BIOREACTOR

Dr Bhavesh Patel
Principal
V P & R P T P Science College, Vallabh Vidyanagar

Back ground

- A Bioreactor is one in which biological reactions takes place.
- Bioreactor is also known as fermentor
- Fermentation is an important reaction carried out by biological system, importantly by microorganisms.
- Fermentation can be defined as an oxidation reduction energy yielding process where both oxidizing and reducing agents are organic compound.
- The process of fermentation was first observed by Louis Pasteur who gave the concept of "Germ theory of fermentation". According to him the fermentation is mediated by germs i.e. microorganism. (the process was originally considered as a chemical process)

Back ground

- Previously the process of fermentation was considered as an anaerobic process where a sugar or starch containing substrate is converted in to alcohol with the help of yeast.
- Present definition of industrial fermentation is "Bioconversion of any substrate in to its product"
- Hence, any process that is mediated by biological system is known as fermentation.
- Presently we are producing many products at industrial scale like alcohol, amino acids, vitamins, antibiotics, organic acids etc. (students must try to prepare a comprehensive list of products produce along with producer organisms)

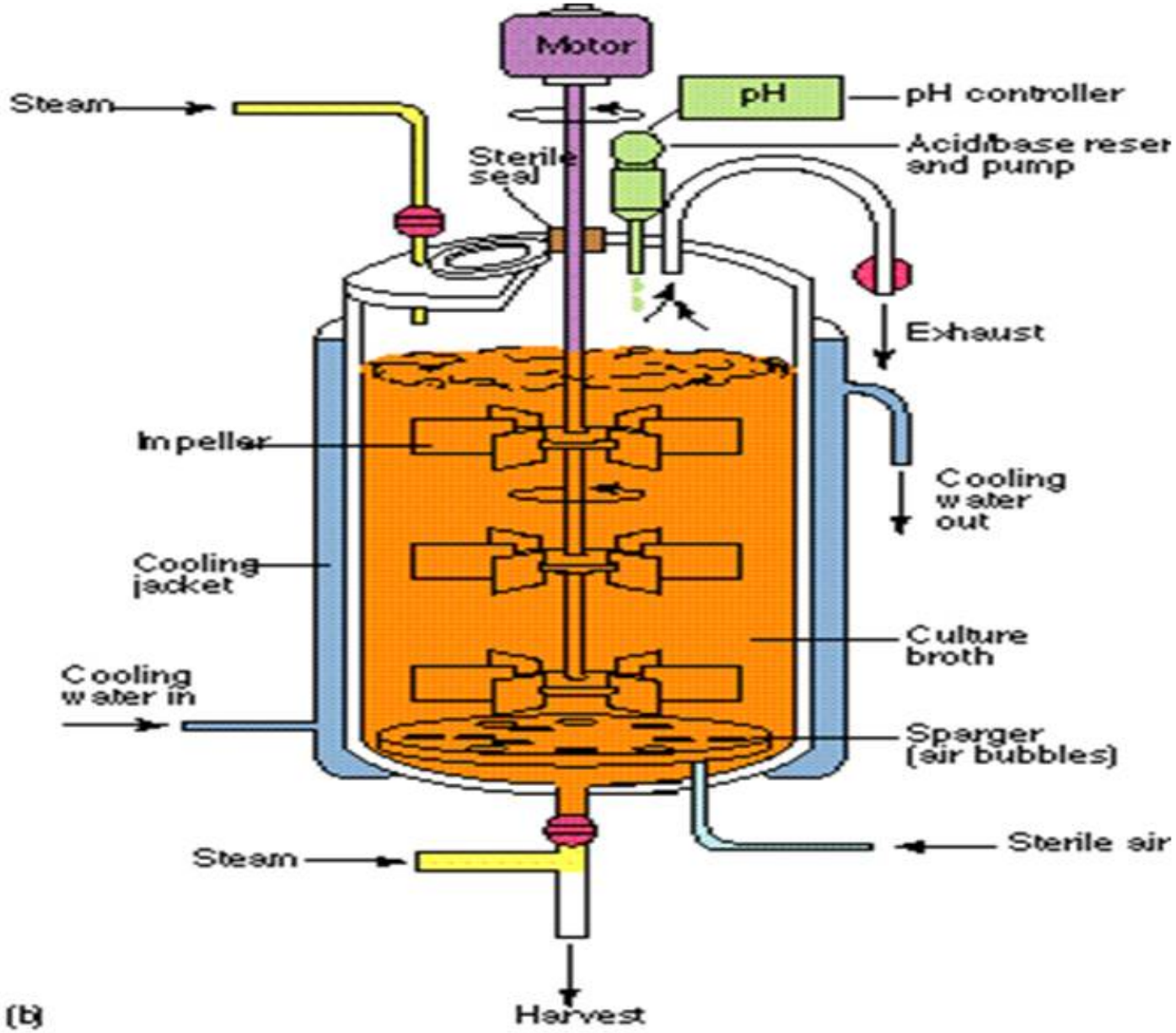
Types of Fermentation

- Fermentation is of two types namely, Surface and submerged
- As the name indicate in surface fermentation the organisms tends to grow on surfaces. This type of process is mainly applicable to fungi and involve the use of solid or semisolid substrate. The present day process which involves the concept of surface fermentation is solid state fermentation.
- The other type of fermentation involves the cultivation of organisms beneath the surface (submerged). Such process mainly involves cultivation of bacteria using liquid media.
- Submerged cultivation can be carried out by two ways i.e. Batch cultivation and Continuous cultivation.

Design of a Batch Reactor

- Presently majority of process at industrial scale is carried out by submerged batch cultivation.
- Use of solid state reactor or continuous reactor is limited.
- Hence, it is important to study design of a batch reactor.
- A bioreactor must provide all the conditions optimum for the growth and product formation. This includes temperature, nutrients, pH, gaseous environment etc.
- The design of a reactor mainly depends on organism involved, media used, environmental conditions etc.

Typical Batch Reactor



Typical Batch Reactor

Ideal Batch reactor

- An ideal batch reactor is one which provides all the conditions optimum for the growth and product formation.
- Creating an ideal reactor is rarity
- For constructing an ideal reactor following is to be kept in consideration
- Size of the reactor:

  Nothing is ideal as far as size is concern. The size of the reactor must be decided keeping in mind –

  a. market demand of the product

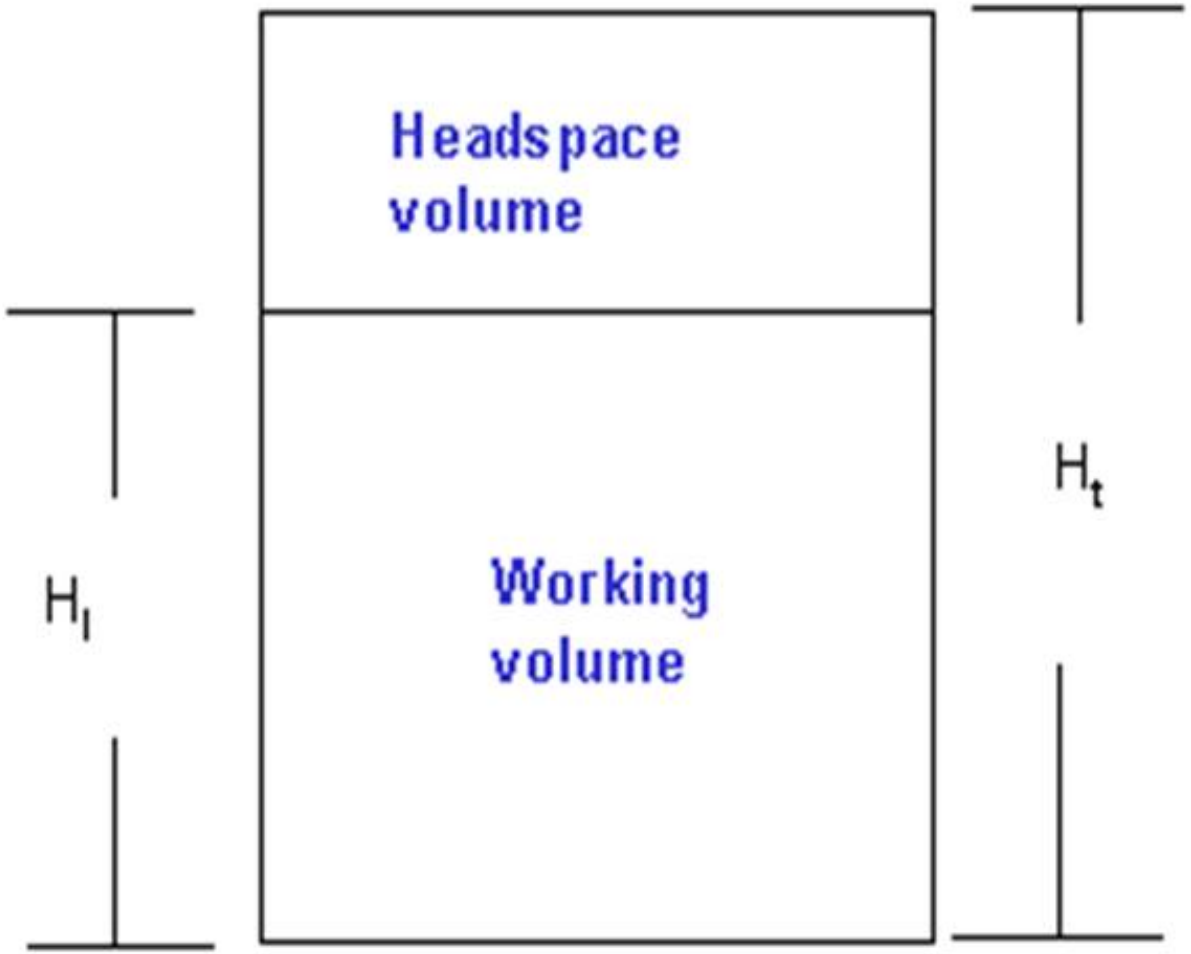
  b. availability of raw materials, manpower, electricity, water, transportation facility, storage facility

  c. Head space

Headspace volume

- A bioreactor is divided in a working volume and a head-space volume.
- The working volume is the fraction of the total volume taken up by the medium, microbes, and gas bubbles.
- The remaining volume is called the headspace.
- Typically, the working volume will be 70-80% of the total fermenter volume.
- This value will however depend on the rate of foam formation during the reactor. If the medium or the fermentation has a tendency to foam, then a larger headspace and smaller working volume will need to be used.

Headspace volume



Material of Fabrication

- An ideal batch reactor is to be made up of with material having following properties:

  1. Must withstand the pressure

  2. Noncorrosive, non explosive

  3. Should not release any ions that affects the growth and product formation

  4. Economic, long-lasting, easy to sterilize.

- Materials of choice : Glass, Wood, Copper, Iron, SS

Main hole and Harvest line

- An ideal batch reactor must have a main hole for the purpose of observing the reaction going on in the reactor. It is normally situated at the top of the reactor (2-3)
- Similarly a harvest line is to be there for harvesting broth after the completion of fermentation process. For the ease of harvesting it is normally situated at the bottom of reactor.

Agitation system (Impeller or Agitator)

❖The function of the agitation system is to

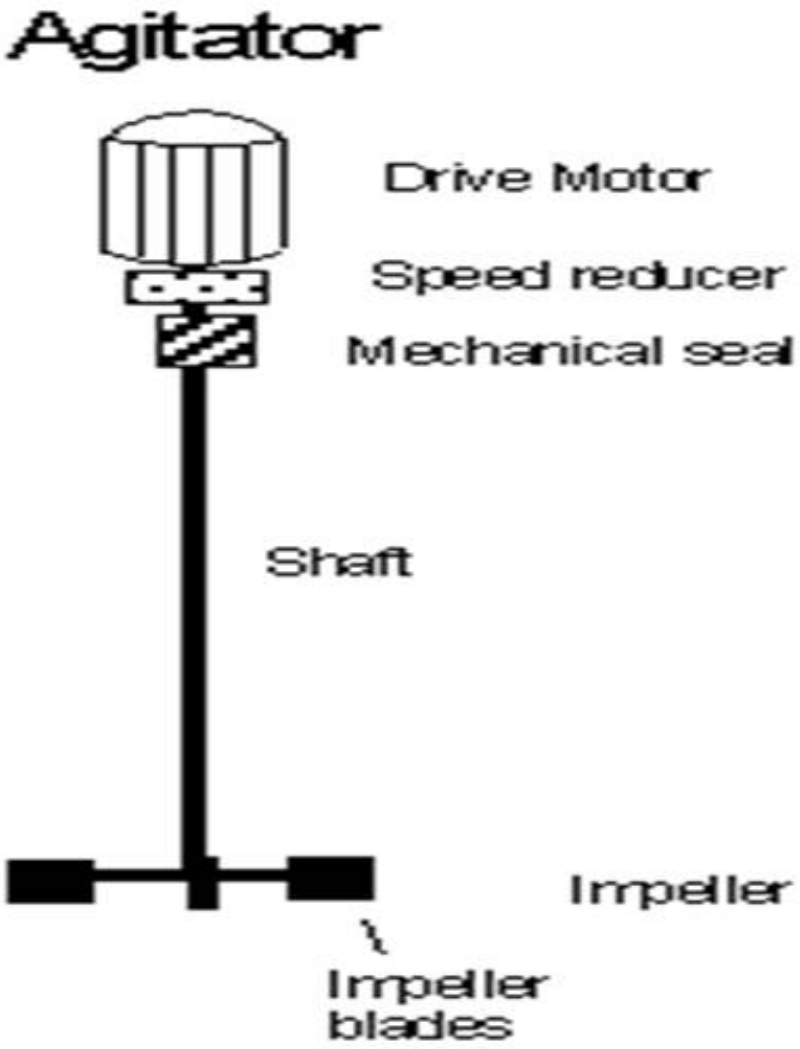
- provide good mixing and thus providing uniform environment surrounding each organisms.
- provide the appropriate shear conditions required for the breaking up of bubbles.

❖The agitation system consists of the agitator and the baffles.

❖The baffles are used to break the liquid flow to increase turbulence, mixing efficiency and avoiding vortex formation.

Agitation system

- **The agitator consists of the components shown in the following diagram:**



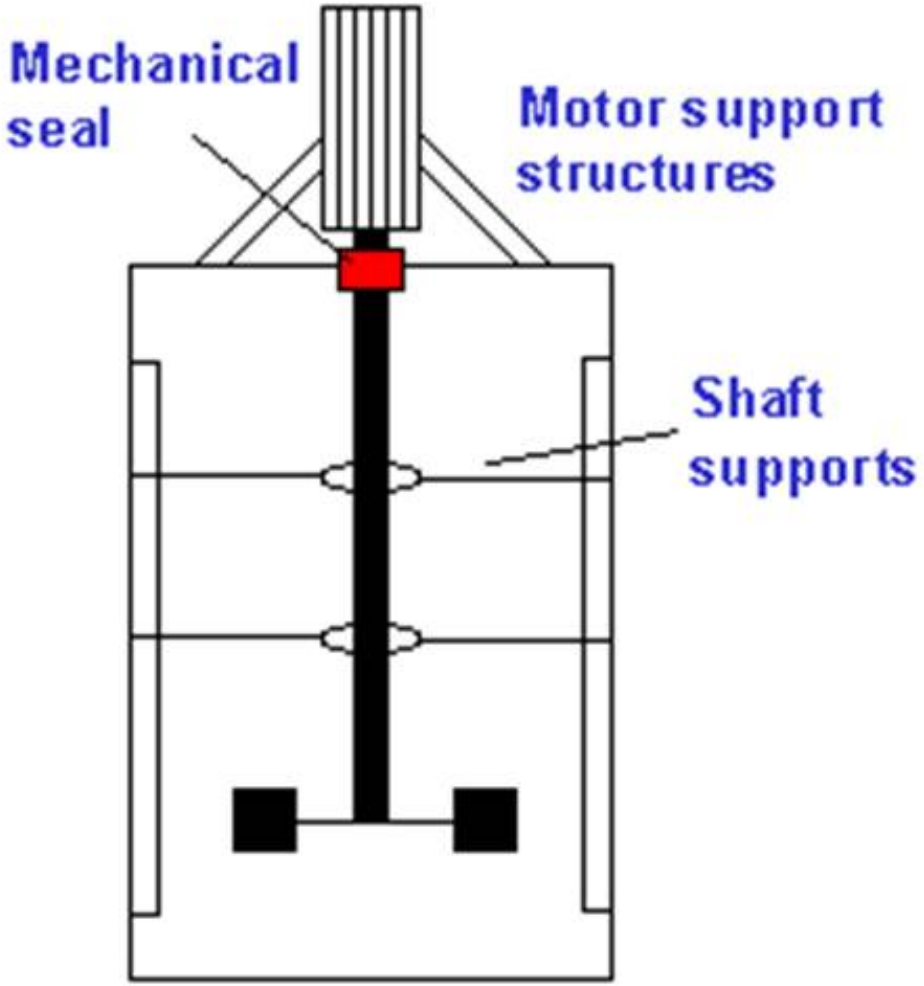
Agitation system

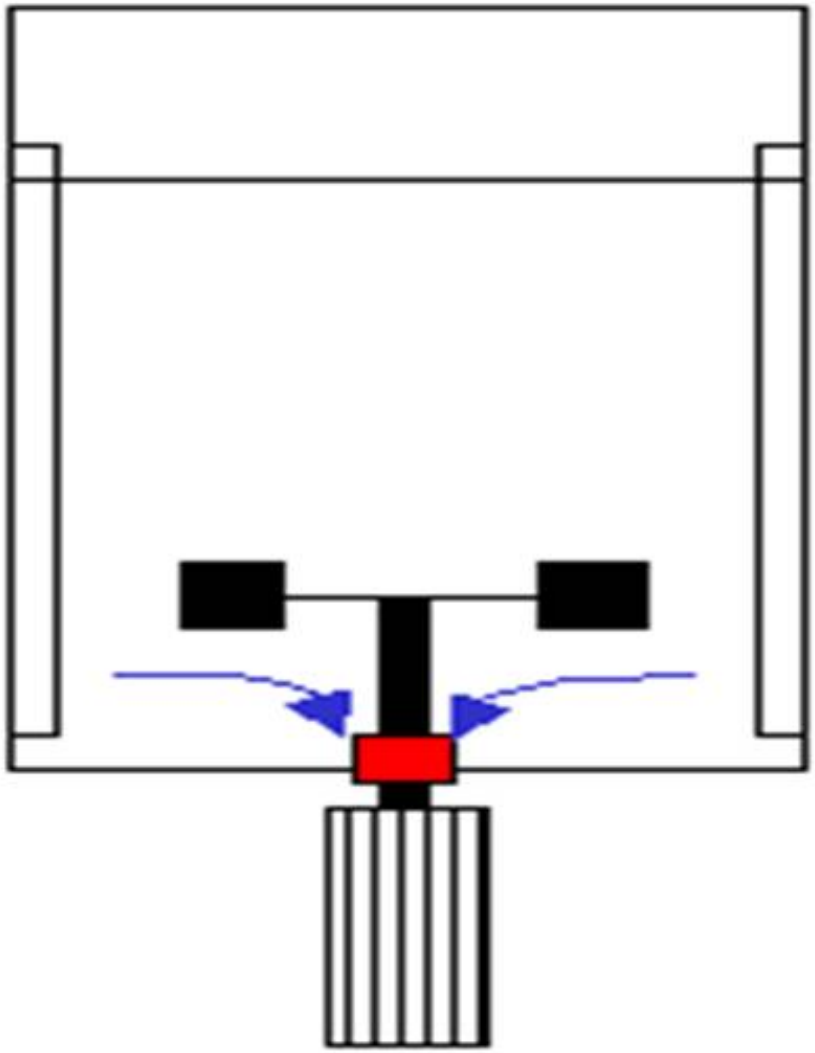
- The number of impellers will depend on the height of the liquid in the reactor. Each impeller will have between 2 and 6 blades. Most microbial fermentations use a Ruston turbine impeller.
- A single phase (ie. 240 V) agitator drive motor can be used with small reactors. However for large reactors, a 3 phase motor (ie 430 V) should be used. The latter will tend to require less current and therefore generate less heat.
- Speed control or speed regulator devices are used to control the agitation speed.
- Number of impellers, number of blade in each impeller and impeller speed depends on organism used, media employed.

Agitation system: Top entry and bottom entry impellers

- The impeller shaft can enter from the bottom of the tank or from the top. A top entry impeller ("overhung shaft") is more expensive to install as the motor and the shaft will need to be structurally supported.
- A reactor with bottom entry impeller however will need higher maintenance due to damage of the seal by particulates in the medium and by medium components that crystallize in the seal when reactor is not in use.
- Bottom entry agitators tend to require more maintenance than top entry impellers due to the formation of crystals and other solids in the seals.

Agitation system: Top entry and bottom entry impellers





Agitator design and operation

❖ Agitators are classified as having radial flow or axial flow characteristics.

❖ With radial flow mixing, the liquid flow from the impeller is initially directed towards the wall of the reactor; ie. along the radius of the tank.

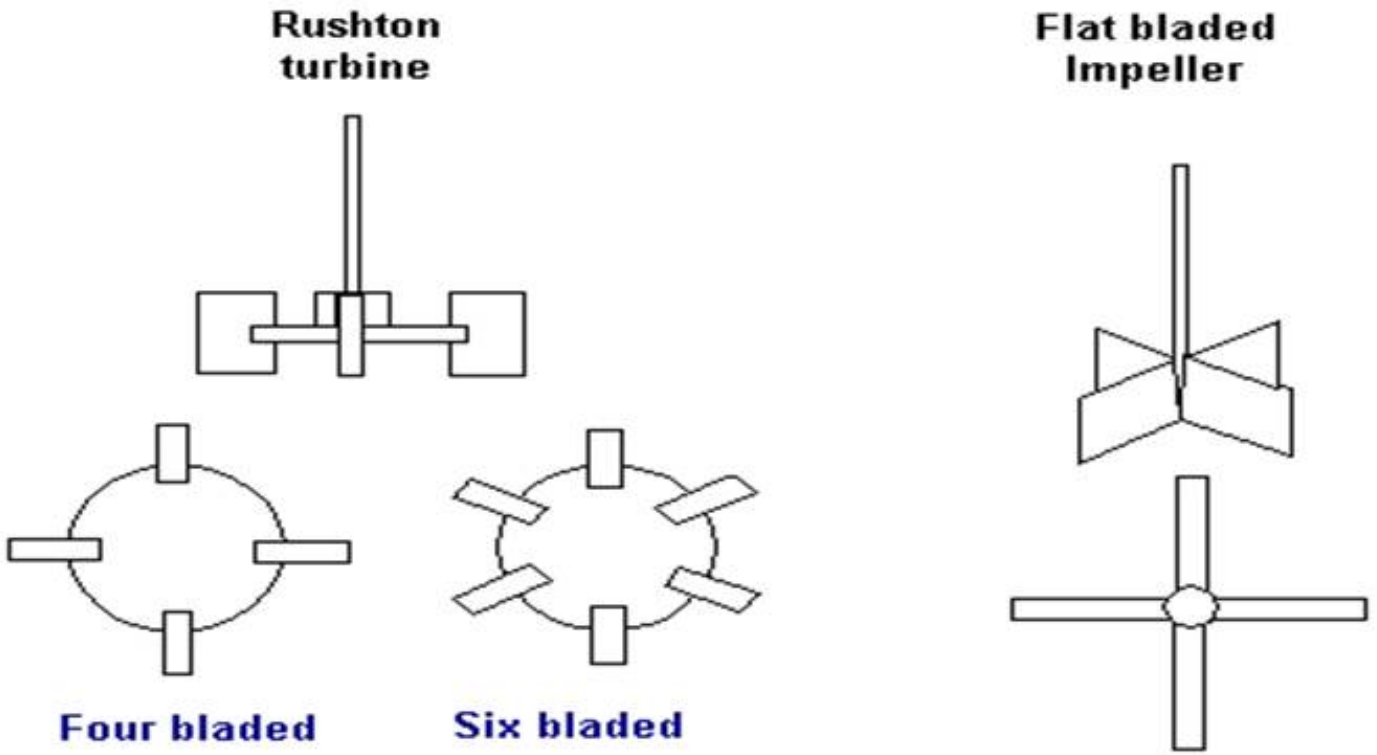
❖ With axial flow mixing, the liquid flow from the impeller is directed downwards towards the base of the reactor, ie. in the direction of the axis of the tank.

❖ Radial flow impellers are primarily used for gas-liquid contacting (such as in the mixing of sparged bioreactors) and blending processes.

❖ Axial flow impellers provide more gentle but efficient mixing and are used for reactions involving shear sensitive cells and particles.

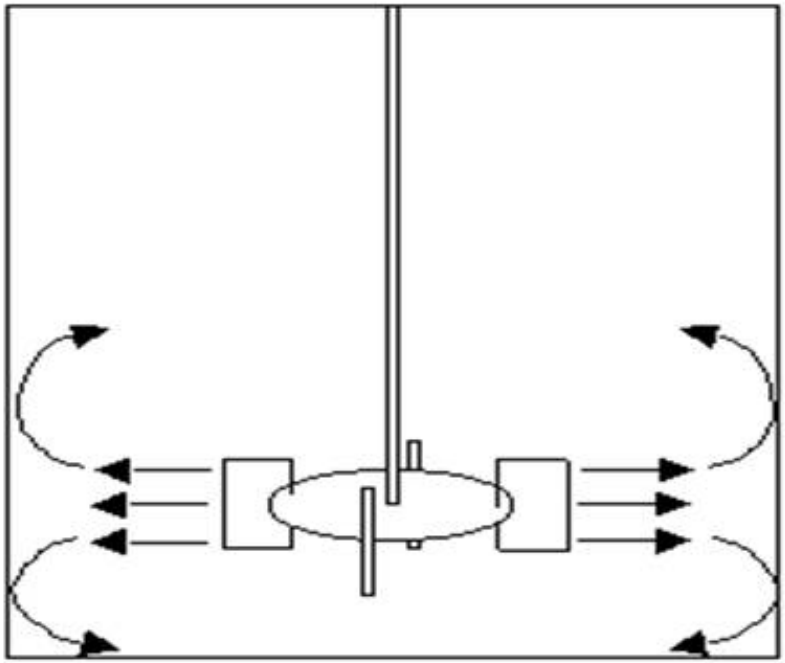
Radial flow impellers

- **Radial flow impellers contain two or more impeller blades which are set at a vertical pitch:**



Agitator design

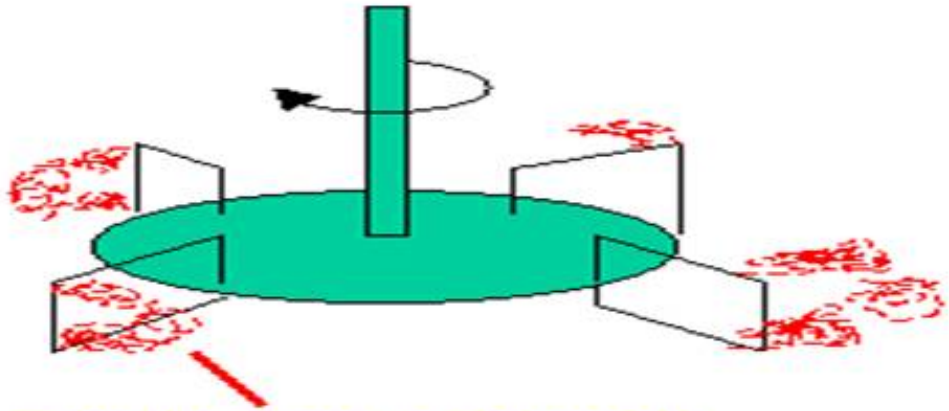
- **The liquid flow from the blades is directed towards the walls of the reactor; ie. along the radius of the tank.**



With radial flow impellers, the liquid is pushed towards the wall of the tank; that is, along the radius of the reactor

Agitator design

- Radial flow mixing is not as efficient as axial flow mixing.
- For radial flow impellers, a much higher input of energy is required to generate a given level of flow.
- Radial flow impellers do and are designed to, generate high shear conditions. This is achieved by the formation of vortices in the wake of the impeller:

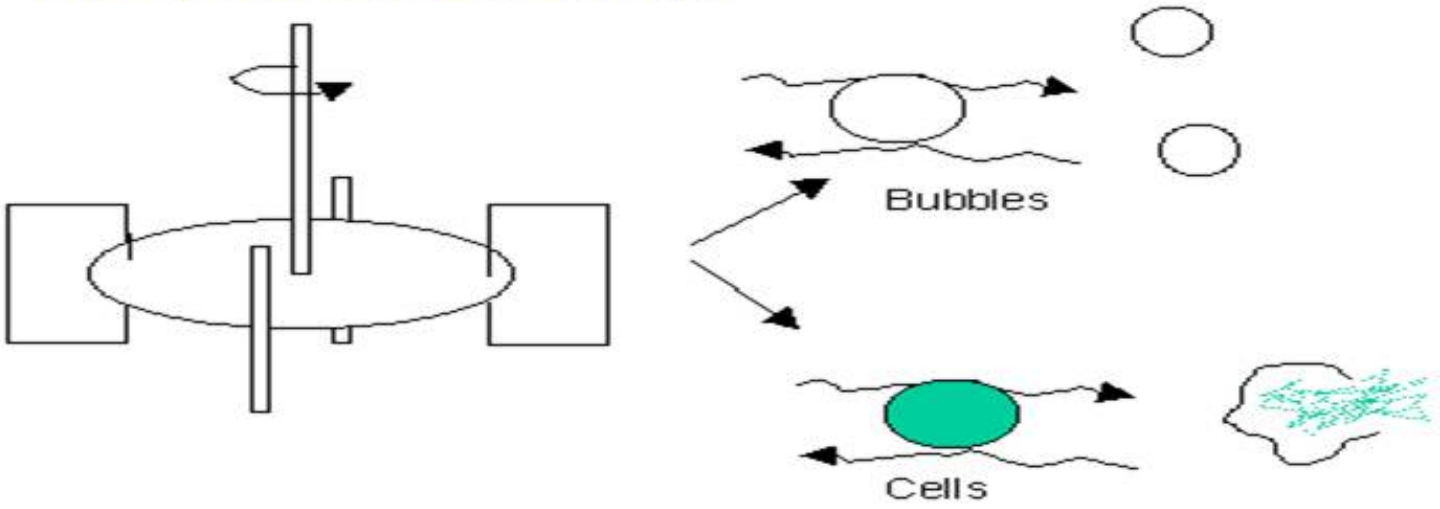


Eddies form in the wake of the impeller blades and generate a high shear environment

Agitator design

- The high shear is effective at breaking up bubbles. For this reason, radial flow impellers are used for the culture of aerobic bacteria.
- High shear can also damage shear sensitive materials such as crystals and precipitates shear sensitive cells such as filamentous fungi and animal cells.

Radial flow impellers are effective at generating high shear conditions. This aids in breaking up bubbles but can also lead to cell damage



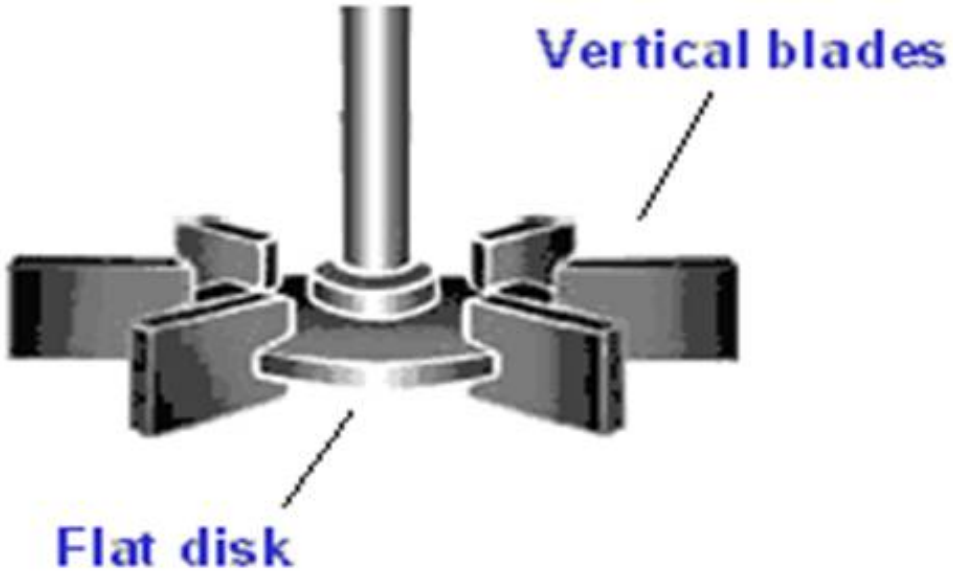
With radial flow impellers, vertical (or axial) mixing is achieved with the use of baffles

Radial flow impellers - Rushton turbine

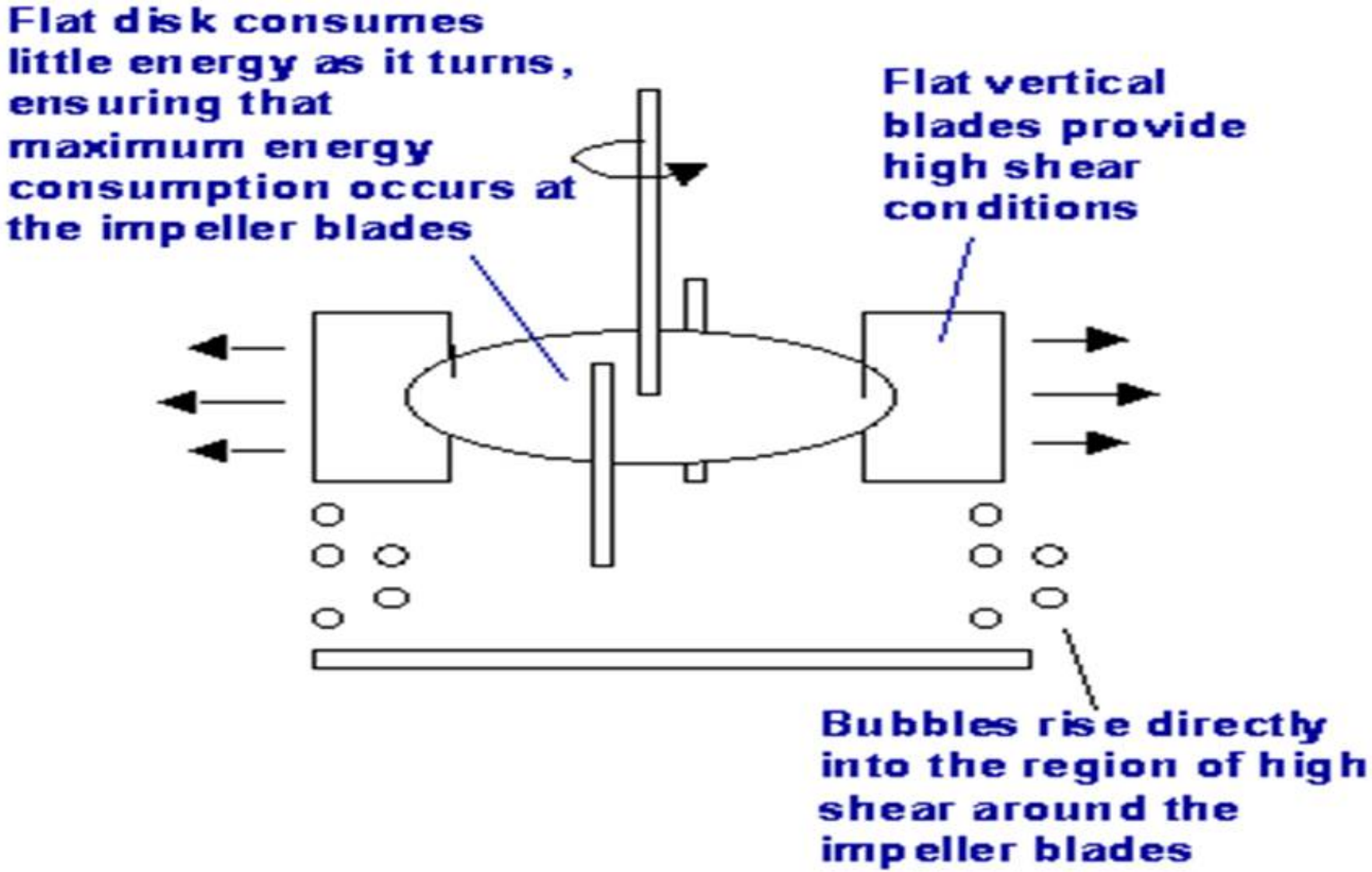
- The most commonly used agitator in microbial fermentations is the Rushton turbine.
- Like all radial flow impellers, the Rushton turbine is designed to provide the high shear conditions required for breaking bubbles and thus increasing the oxygen transfer rate.
- The Rushton turbine has a 4 or 6 blades which are fixed onto a disk.
- The diameter of the Rushton turbine should be 1/3 of the tank diameter.

Radial flow impellers

- A Rushton turbine is often referred to as a disk turbine.
- The disk design ensures that most of the motor power is consumed at the tips of the agitator and thus maximizing the energy used for bubble shearing.

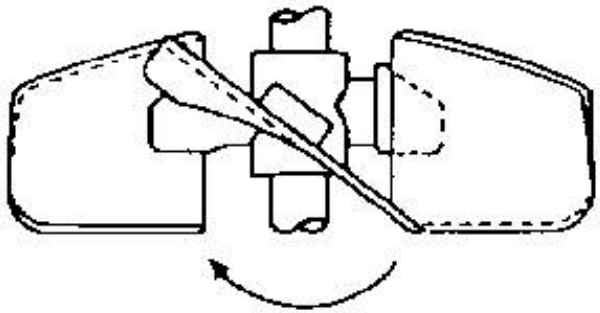


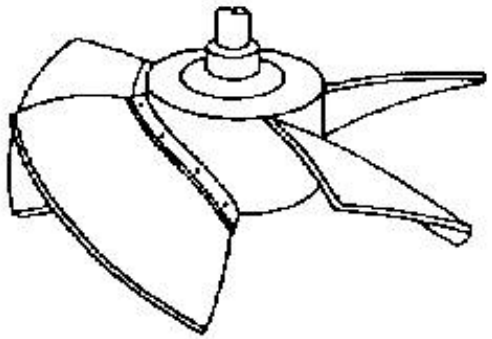
Radial flow impellers



Axial flow impellers

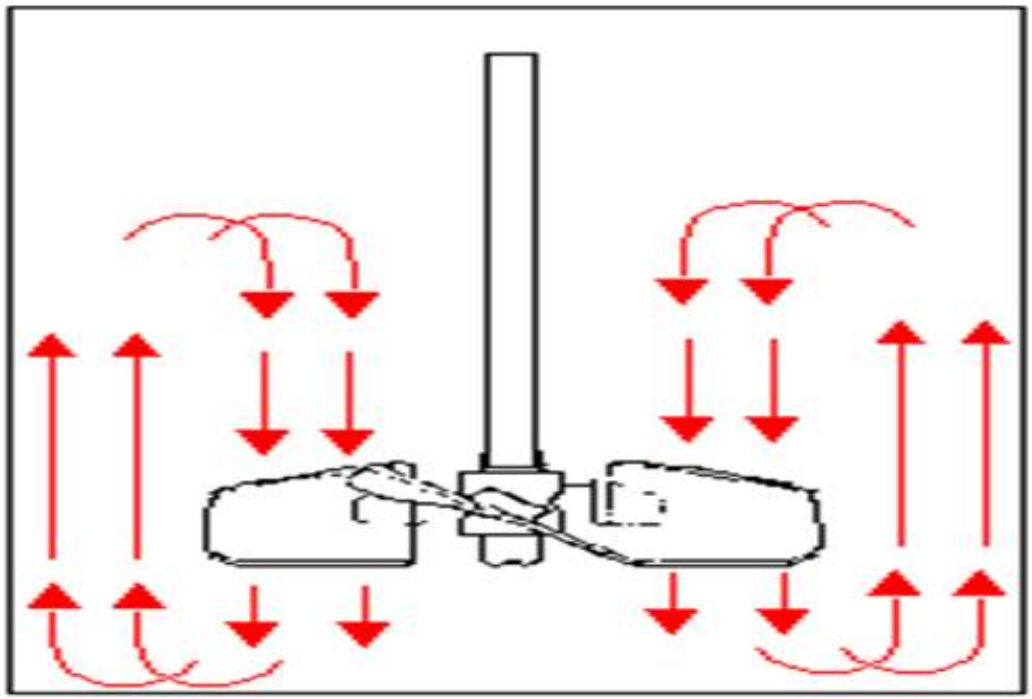
Axial flow impeller blades are pitched at an angle and thus direct the liquid flow towards the base of the tank. Examples of axial flow impellers are marine impellers and hydrofoil impellers.





Axial flow impellers

- **The resultant flow pattern is thus predominantly vertical; ie. along the tank axis.**



With axial impellers, the liquid is pushed in a downward direction; that is, along the axis of the reactor.

Axial flow impellers

- Axial flow mixing is considerably more energy efficient than radial flow mixing.
- They are also more effective at lifting solids from the base of the tank.
- Axial flow impellers have low shear properties. The angled pitch of the agitators coupled with the thin trailing edges of the impeller blades reduces formation of eddies in the wake of the moving blades.

Axial flow impellers

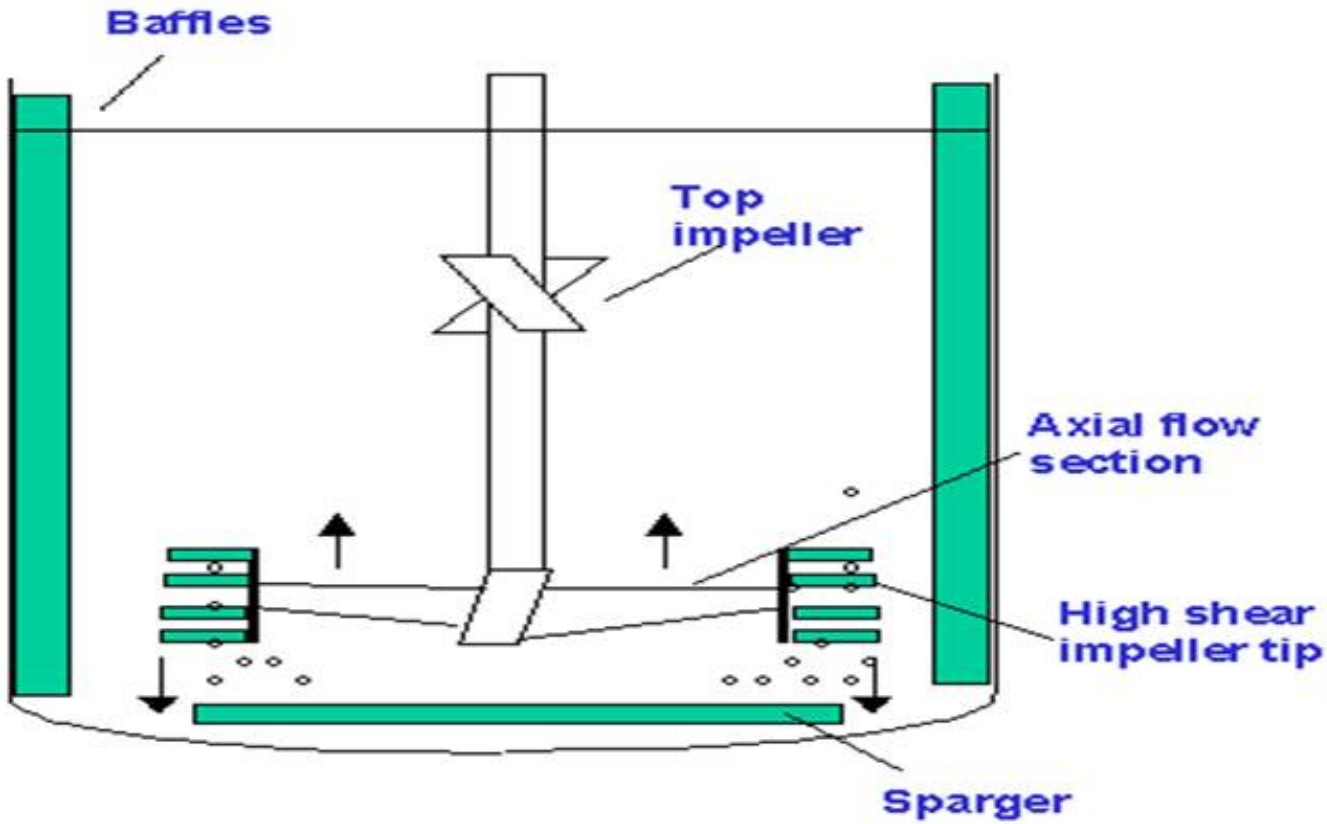
- Axial flow impellers are used for mixing shear sensitive processes such as crystallization and precipitation reactions.

- They are also used widely in the culture of animal cells.

- Their low shear characteristics generally makes them ineffective at breaking up bubbles and thus unsuitable for use in aeration of bacterial fermentations

Axial flow impellers - Intermig Impeller

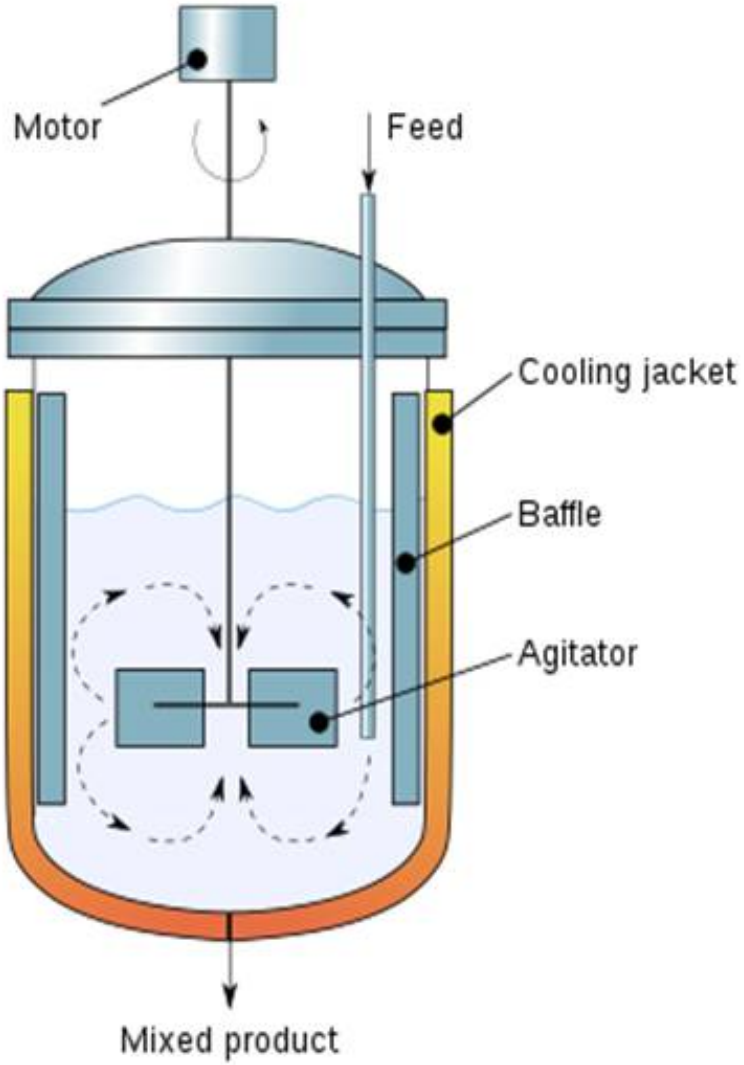
- Intermig impeller is a axial flow which is used for microbial fermentations.
- The impeller is shown below:

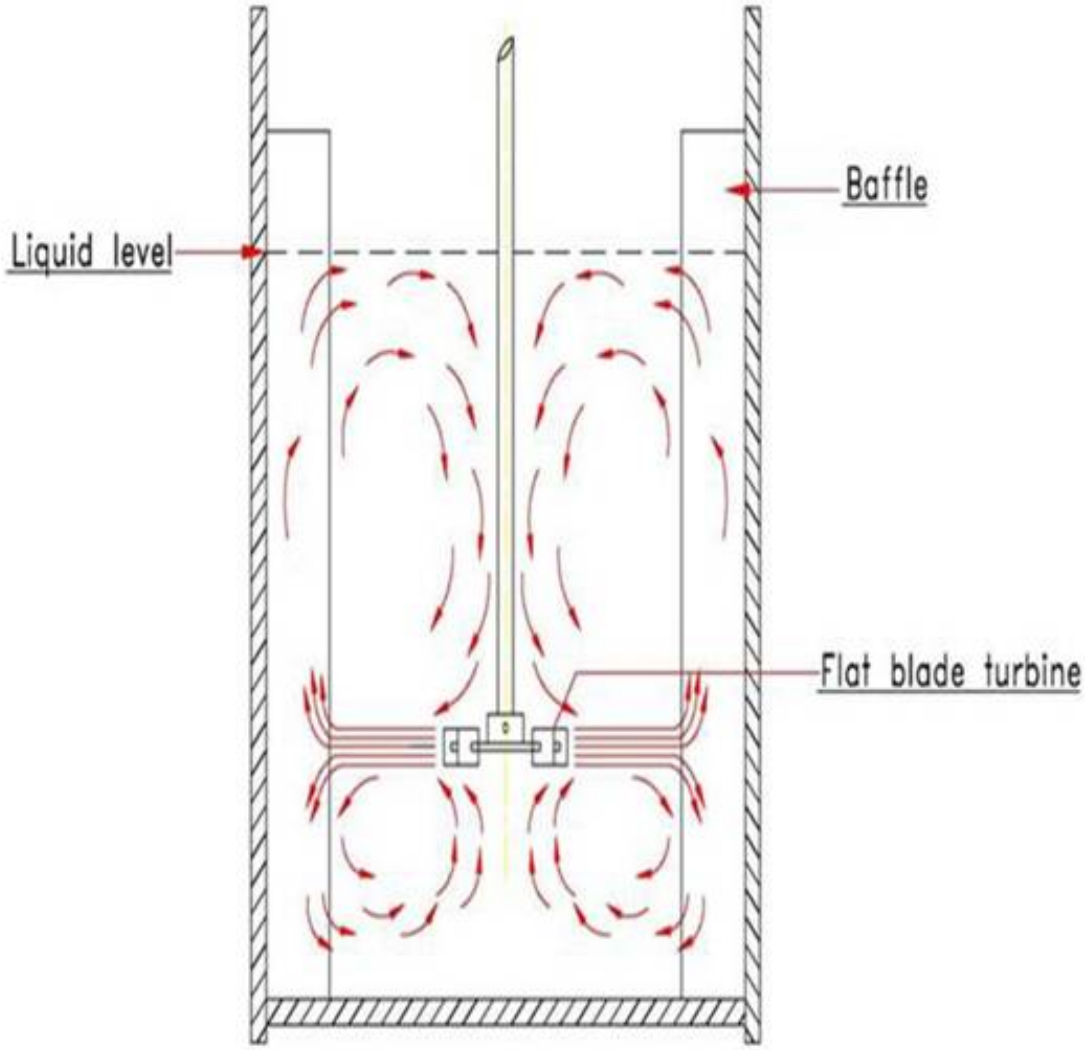


Intermig Impeller

- The agitation system has two impellers. The bottom impeller has a large axial flow section. The tips of the impeller contain finger like extensions which create a turbulent wake for breaking bubbles.
- As the high shear region exists only at the tip, the overall shear conditions in the reactor are lower than would be generated by a radial flow impeller such as a Rushton Turbine.
- Intermig impellers are used widely for agitation and aeration in fungal fermentations.

Baffle and its function

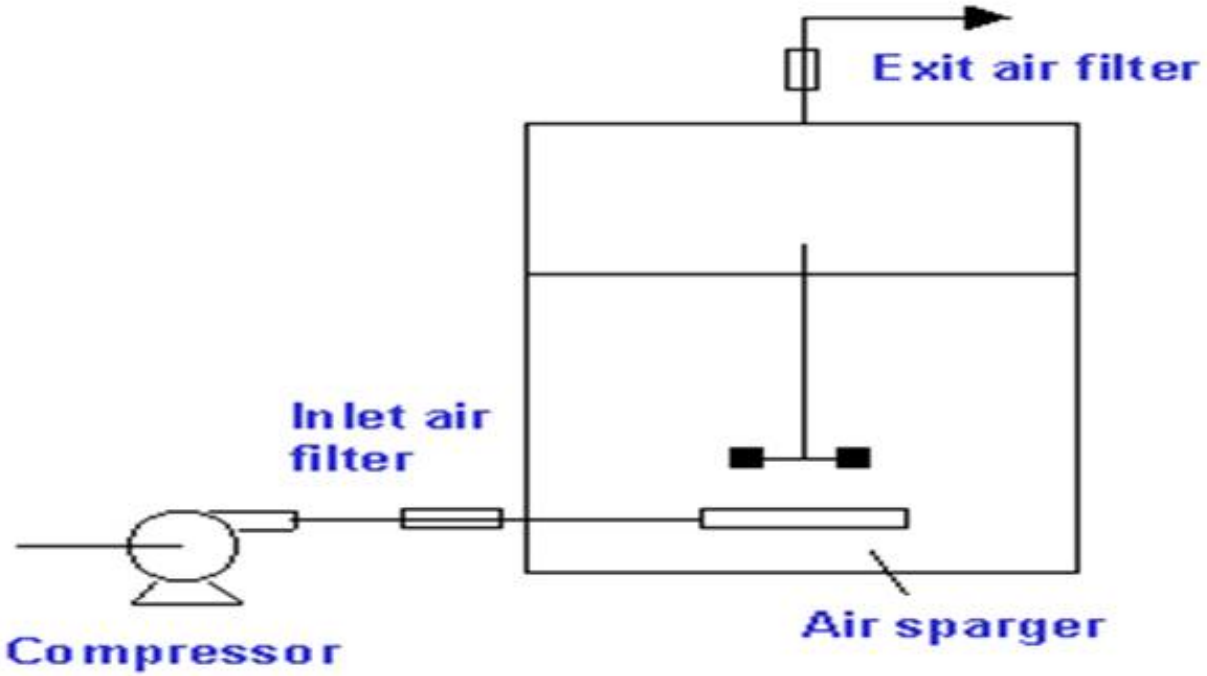




Aeration - Oxygen delivery system

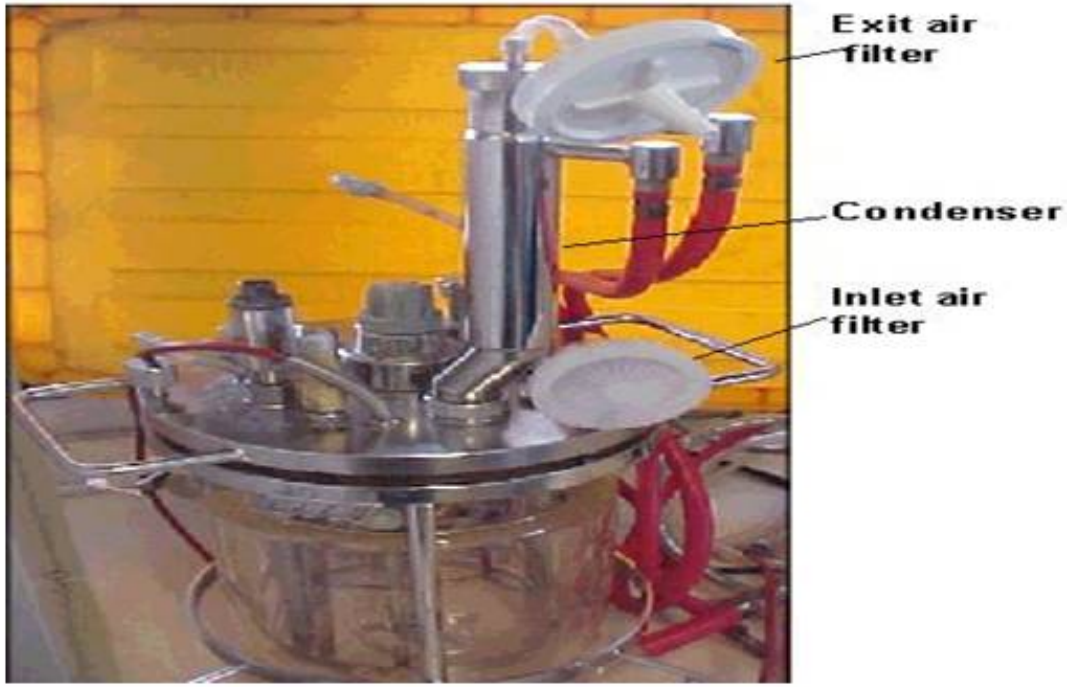
❖ The oxygen delivery system consists of

- a compressor
- inlet air sterilization system
- an air sparger , exit air sterilization system



Oxygen delivery system - Compressor

- A compressor forces the air into the reactor. The compressor will need to generate sufficient pressure to force the air through the filter, sparger holes and into the liquid.
- Air compressors used for large scale bioreactors typically produce air at 250 kPa. The air should be dry and oil free so as to not block the inlet air filter or contaminate the medium.



Oxygen delivery system - Air sterilization system

- Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor.
- The exit air on the other hand is sterilized to prevent organisms in the reactor from contaminating the air.
- A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 litres), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block.
- For larger laboratory scale fermenters (up to 1000 litres), pleated membrane filters housed in polypropylene cartridges are used.

Sterilisation of the air

Sterilization of the air

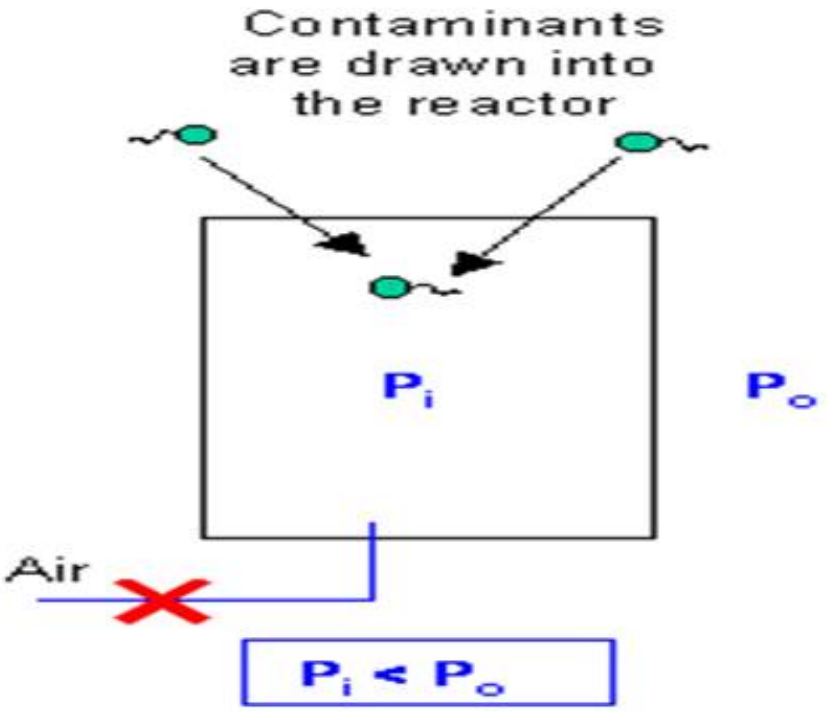
- By pleating the membrane, it is possible to create a compact filter with a very large surface area for air filtration. Increasing the filtration area decreases the pressure required to pass a given volume of air through the filter.
- Sterilization of the inlet and exit air in large bioreactors (> 10,000 litres) can present a major design problem. Large scale membrane filtration is a very expensive process. The filters are expensive as they are difficult to make and the energy required to pass air through a filter can be quite considerable.
- Heat sterilization is alternative option. Steam can be used to sterilize the air. With older style compressors, it was possible to use the heat generated by the air compression process to sterilize the air. However, compressors are now multi-stage devices which are cooled at each stage and disinfecting temperatures are never reached.

Air sterilization system - Positive pressure

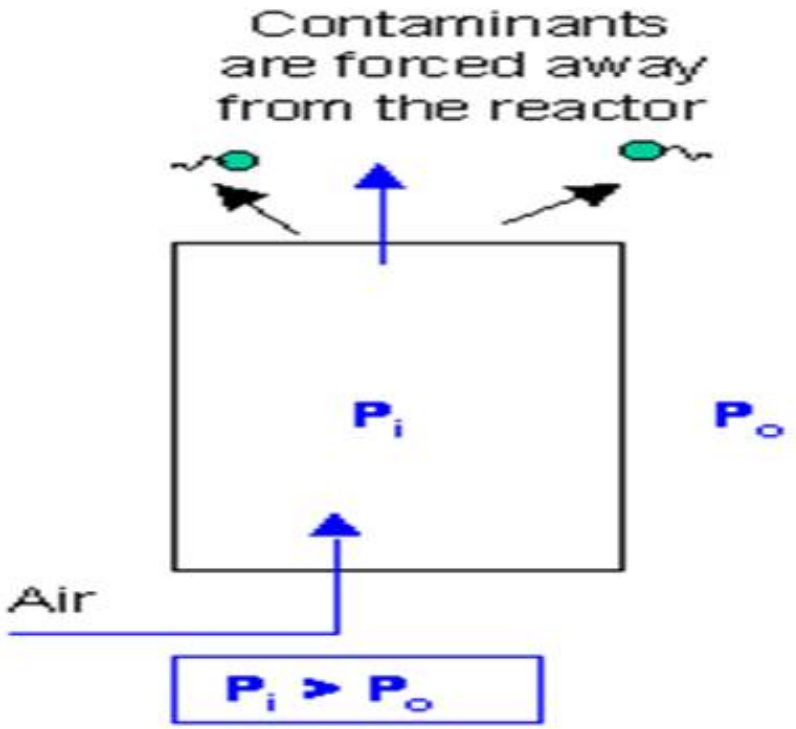
- During sterilization the concept of "maintaining positive pressure" will often be used.
- Maintaining positive pressure means air must be pumped into the reactor.
- In this way the reactor is always pressurized and thus aerial contaminants will not be "sucked" into the reactor.
- It is very important that positive pressure is maintained when the bioreactor is cooled following sterilization. Without air being continuously pumped into the reactor, a vacuum will form and contaminants will tend to be drawn into the reactor.

Air sterilization system - Positive pressure

❖Maintaining positive pressure at all stages of the fermentation setup and operation is an important aspect of reducing the risk of contamination



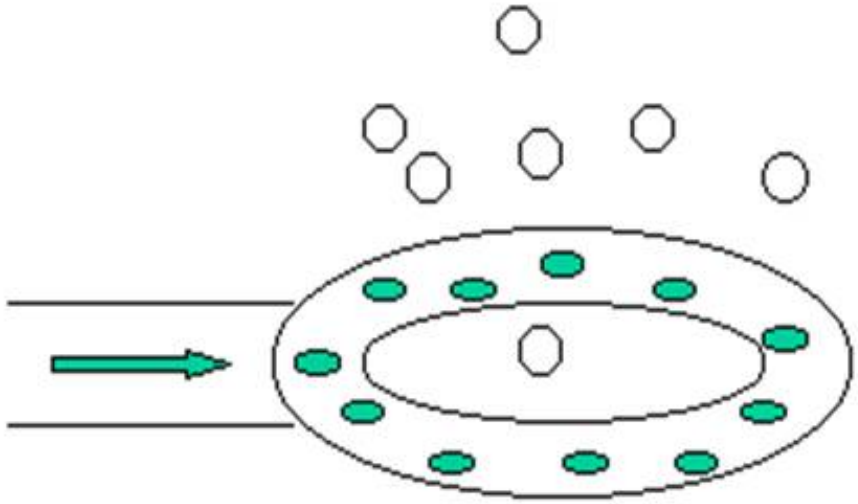
Without aeration, a vacuum forms as the reactor cools.



With aeration, positive pressure is always maintained and contaminants are pushed away from the reactor

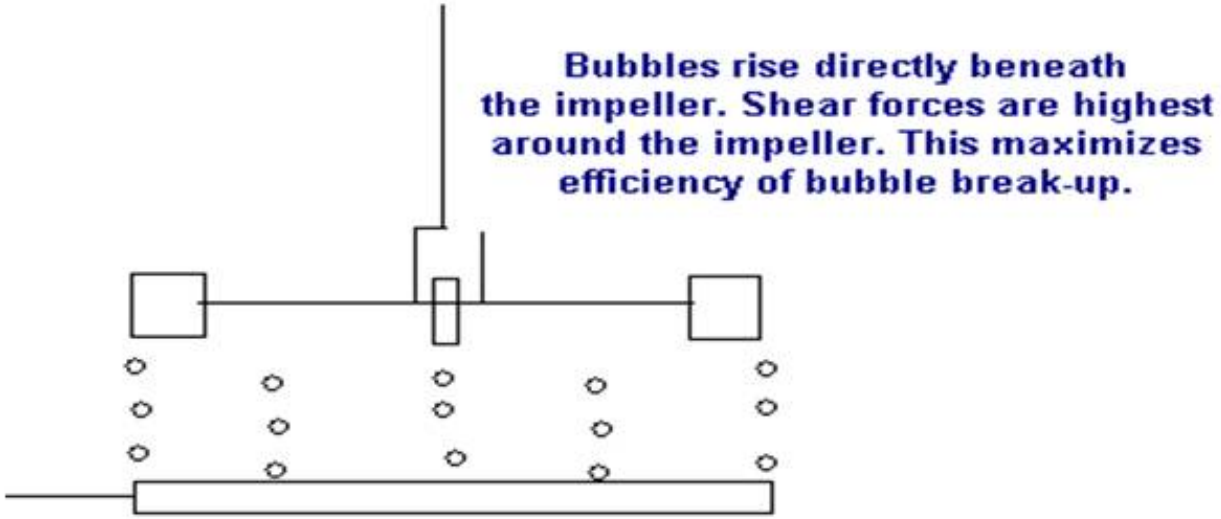
Oxygen delivery system - Sparger

- The air sparger is used to break the incoming air into small bubbles.
- Although various designs can be used such as porous materials made of glass or metal, the most common type of filter used in modern bioreactors is the sparger ring:



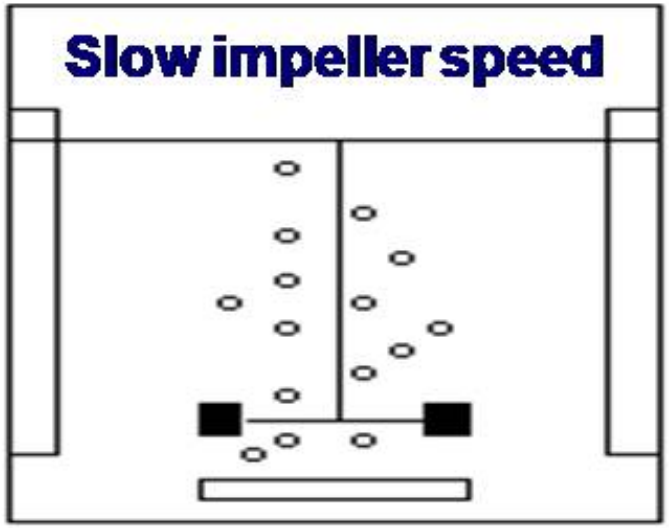
Oxygen delivery system - Sparger

- A sparger ring consists of a hollow tube in which small holes have been drilled. A sparger ring is easier to clean than porous materials and is less likely to block during a fermentation.
- The sparger ring must be located below the agitator and will have approximately the same diameter as the impeller.
- Thus, the bubbles rise directly comes in contact with the impeller blades, facilitating bubble break up.

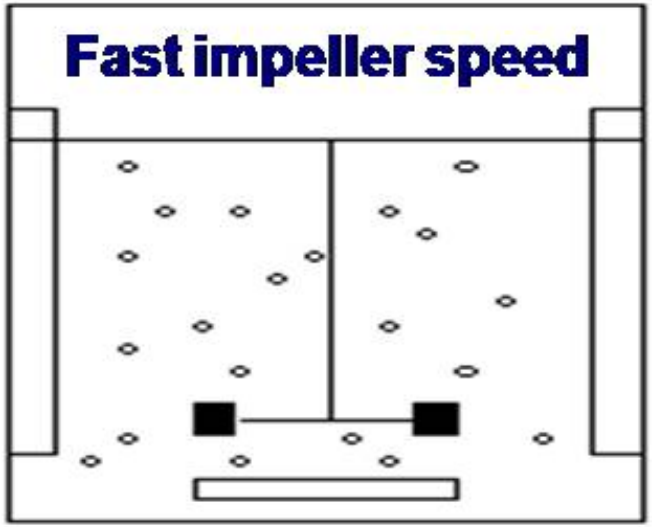


Oxygen delivery system - Effect of impeller speed

- **The shear forces that an impeller generates play a major role in determining bubble size. If the impeller speed is to slow then the bubbles will not be broken down. In addition, if the impeller speed is too slow, then the bubbles will tend to rise directly to the surface due to their buoyancy.**



The bubbles will not be sheared into smaller bubbles and will tend to rise directly towards the surface



Smaller bubbles will be generated and these bubbles will move with throughout the reactor increasing the gas hold up and bubble residence time

Oxygen delivery system - Effect of impeller speed

- Another consequence of too slow an impeller speed is a flooded impeller.

- Under these conditions, the bubbles will accumulate and coalesce under the impeller, leading to the formation of large bubbles and poor oxygen transfer rates.

- A similar phenomenon will happen when aeration rate is too high.

- In this case, the oxygen transfer efficiency will be low

Foam control system

- Foam control is an essential element of the operation of a sparged bioreactor. The following photograph shows the accumulation of foam in a 2 liter laboratory reactor.

Foam control system

- Excessive foam formation can lead to blocked air exit filters and to pressure build up in the reactor.
- The latter can lead to a loss of medium, damage to the reactor and even injury to operating personnel.
- Foam is typically controlled by chemical agents or physical methods.
- Excessive antifoam addition can however result in poor oxygen transfer rates.

- **The antifoam requirement will depend on**
  - **The nature of the medium.**

    Media rich in proteins will tend to foam more readily than simple media.
  - **The products produced by the fermentation.**

    Secreted proteins or nucleic acids released as a result of cell death and hydrolysis have detergent like properties.
  - **The aeration rate and stirrer speed.**

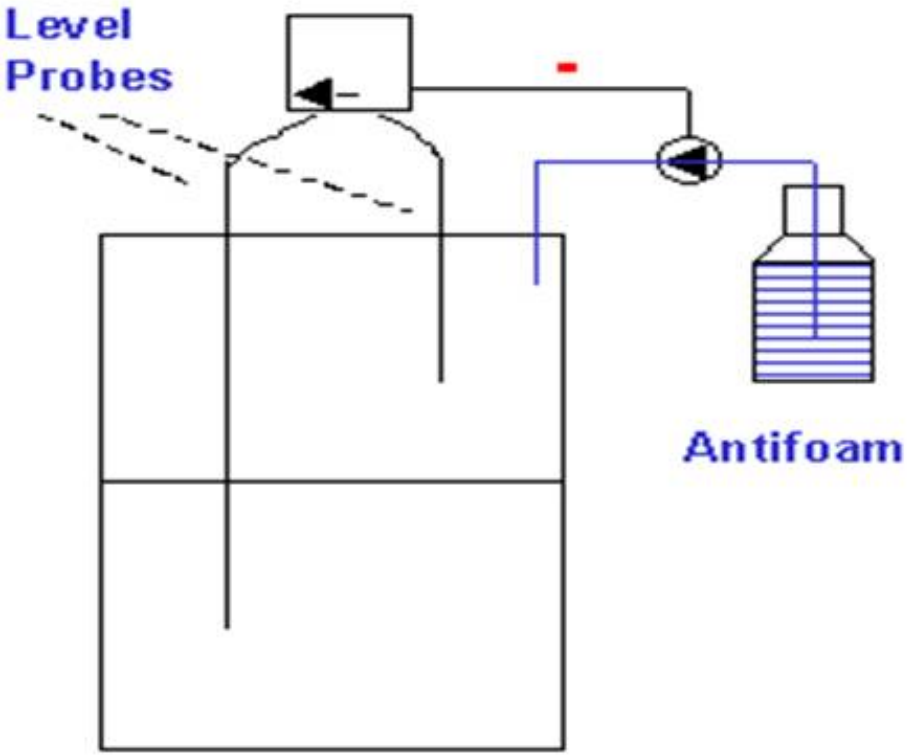
    Increasing the aeration rate and stirrer speed increases foaming problems.
  - **The head space volume**

    The larger headspace volume, then the greater the tendency for the foam to collapse under its own weight. For example, for fermentations in which high levels of foam is produced, a 50% headspace volume may be required.

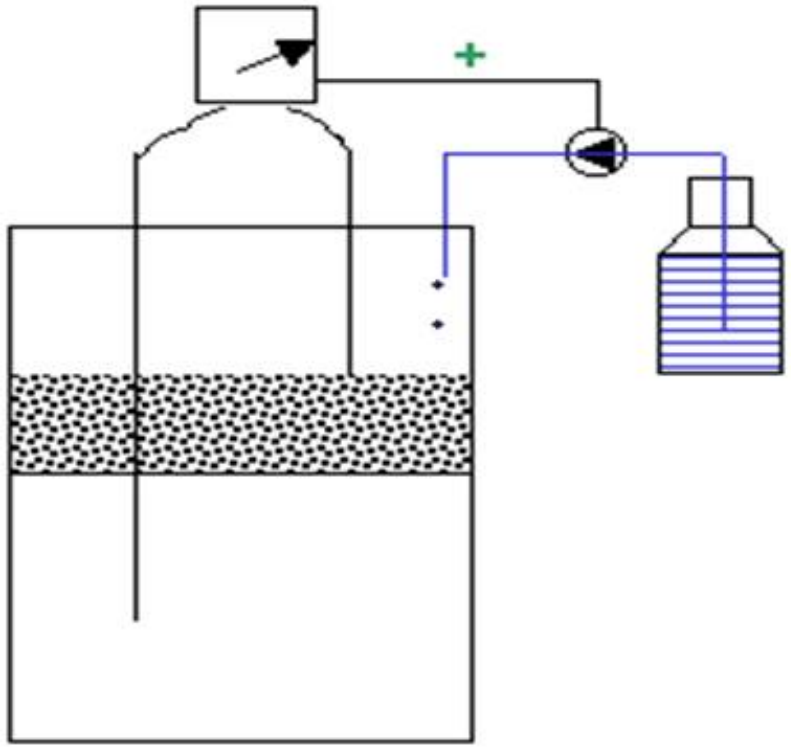
An ideal anti foam agent

- ❖ It should be non toxic for the organism and human
- ❖ It must be effective in small quantity
- ❖ It must suppress the foam immediately
- ❖ It should be non corrosive, non explosive and stable at normal conditions
- ❖ It must be sterilize before adding to the process
- ❖ Foam is typically controlled with aid of antifoaming agents based on silicone or on vegetable oils or alcohol. The physical way to control the foam is to put an extra pair of impeller in the head space. In many cases ultrasonic foam breaking devices are also used.

Foam is typically detected using two conductivity or "level" probes.



When the upper level probe is above the foam level, no current will pass between the level probes and the antifoam pump remains turned off.



When the upper level probe is immersed in the foam layer, a current is carried in the foam. This causes the antifoam to turn on.

Foam control system

- One probe is immersed in the fermentation liquid while the other placed above the liquid level.

- When the foam reaches the upper upper probe, a current is carried through the foam.

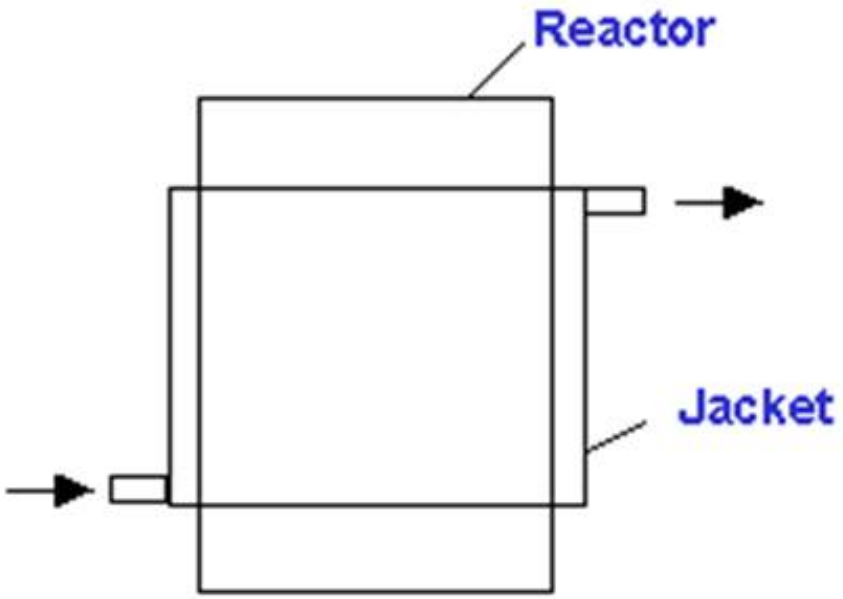
- The detection of a current by the foam controller results in the activation of a pump and the antifoam is then added until the foam subsides.

Temperature control system

- The temperature control system consists of
  - temperature probes
  - heat transfer system

- Typically the heat transfer system will use a "jacket" to transfer heat in or out of the reactor. The jacket is a shell which surrounds part of the reactor. The liquid in the jacket does not come in direct contact with the fermentation fluid.

Temperature control system



❖ The jacket will typically be "dimpled" to encourage turbulence in the jacket and thus increase the heat transfer efficiency.

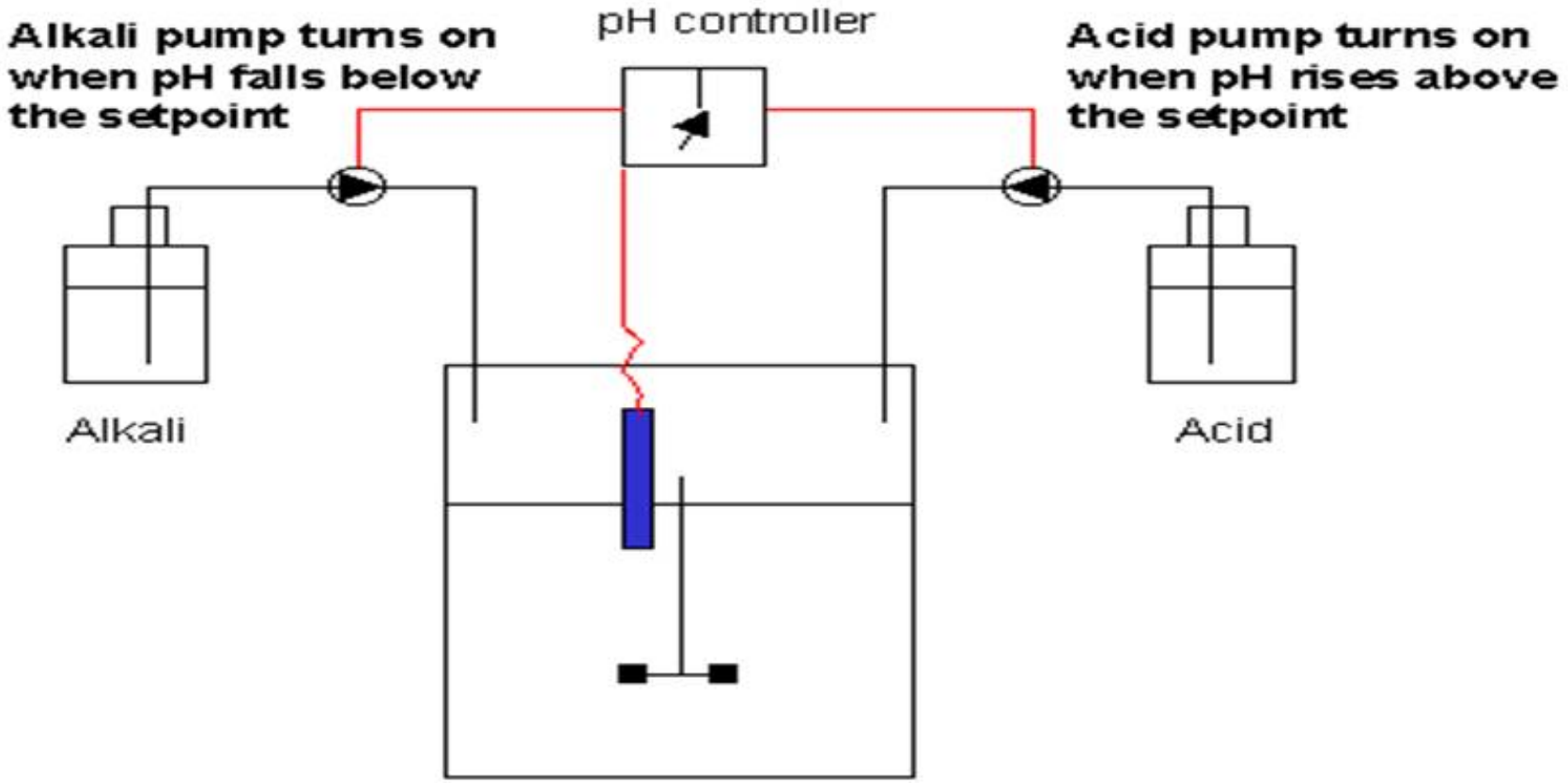
❖ An alternative to using jackets are coils. Coils have a much higher heat transfer efficiency than jackets. However coils take up valuable reactor volume and can be difficult to clean and sterilize.

Temperature control system

❖ The heating/cooling requirements are provided by the following methods:

	Laboratory scale	**Pilot and production scale**
Heating	Electric heaters	Steam generated in boilers
Cooling	Tap water or refrigerated water baths	Cooling water produced by cooling towers or frigerants such as ammonia.

pH control system



The pH control system consists of

- **a pH probe**
- **Alkali/ acid delivery system**

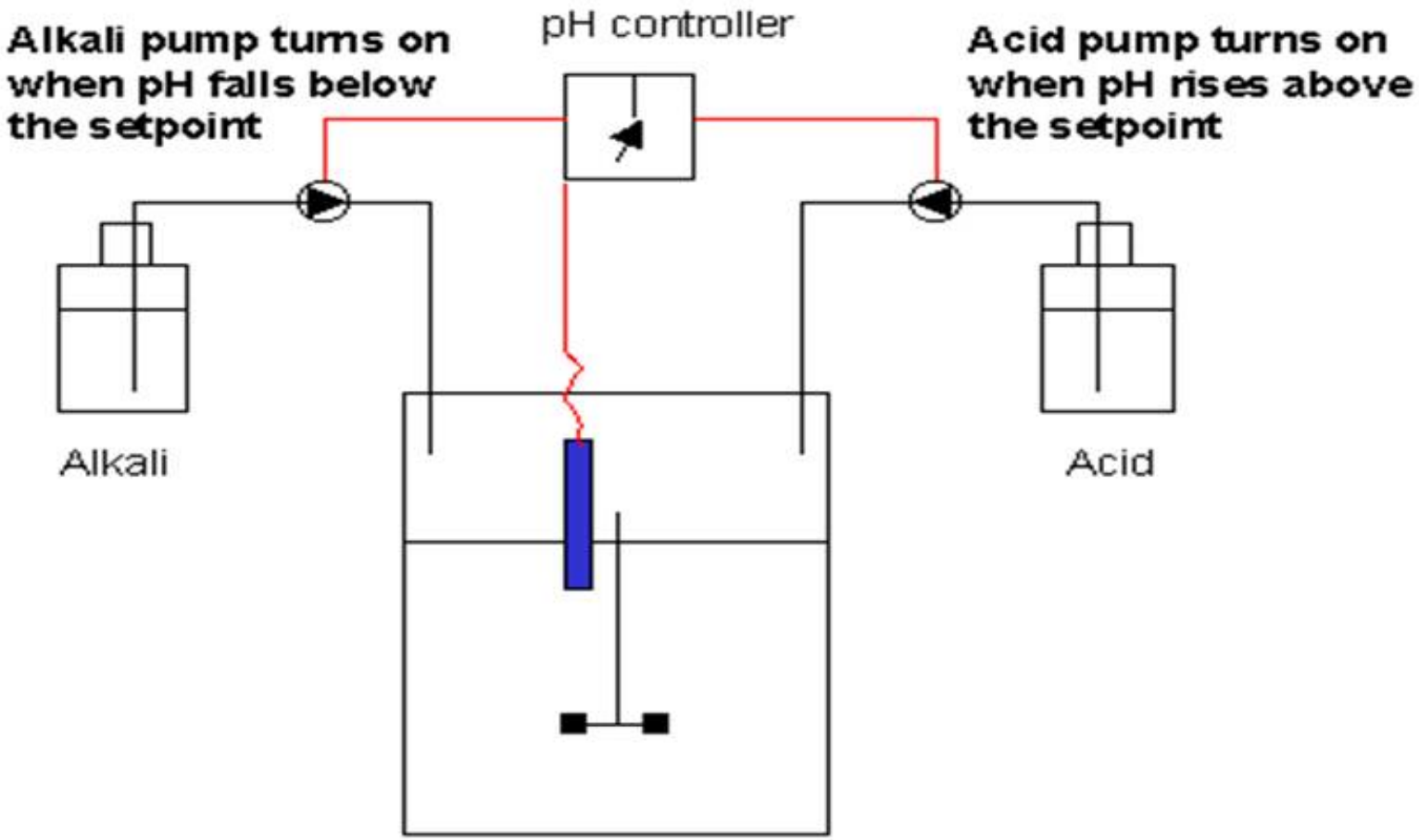
pH control system - Neutralizing agents

- The neutralizing agents used to control pH should be non-corrosive. They should also be non-toxic to cells when diluted in the medium.
- Potassium hydroxide is preferred to NaOH, as potassium ions tend to be less toxic to cells than sodium ions. However KOH is more expensive than NaOH. Sodium carbonate is also commonly used in small scale bioreactor systems.
- Hydrochloric acid should never be used as it is corrosive even to stainless steel.
- Likewise sulphuric acid concentrations should not be between 10% and 80% as between this range, sulphuric acid is most corrosive.

Neutralizing agents

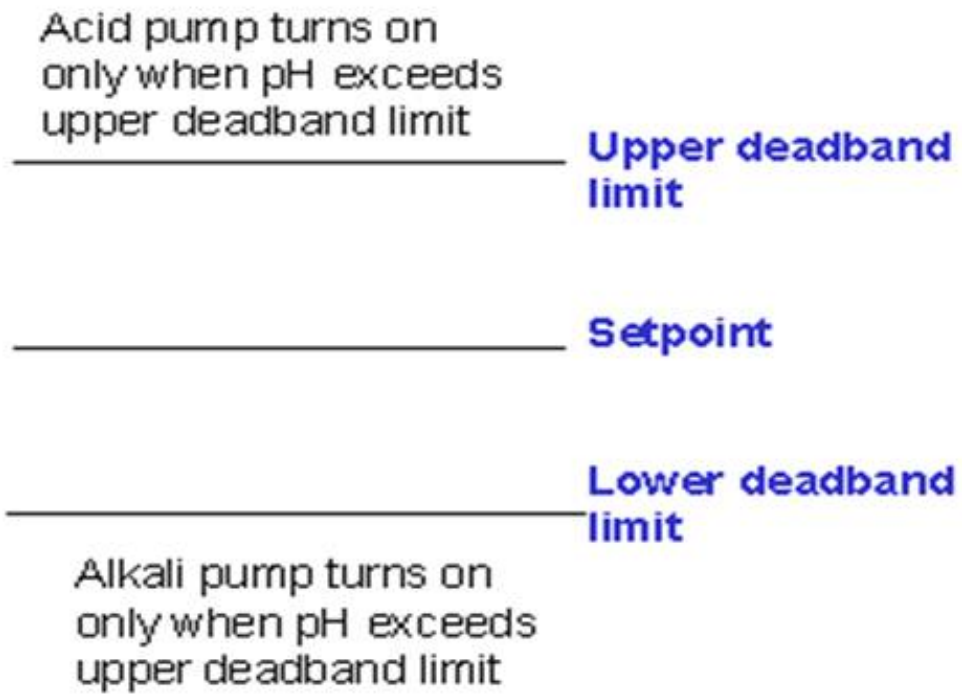
- For fermentations that produce large amounts of acids, for example lactic acids fermentation using media containing high sugar concentrations, high concentrations of alkali (4 M and above) are preferred. This will prevent dilution of the medium due to the addition of excessive addition of the alkali solution.
- For laboratory fermenters, a peristaltic pump is used to add the pH adjusting agents. Silicone tubing is often used. However, note that silicone tubing will decay in the presence of high alkali concentrations. Thick walled slicone tubing should be used.
- Alternatively Tygon or Neoprene tubing can be used. Tygon is not autoclavable but can be sterilized by passing the NaOH through the tubing for about 1 hour. Neoprene is autoclavable but is not transparent or translucent as is Tygon or silicone.

pH control system – Set point and dead band



Set point and dead band

- **The pH control system (and indeed all other fermenter control systems) are designed to have a dead band. A dead band is used to prevent excessive alkali and acid addition.**
- **The pH control dead band is shown in the following diagram:**



Set point and dead band

- The set point is the pH at which the fermenter is being attempted to be controlled at. For example, if the fermentation is to be run at a constant pH of 6.5, then the set point is set to 6.5
- If for example, a 5% dead band is used, then the upper dead band limit will be : 1.05 x 6.5 = 6.83
- and the lower dead band limit will be : 0.95 x 6.5 = 6.18
- If the dead band is too small, then it is possible that pH will often overshoot and undershoot the dead bands leading to excessive alkali and acid addition. The trade off is that a wide dead band will lead to less precise pH control.
- As many fermentations tend to produce acids rather than substances that increase the pH, acid addition is often not required. Indeed not all fermentations need continuous pH control.

Sample line

- There must be a sterile sample line to withdraw the sample intermittently.
- The sample withdrawn is useful in –
  - Determining viable count
  - Contamination, if any
  - Mutation/back mutation, if any
  - Product yield
  - Nutrition status of broth

- Sample gives significant information about the completion of process by showing two or more consecutive reading same in terms of product yield

Cleaning and sterilization facilities.

- Small scale reactors are taken apart and then cleaned before being re-assembled, filled and then sterilized in an autoclave.
- However, reactors with volumes greater than 5 liters cannot be placed in an autoclave and sterilized. These reactors must be cleaned and sterilized "in place". This process is referred to "Clean in Place".
- CIP involves the complete cleaning of not only the fermenter but also all lines linked to the internal components of the reactor. Steam, cleaning and sterilizing chemicals, spray balls and high pressure pumps are used in these processes. The process is usually automated to minimize the possibility of human error.