bioreactors



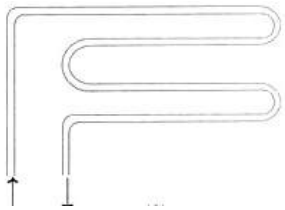
Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out. These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells and microbial flocs. For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas too dense ones may settle at the bottom), and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enables good efficiency of bioprocessing.

Packed Bed Bioreactors



A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor .The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilized biocatalyst. The products obtained in the packed bed bioreactor are released into the fluid and removed. While the flow of the fluid can be upward or downward, down flow under gravity is preferred. The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth. Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

Photo-Bioreactors



These are the bioreactors specialized for fermentation that can be carried out either by exposing to sunlight or artificial illumination. Since artificial illumination is expensive, only the outdoor photo-bioreactors are preferred. Certain important compounds are produced by employing photo-bioreactors e.g., p-carotene, asthaxanthin. They are made up of glass or more commonly transparent plastic. The array of tubes or flat panels constitutes light receiving systems (solar receivers). The culture can be circulated through the solar receivers by methods such as using centrifugal pumps or airlift pumps. It is essential that the cells are in continuous circulation without forming sediments. Further adequate penetration of sunlight should be maintained. The tubes should also be cooled to prevent rise in temperature. Photo-bioreactors are usually operated in a continuous mode at a temperature in the range of 25-40°C. Microalgae and cyanobacteria are normally used. The organisms grow during day light while the products are produced during night.

Downstream Processing in Biochemical Engineering

- Process industries manufacture different products from a variety of raw materials
- The raw materials are pretreated and conversion takes place in a reactor and separation of product of interest and its purification takes place in subsequent steps
- All the steps that are prior to the reactor form "upstream processing"
- All the steps after the reactor form "downstream processing"
- In all the unit operations involved in downstream and upstream processing only physical changes occur and do not involve chemical changes
- Unit operations for separation and purification during downstream processing include:
    - distillation, absorption,
    - extraction, crystallization,
    - drying, mixing,
    - evaporation
- Both refer to the separation or purification of biological products, but at different scales of operation and for different purposes
- Downstream processing implies manufacture of a purified product for a specific use in marketable quantities
- Analytical bioseparation refers to purification for the sole purpose of measuring a component or components of a mixture, and may deal with sample sizes as small as a single cell

Complexity Of Downstream Processing

- the desired product is generally present in low concentrations and
- it is present along with several impurities or undesired components
- The economics of downstream processes are determined by the required purity of the product which in turn depends on the applications of the product.

As a result downstream processing mostly contributes 40-90 % of total cost

Introduction to downstream processing

Downstream processing refers to separation and purification processes of fermentation/enzyme reaction into desired products. It accounts for up to 60% of the total production costs, excluding the purchased raw materials cost. Fermentation can be cells themselves, components within fermentation broth or those trapped in cells. Normally in bioprocessing, the product of interest is the cell itself. Cells are separated from the fermentation broth, then washed and dried. For extracellular products, when cells are separated, the products in the dilute aqueous medium required to be recovered and purified. For intracellular products, they can be released by rupturing the cells and then recovered and purified. For enzyme reactions, the procedures are the same as that of the extracellular products.

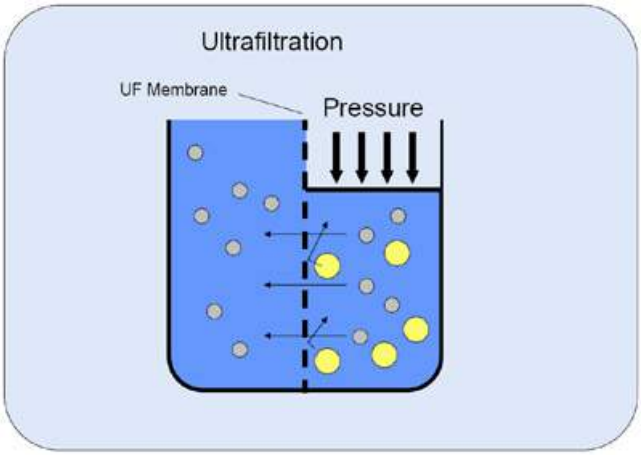
Solid-Liquid Separation

- First procedure/step in downstream processing
- Solid particles to be separated consist of cellular mass;

- with specific gravity between 1.05-1.1 (not much greater than the broth)
- shape may be spheres, ellipsoid, rods, filaments or flocculent.

There are 2 techniques for solid-liquid separation:
1. Filtration/ultrafiltration
2. centrifugation

Filtration/Ultrafiltration



- It is a technique where a mixture of solid and liquid is separated by forcing through a filtering medium on which solids are deposited.

Categories of filtration depend on:
1. filtering medium used
2. range of particle sizes removed
3. pressure differences
4. principle of filtration: conventional filtration, microfiltration, ultrafiltration, reverse osmosis.

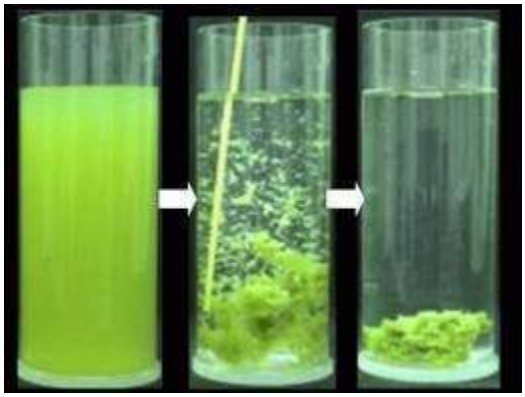
For a conventional filtration:
size of particles: $dp = 10'$m
effective for dilute suspension, large and rigid particles

Two types of filters used:
1. pressure
2. vacuum

Filter aid: Filter aids such as diatomaceous earth, perlite and cellulose enables a liquid to pass through while retaining the haze-causing particles.

Sedimentation: Sedimentation is the tendency for particles in suspension to settle out of the fluid in which they are entrained and come to rest against a barrier. This is due to their motion through the fluid in response to the forces acting on them. These forces can be due to gravity, centrifugal acceleration or electromagnetism.

Floculation: Flocculation is when microscopic particles bind together to form larger particles in liquids. It occurs naturally or artificially in water as well as other solutions. Flocculation is used in applications such as water purification, sewage treatment, cheese production and brewing.



In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, pressure filters, rotary drum vacuum filters and membrane filters are in use.

1. Filter press: normally used for small scale separation of bacteria and fungi from broth
2. Rotary drum filter: used for large-scale separation, common filter medium includes canvas, wool, synthetic fabrics, metal or fibre glass

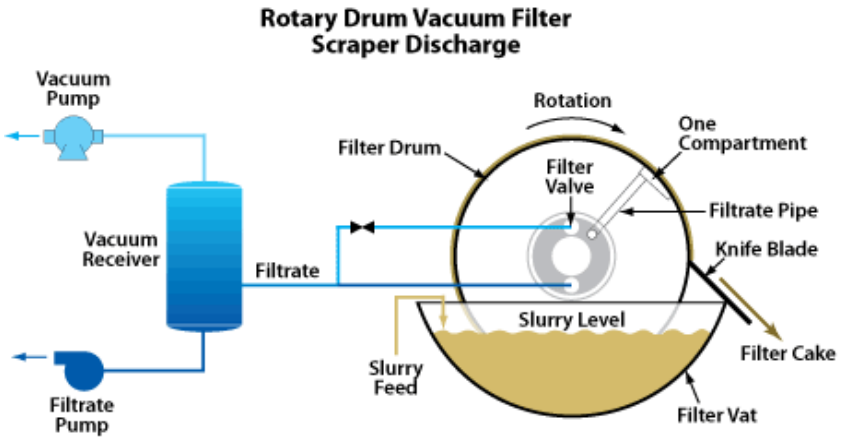
A number of factors will influence the choice of the most suitable filtration technique/equipment and they include;

1. properties of filtrate-viscosity and density
2. nature of solid particles-size, shape, size distribution and packing characteristics
3. solid:liquid ratio
4. need for recovery of the solid/liquid fraction or both
5. scale of operation
6. need for batch/continuous operation
7. need for aseptic conditions
8. need for pressure/vacuum suction to ensure an adequate flow rate of the liquid

Rotary Vacuum Filter

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth .As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

Rotary vacuum filter drum consists of a drum rotating in a tub of liquid to be filtered. The technique is well suited to slurries, and liquids with a high solid content, which could clog other forms of filter. The drum is pre-coated with a filter aid, typically of diatomaceous earth (DE) or Perlite. After pre-coat has been applied, the liquid to be filtered is sent to the tub below the drum. The drum rotates through the liquid and the vacuum sucks liquid and solids onto the drum pre-coat surface, the liquid portion is "sucked" by the vacuum through the filter media to the internal portion of the drum, and the filtrate pumped away. The solids adhere to the outside of the drum, which then passes a knife, cutting off the solids and a small portion of the filter media to reveal a fresh media surface that will enter the liquid as the drum rotates. The knife advances automatically as the surface is removed.



Advantages

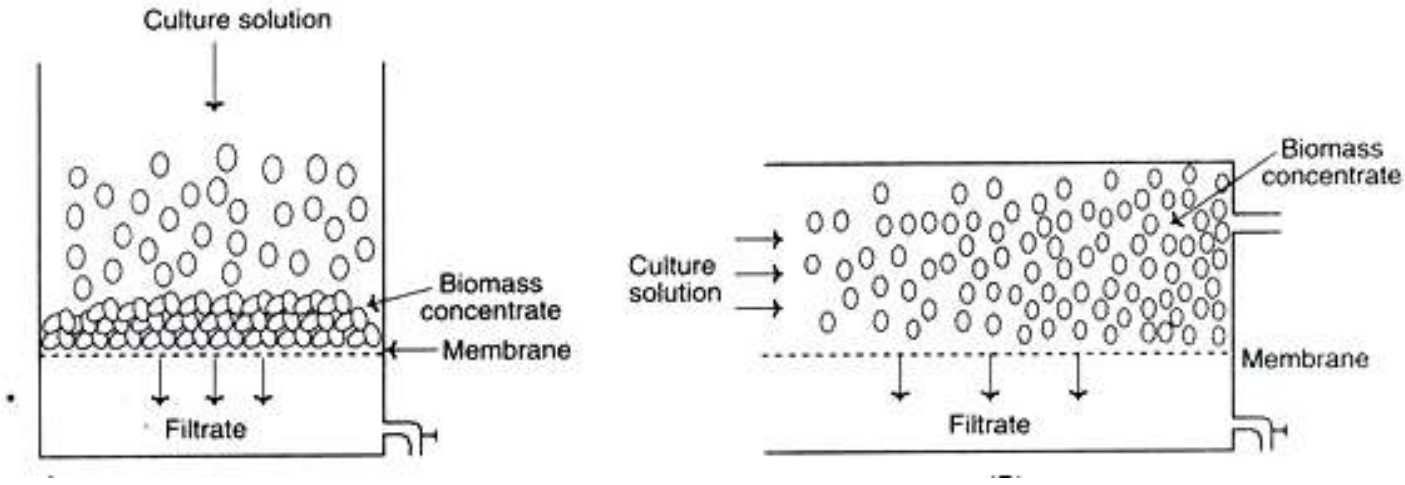
- The rotary vacuum drum filter is a continuous and automatic operation, so the operating cost is low.
- The variation of the drum speed rotating can be used to control the cake thickness.
- The process can be easily modified (pre-coating filter process).
- Can produce relatively clean product by adding a showering device.

Disadvantages

- Due to the structure, the pressure difference is limited up to 1 bar.
- Besides the drum, other accessories, for example, agitators and vacuum pump, are required.
- The discharge cake contains residual moisture.
- High energy consumption by vacuum pump.

Membrane Filters:

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration . In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.



Centrifugation

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale

industrial centrifugation. It is an alternative method when filtration is ineffective (in the case of small particles). Centrifugation is a process which involves the application of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge and is used in industrial and laboratory settings. Requires more expensive equipment and cannot be scaled to the same capacity as filtration equipment. Some centrifuges can be used for separating 2 immiscibly liquids and breaking emulsions.

Centrifuge is any device that applies a sustained centrifugal force—that is, a force due to rotation. Effectively, the centrifuge substitutes a similar, stronger, force for that of gravity. Every centrifuge contains a spinning vessel; there are many configurations, depending on use. A perforated rotating drum in a laundry that throws off excess water from clothes, for example, is a type of centrifuge. A similar type is used in industry to separate fluids from solid matter after crushing.

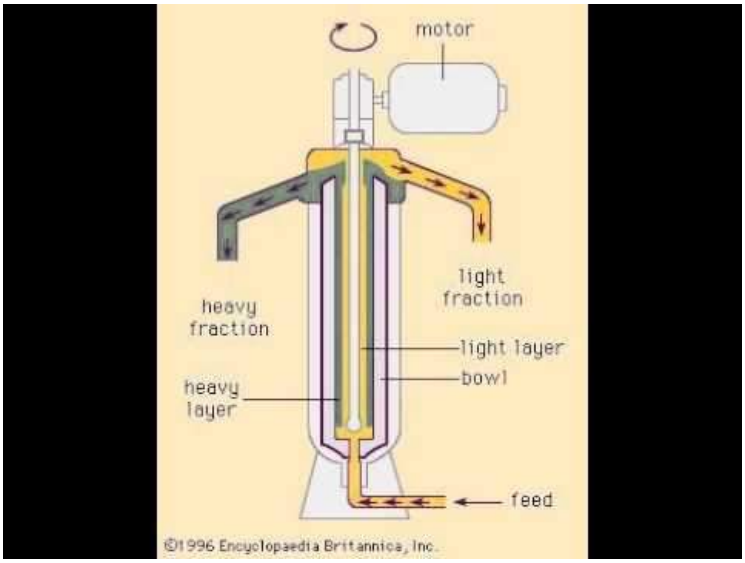
Two basic types of centrifuges (large-scale):
1. tubular centrifuge
2. disk centrifuge

Tubular centrifuge:

The tubular centrifuge is used primarily for the continuous separation of liquids from liquids or of very fine particles from liquids, although in some cases it is employed as a batch-type centrifuge. In general, it is used when higher centrifugal fields are required for separation. The rotating bowl of a tubular centrifuge consists of a long hollow tube (length many times its diameter) as shown schematically

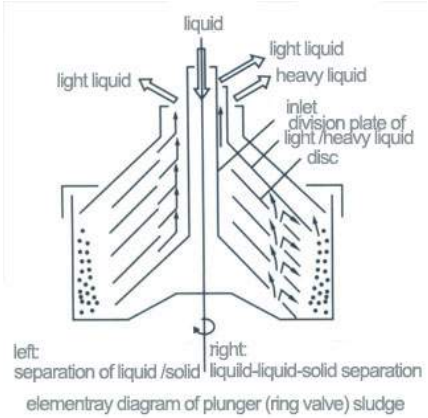
For continuous separation the feed or material to be centrifuged enters at one end near the axis and is removed in two streams containing the separated material. In many cases the separation is not complete, and the separated fractions must be passed through the machine several times. Many different designs for the internal structure of the tube are employed, but, in general, radial vanes are used to bring the feed material up to speed and to slow down the separated streams before they are discharged. The centrifuge is driven by a high-speed motor or an air or steam turbine. The sedimentation takes place as the fluid flows from one end of the tube to the other. When the heavy material consists of very fine particles or molecules and the concentration is very low, the solid material is usually allowed to deposit on the wall. In this case the machine is operated as a batch centrifuge.

The tubular centrifuge is finding an increasing number of applications because of the high centrifugal fields that may be used ($10^5 g$ near the periphery in some cases). A few typical uses are as follows: (1) the purification of vaccines(uncentrifuged vaccines contain a large amount of nonessential and harmful material), (2) purification of lubricating and industrial oils, (3) clarification and purification of food products such as essential oils, extracts, and fruit juices, and (4) separation of immiscible liquids that cannot be separated by gravity.



- hollow cylinder rotating element in a stationary casing
- suspension usually fed through bottom and clarified liquid is removed from top leaving solid deposit on the bowl's wall
- accumulated solids are recovered manually from bowl
- for a typical tubular centrifuge: bowl size: 2 to 5 in diameter, 9 to 30 in. height, max. speed 15,000 to 50,000 rpm

Disk centrifuge:



- often used for bioseparation
- operate continuously
- consists of a short, wide bowl (8 to 20 in.) diameter which turns on a vertical axis
- closely spaced cone-shaped disc in the bowl decrease the distance that a suspended particle has to be moved to be captured on the surface and increases the collection efficiencies
- feed enters the bowl at the bottom, flows into channels and upward past the disks
- solid particles are thrown outward and the clear liquid flows toward the centre of the bowl and discharge through annular slit

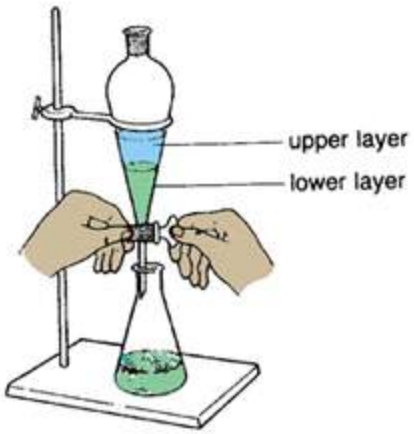
The disk-type centrifuge consists of a vertical stack of thin disks in the shape of cones. The sedimentation takes place in the radial direction in the space between adjacent cones. This greatly reduces the settling distance and hence increases the rate at which the material is separated. The angle of the cones is designed so that upon reaching the inside surface of the cone the heavier material slides down along its surface in a manner that is similar to that of the 37° fixed-angle bottle centrifuge.

The disk-type centrifuge usually operates continuously. The material to be processed enters in one stream and is separated into two purified streams. These centrifuges are used primarily for the separation of liquids in which the solid or immiscible components occur in relatively low concentrations. The familiar cream separator, widely used in the dairy industry and on farms for separating cream from milk, is a typical example of this type of centrifuge. They also are used for the purification of fuel oil, the reclamation of used motor oil, and the removal of soap stock in the refining of vegetable oils.

Liquid-liquid Extraction

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product. The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases.

Solvent Extraction : Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example water and an organic solvent. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel.



It is a unit operation based on differential solubility of a consolute in two immiscible solvents. It is also known as solvent extraction. The application is rather popular due to its inherent flexibility and its suitability for processing heat sensitive-products. The operation is highly dependent on the solubility of the chemical used that leads to the strengths and limitation of the operation. Possible choices of solvents results in the unmatched versatility of extraction as a mass transfer operation. Characteristics of the operation of particular relevance to bioseparations are the effectiveness of extraction at low temperatures and the tendency of organic solvents to denature most proteins irreversibly. Extraction in the pharmaceutical industry has been used primarily in the isolation of antibiotics from fermenter broth, in the preparation of natural and synthetic vitamins and the preparation of drugs from naturally occurring materials.
Contrary to extraction, applications in most other industries, very dilute aqueous solutions are usually fed into the process. Extraction may be carried out in batch or staged-batch sequence such in mixer-settler unit, however, most pharmaceutical extractions have been carried out in differential units. Fractional extraction processes involve two or more consolute components and are designed to enrich the extract in the desired product component and to isolate the product from the aqueous feed solution. Conventional liquid-liquid extraction is not normally useful for the isolation of protein products because of the irreversible denaturation of these macromolecules that typically occurs in organic solvents. Immiscible aqueous-phase solutions can be prepared by the addition of polymers or polymers with an addition of salts to water.

Cell Rupture/Disruption

Disruption of cellular materials is usually difficult due to the strength of the cell walls and high osmotic pressure inside. Techniques of rupturing cells have to be powerful and mild such that the desired components are not damaged. Cells can be ruptured either mechanically or chemically.

Mechanical methods:

1. Liquid shear (homogenisation)
   - it is actually a positive displacement pump with an adjustable orifice valve

- used normally for large scale cell disruption

- pump pressurises the cell suspension to approximately 400 to 500 bar then releases it through a spacial discharge valve{creating very high shear rates
- cooling to 4±C is necessary to compensate for heat generated during the
- adiabatic compression and homogenisation steps

2. Solid shear (pressure extrusion)

- using frozen microorganisms at temperature of -25±C through small orifice
- normally lab scale procedure
- disruption is due to combination of liquid shear through narrow orifice

with the presence of ice crystals

- 90% cell disruption is able to achieved with a single passage of *S.cerevisiae*

using a throughput of 10kg yeast cell paste per hour

- ideal for microbial products which are very temperature labile

3. Agitation with abrasives
4. Freeze-thawing
5. Ultra-sonicator

Cell disruption methods for intracellular products

Chemical methods:

1. Detergent

Detergents that are used for disrupting cells are divided into anionic, cationic and non-ionic detergents. The common thing for all detergents is that they directly damage the cell wall or membrane, and this will lead to release of intracellular content . One of the most commonly used anionic detergent is sodium dodecyl sulfate (SDS) which reorganizes the cell membrane by disturbing protein-protein interactions. Another commonly used compound for cell lysis is Triton X100, which is non-ionic detergent. Its mechanism of action is to solubilize membrane proteins. In addition to these chemical compounds, for example cationic detergent ethyl trimethyl ammonium bromide can also be used for cell disruption. It is speculated that it acts on cell membrane lipopolysaccharides and phospholipids .The disadvantage of using detergents for cell lysis is that many proteins will be denatured in lysis process. Detergents may also disturb subsequent downstream processing steps. Thus additional purification step may be required after cell lysis, which limits their utilization in large scale processes. However, detergents are commonly used for cell lysis in laboratory for example once DNA, RNA or proteins are extracted from cells.

2.Osmotic shock

The proper functionality of cell's processes usually requires strictly defined chemical conditions. This means e.g. that cell's internal pH or salt concentrations should not deviate significantly from the optimal values. The optimal conditions and ability to withstand suboptimal conditions are species specific. Cells have an ability to actively control the internal conditions but sudden and major changes in cell's surrounding environment might lead to extreme shock which results

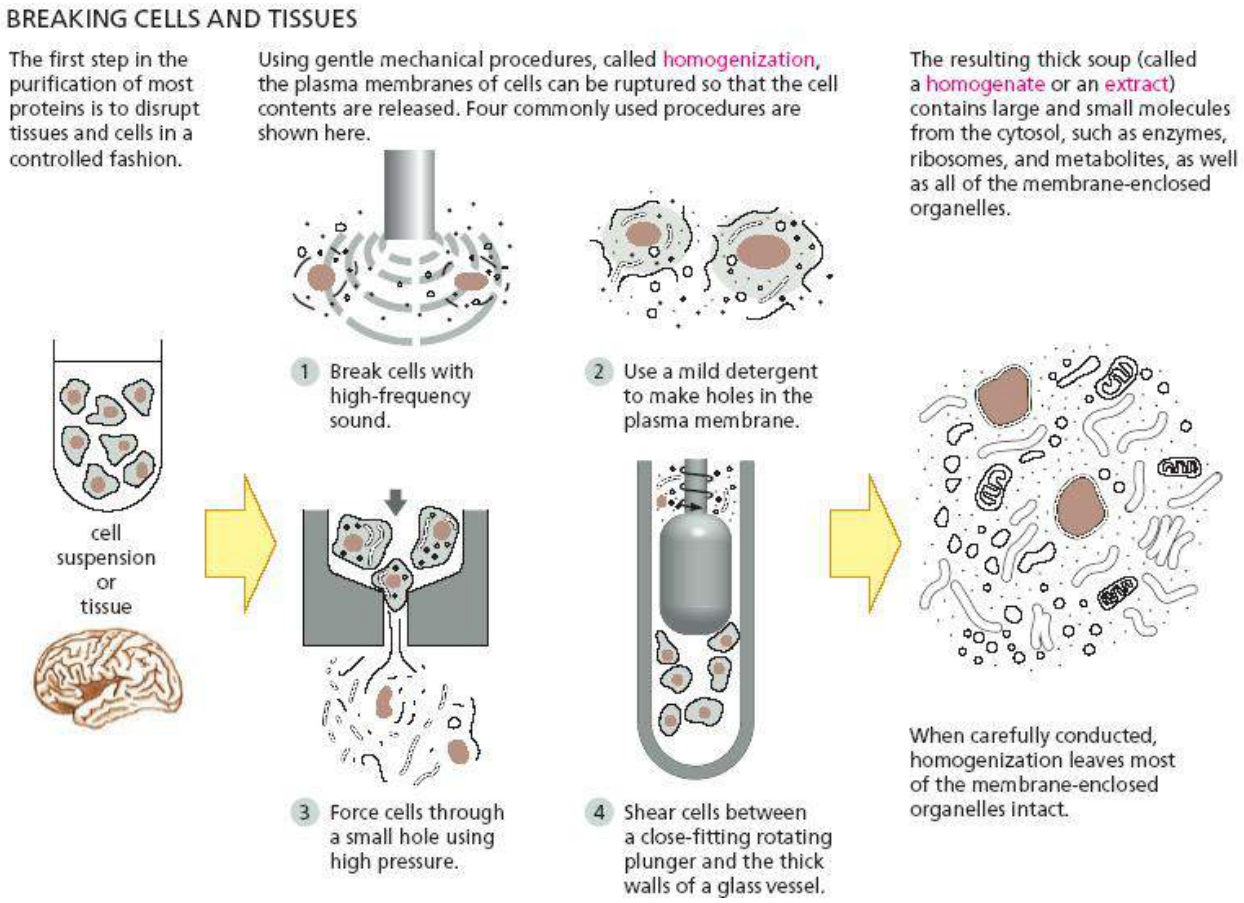
in cell death and disruption. Osmotic shock is a technology which can be utilized in biotechnical applications to cause cell lysis. In this technology, cells are first exposed to either high or low salt concentration. Then the conditions are quickly changed to opposite conditions which leads to osmotic pressure and cell lysis . The reason for that is that water quickly flows from low salt concentration conditions towards conditions with high salt concentration. Thus, if the cells are first exposed to high salt concentration solution, water flows into cell after exposure to low salt concentration. As a result, pressure in cell increases and cell explodes. Conversely, if cell are exposed to high salt concentration (~1 molar solution) after exposure to low concentration, water flows out of the cell which leads to cell disruption. Osmotic shock is not commonly used method for cell disruption because of its low efficiency. The efficient disruption would commonly require for example enzymatic pre-treatment to weaken the cells. In addition, this technology requires addition of high amounts of salts and water usage is high. Also product may be diluted which increases downstream processing costs.

3. Alkali treatment

One additional method for chemical cell disruption is the utilization of chemical solvents. Solvents which can be used for cell lysis include for example some alcohols, dimethyl sulfoxide, methyl ethyl ketone or toluene . These solvents extract cell wall's lipid components which leads to release of intracellular components. This method can be used with wide range of production organisms but the problem can be that some proteins are denatured. However, the advantage is that by the choice of solvent, it might be possible to select the relished product. This method is not generally applied in large scale processes. In addition to solvents, cell lysis can be achieved by hydrolysing the cell wall by alkali compound (pH 10.5-12-5). Disadvantage of this method is that chemical costs for neutralization of alkali are high. In addition, the product may not be stable in alkali conditions.

4. Enzyme treatment

Another strategy to achieve cell lysis is to use digestive enzymes which will decompose the microbial cell wall. Different cell types and strains have different kind of cell walls and membranes, and thus the used enzyme depends on microbe. For example, lysozyme is commonly used enzyme to digest cell wall of gram positive bacteria. Lysozyme hydrolyzes β-1-4-glucosidic bonds in the peptidoglycan. The cell wall of gram negative bacteria differs from the cell wall of gram positive bacteria so lysozyme is not very efficient in the case of gram negative cell wall. The cell wall of yeast and fungi differs significantly from the cell wall bacteria. One commonly used enzyme mixture for degradation of cell wall of yeast and fungi is Zymolyase. It has for example β-1,3 glucanase and β-1,3-glucan laminaripentao-hydrolase activities (Zymolyase | Yeast lytic enzyme). In addition, the enzymes that are commonly used for degradation of cell wall of yeast and fungi include different cellulases, pectinases, xylanases and chitinases. The utilization of enzymes in cell lysis process is one of the gentlest methods.However, the enzyme's high price and limited availability limits their utilization in large scale processes. In addition, the added enzyme may complicate downstream processing (e.g. purification). However, these drawbacks could be minimized by immobilization of enzymes.



Adsorption:

The biological products of fermentation can be concentrated by using solid adsorbent particles. In the early days, activated charcoal was used as the adsorbent material. In recent years, cellulose-based adsorbents are employed for protein concentration.And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

Drying

- Air-drying is an ancient preservation method
- Foods are exposed to a continuously flowing stream of hot air
- It involves simultaneous mass and heat transport

- Moisture availability has a great impact on the transfer of heat to microorganisms
- Consumer demand has increased for processed products that keep more of their original characteristics
- This requires the development of operations that minimize the adverse effects of processing
- There have been various advances in the drying of foods with respect to quality, rehydration, and energy minimization
- Some of the improvements and advancements made leading to the new developments in drying are discussed

Intermittent batch drying

- By varying the operating conditions of a drying process
  - Airflow rate
  - Temperature
  - Humidity or
  - Operating pressure
- It can be monitored in order to reduce the operating cost e.g. thermal input and power input
- The objective is to obtain high energy efficiency without subjecting the product beyond its permissible temperature limit and stress limit while maintaining high moisture removal rate

Hybrid drying techniques

- May include either use of
  - More than one dryer for drying of a particular product (multi-stage drying)
  - More than one mode of heat transfer
  - Various ways of heat transfer or
  - Multiprocessing dryers
- For particulate drying
  - Variants of fluid bed or
  - Fluid bed with some other techniques can be used in series to achieve faster drying
- For liquid feedstock
  - Generally spray drying is followed by the fluid bed dryer
- To reduce moisture content to an acceptable level which is not possible by spray dryer alone

Modified atmosphere drying

- The presence of oxygen results in various unwanted characteristics in dried food materials
  - oxidation of the drying material
  - destruction of its bioactive compounds
  - browning
- O_2 can be replaced by N_2 or CO_2
- In addition, it increases the effective moisture diffusivities of some food products

Superheated steam drying

- Superheated steam does not contain oxygen, hence oxidative or combustion reactions are avoided
- It also eliminates the risk of fire and explosion hazard

- It allows pasteurization, sterilization and deodorization of food and bio-products
- Net energy consumption can be minimized if the exhaust (also superheated steam) can be utilized elsewhere in the plant and hence is not charged to the dryer

- Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product.

- Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

Purification

Crystallization

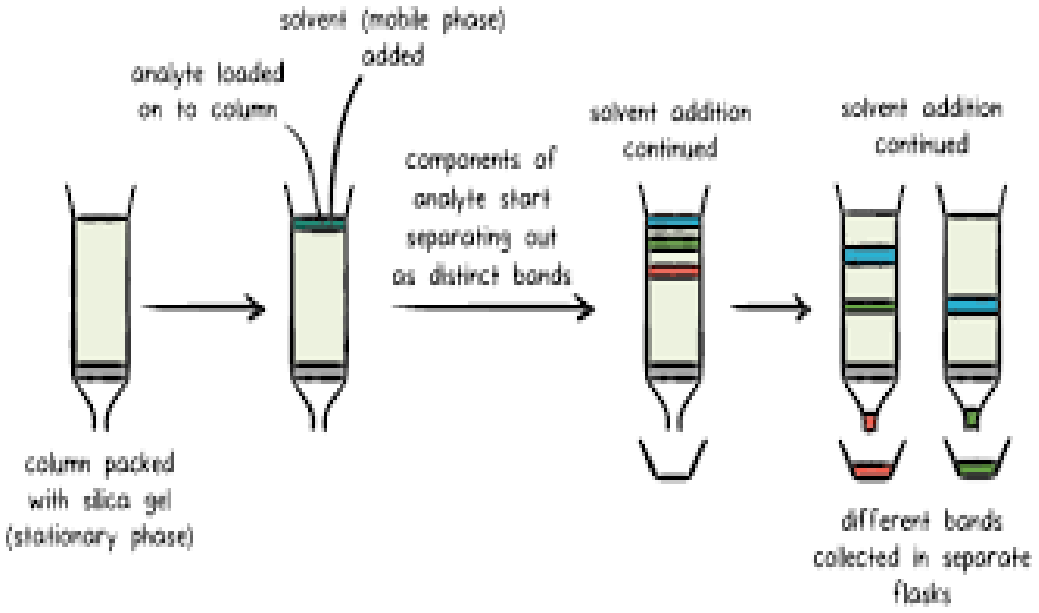
Crystallization is a separation and purification method widely used for final purification of components. Crystallization consists of two stages: formation of nuclei and growth of crystals. For crystallization to occur the solution should be first supersaturated. The first step of crystallization is formation of nucleation where crystals are formed when particles gather into clusters. There are two different nucleation formations – primary and secondary. The second step of crystallization is crystal growth where nucleus size increases after the critical cluster size is achieved. Crystallization is widely used in chemical industry due to rather low operation temperatures and energy consumptions. Main crystallization processes are cooling and evaporative crystallization. Crystallization, one of the oldest of unit operations in the portfolio of industrial separations, is a separation and purification method widely used for final purification of components. Crystallization is especially used for high quality products, which have high purity grade. The oldest crystallization techniques are applied in salt and sugar production.

Crystallization consists of two stages: formation of nuclei and growth of crystals. For crystallization to happen the solution should be first supersaturated thus the solution must contain more dissolved material than it would under normal conditions. Several methods can be used to obtain supersaturation such as solvent evaporation, cooling, chemical reaction and addition of a second solvent to reduce the solubility of the solute, solvent layering and sublimation along with other methods.
The clusters become stable nuclei after achieving the critical cluster size. There are two different nucleation formations – primary and secondary .Primary nucleation is spontaneous and homogeneous in the absence of foreign particles. Thus, in the presence of foreign particles nucleation formation is heterogeneous and then the formation is induced by foreign particles and occurs lower supersaturation concentration compared to homogeneous primary nucleation.

Secondary nucleation appears when crystals already exist in the solution and it is caused by collision of crystals. This type of nucleation formation is typical mechanism in industrial crystallization because of low supersaturation concentration.

Chromatography- more notes as to how compounds are separated

Chromatography is an analytical technique commonly used for separating a mixture of chemical substances into its individual components, so that the individual components can be thoroughly analyzed. There are many types of chromatography e.g., liquid chromatography, gas chromatography, ion-exchange chromatography, affinity chromatography, but all of these employ the same basic principles. In all chromatography there is a mobile phase and a stationary phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. At different points in the stationary phase the different components of the compound are going to be absorbed and are going to stop moving with the mobile phase. This is how the results of any chromatography are gotten, from the point at which the different components of the compound stop moving and separate from the other components.



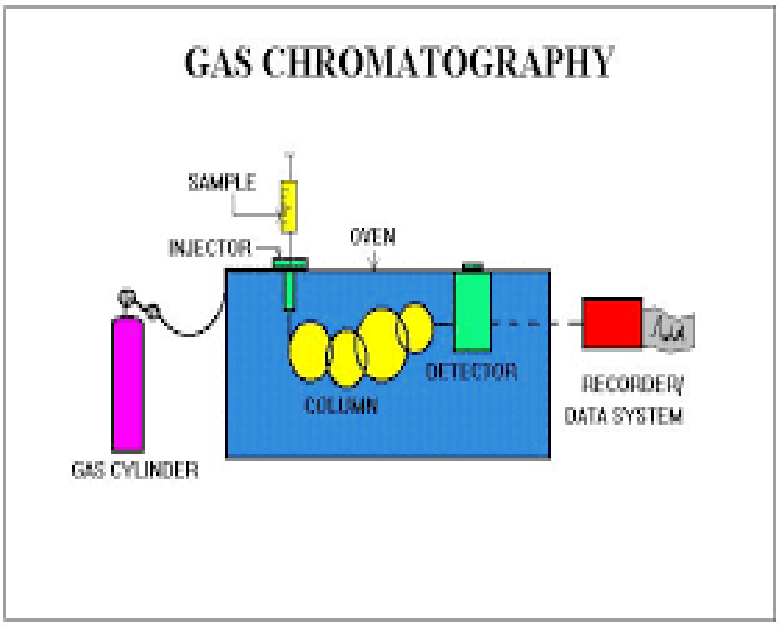
- The analyte is the substance to be separated during chromatography. It is also normally what is needed from the mixture.
- Analytical chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a sample.
- A bonded phase is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.

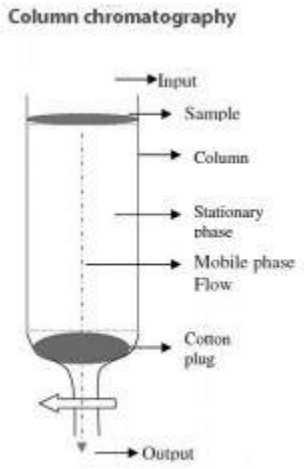
- A chromatogram is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.

- Separation of various compounds by the flow of a liquid in a porous, solid, sorptive medium.
- Separation occurs by the principle of different migration properties of solutes in a particulate adsorptive medium-components are separated in the form of distinct bands.
- The operation is time dependent-components are separated at increasing time in-terval of fluid flow.
- Separation is based on different adsorptive characteristics of solute materials on absorbent particles.
- Fluid flow carries the least adsorptive material to the far end of the column, and the most adsorptive material is retained on top of the column.
- Different bands are obtained at different locations of the column depending on the adsorptive characteristics of the solute compounds.
- The forces between solute molecules and adsorbent particles vary depending on the type of chromatography.

The types of chromatographic methods are given below:

1. **Adsorptive chromatography**: It is based on the adsorption of solute molecules onto solid particles, such as alumina and silica gel, by weak van derWaals forces and stearic interaction.
2**. Liquid-liquid partition chromatography**: It is based on the different partition coefficients (solubility)of solute molecules between an adsorbed liquid phase and passing solution.
3. **Ion-exchange chromatography**: It is based on the adsorption of ions (electrically charged compounds)on ions-exchange resin particles by electrostatic forces.
4. **Gel Filtration chromatography**: It is based on the penetration of solute molecules into small pores of packing particles on the basis of molecular size and shape of the solute molecules.
5. **Affinity chromatography**: It is based on the specific chemical interaction between solute molecules and ligands (a functional molecule covalently linked to a support particle)bound on support particles. Ligand-solute interaction is very specific, such as enzyme-substrate interaction, which may depend on covalent, ionic forces or hydrogen-bond formation. Affinity binding may be molecular size and shape specific.
6. **Hydrophobic chromatography**: It is based on hydrophobic interactions between solute molecules such as proteins and functional groups like alkyl residues on support particles.
7. **High-pressure liquid chromatography**: It is based on the general principles of chromatography, with the only different being high liquid pressure applied to the packed column. Due to high-pressure liquid (high liquid flow rate) and dense column packing. HPLC provides fast and high resolution of solute molecules.





Product Polishing

It is the last processing steps which end with packaging of the product. It includes Crystallization, desiccation and spray drying. Sometime it also includes operations to sterilize the product by removing contaminants which otherwise affect product safety