Shroff S.R. Rotary Institute of Chemical Technology (SRICT)

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Fermentation

Syllabus

- Principles of surface and solid state fermentation,
- Design of different fermentors and the biochemical engineering aspects.
- Process control of fermentations.
- Fermentation technology of industrial chemicals, organic acids, amino acids, vitamins, polysaccharides, antibiotics, etc.
- Enzyme fermentation and technology including immobilization and enzyme reactors.
- Fermentative animals, and other developments

- Fermentation is derived from the latin word *fervere,* meaning to boil.
- The term fermentation is used in biochemical sense to mean energy generation process through organic compounds which act as electron donors as well as terminal electron acceptors.
- Industrial microbiologists describe fermentation as any process for the production of product by mass culture of a micro-organism.
- The first industrial process for production of a microbial metabolite, alcohol, was by the action of yeast on malt or fruit extracts.
- **Fermentation** is a metabolic process that produces chemical changes in organic substrates through the action of enzymes
- In microorganisms, fermentation is the primary means of producing ATP by the degradation of organic nutrients anaerobically
- The science of fermentation is known as zymology.

Basic Design of a Fermenter

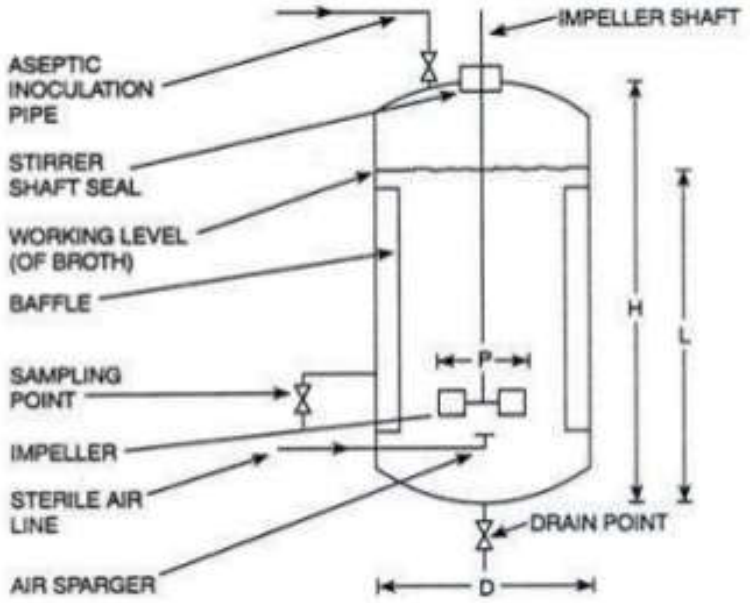
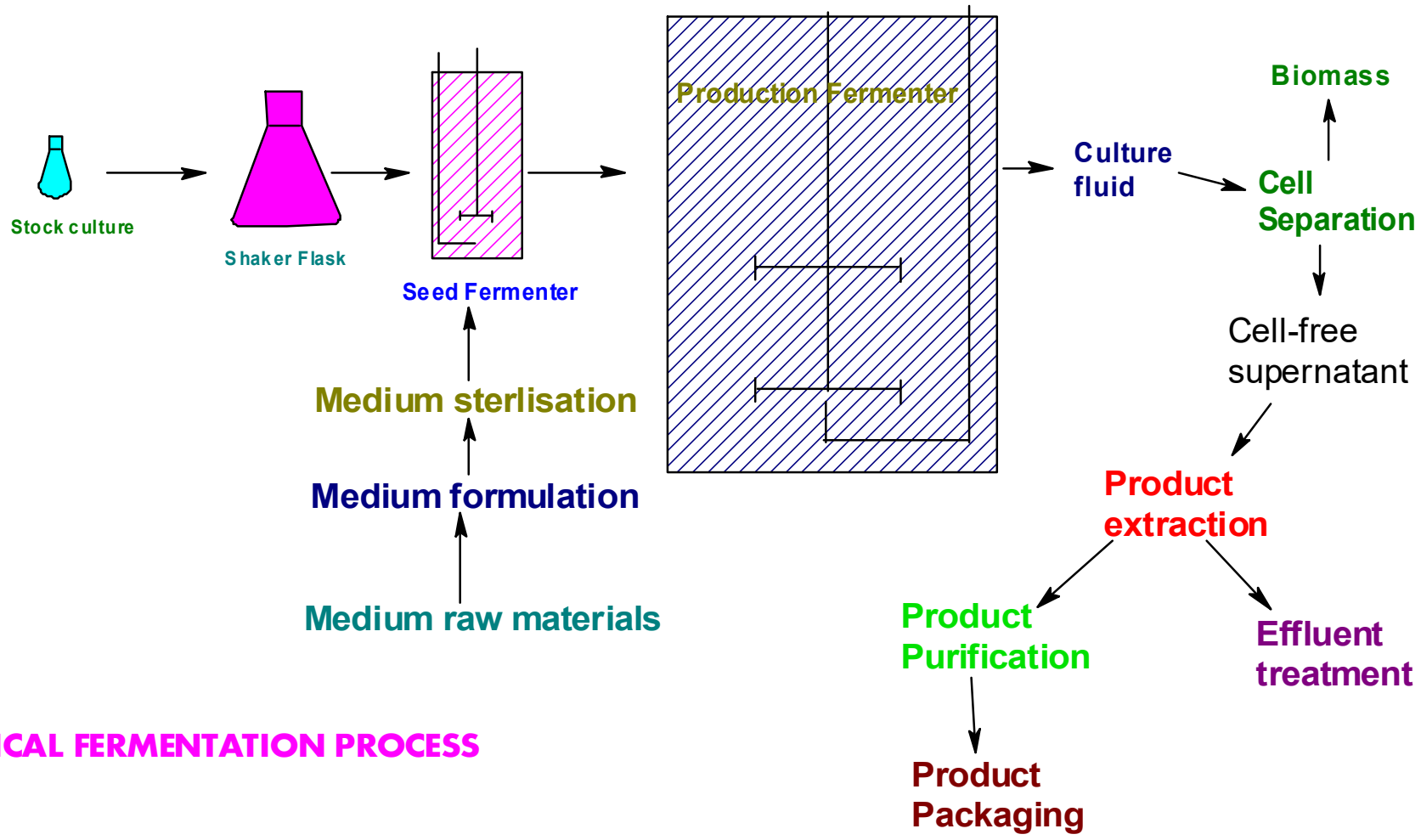


FIG. 14.3. Diagram of a fermenter with one multi-bladed impeller. H, fermenter height; L, liquid height; D, tank diameter; P, impeller diameter.

Components of a Fermentation Process:

- Regardless of the type of fermentation the process is divided into six basic parts (except transformation processes where microbial cells are used to convert compounds to structurally related more valuable compounds):

1. **The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter**
2. **The sterlization of the medium, fermenters and ancillary equipment**
3. **The production of an active, pure culture in sufficient quantity to inoculate the production vessel**
4. **The growth of the organism in the production fermenter under optimum conditions for product formation**
5. **The extraction of the product and its purification**
6. **The disposal of effluents produced by the process**



TYPICAL FERMENTATION PROCESS

- **Major types of fermentations**:
  i. Those that produce microbial cells (biomass) as the product
  ii. Those that produce microbial enzymes
  iii. Those that produce microbial metabolites
  iv. Those that produce recombinant product(artificially produced & often purified product)
  v. Those that modify a compound which is added to the fermentation – the transformation process
  - **Microbial biomass**
    - Two major process
      - Production of Yeast for the baking industry
      - Production of microbial cells as human / animal food
  - **Microbial Enzymes**
    - Produced from-
      - Animals
      - Plants
      - Microbes

- Microbial system for enzymes offers:
    - Ease of production
    - Advantage of mass production by well established fermentation techniques
    - Enzymes of animal origin can be synthesized with microorganisms (recombinant technology)
    - Easy to control and modify

• Microbial metabolites:

- There are a number of stages in the growth of microbial culture
    - Lag phase: On inoculation of a culture into a nutrient medium growth does not appear to occur. This phase is called the time of adaptation.
    - Log or Exponential Phase: The cells grow at a constant maximum rate after the lag phase. During this phase the products produced are essential for the growth of the cells such as proteins, lipids , carbohydrates, amino acids, nucleotides, etc. These primary products of metabolism. The phase is also referred as Trophophase. Products of commercial importance are produced by fermentation such as citric acid, ethanol, vitamins, polysaccharides, lysines, glutamic acid, etc
    - Death Phase: The period in which the viable cell numbers declines. In this phase, secondary metabolites which do not have any function in cell metabolism. The phase under which these secondary metabolites are produced is referred as idiophase
- The primary and the secondary metabolites are interrelated in the sense that the secondary metabolites are produced from the products of primary metabolites. Many secondary metabolites are antimicrobial, enzyme inhibitors, growth promotors and pharmacological active.

- **Recombinant Products**:
  - A large number of potential fermentation products are possible by using the recombinant technology. The genes of higher organisms are introduced in microbes such as *Escherichia Coli, Saccharomyces cerevisiae* and the filamentus fungi to produce products such as insulin, interferon, human serum albumin, epidermal growth factor, calf chymosin and bovine somatostatin.
- **Transformation Processes:**
  - Microbial cells are used to convert compounds to structurally related more valuable compounds. This is done by taking advantage of the fact that they behave as chiral catalysts where high positional specificity and stereospecificity are shown in their transformations. Microbial process offer low temperature and pressure transformations and are essentially non-polluting. Production of vinegar (conversion of ethanol to acetic acid) is a fine example of this process. Other high value products include prostaglandins, steroids and antibiotics.

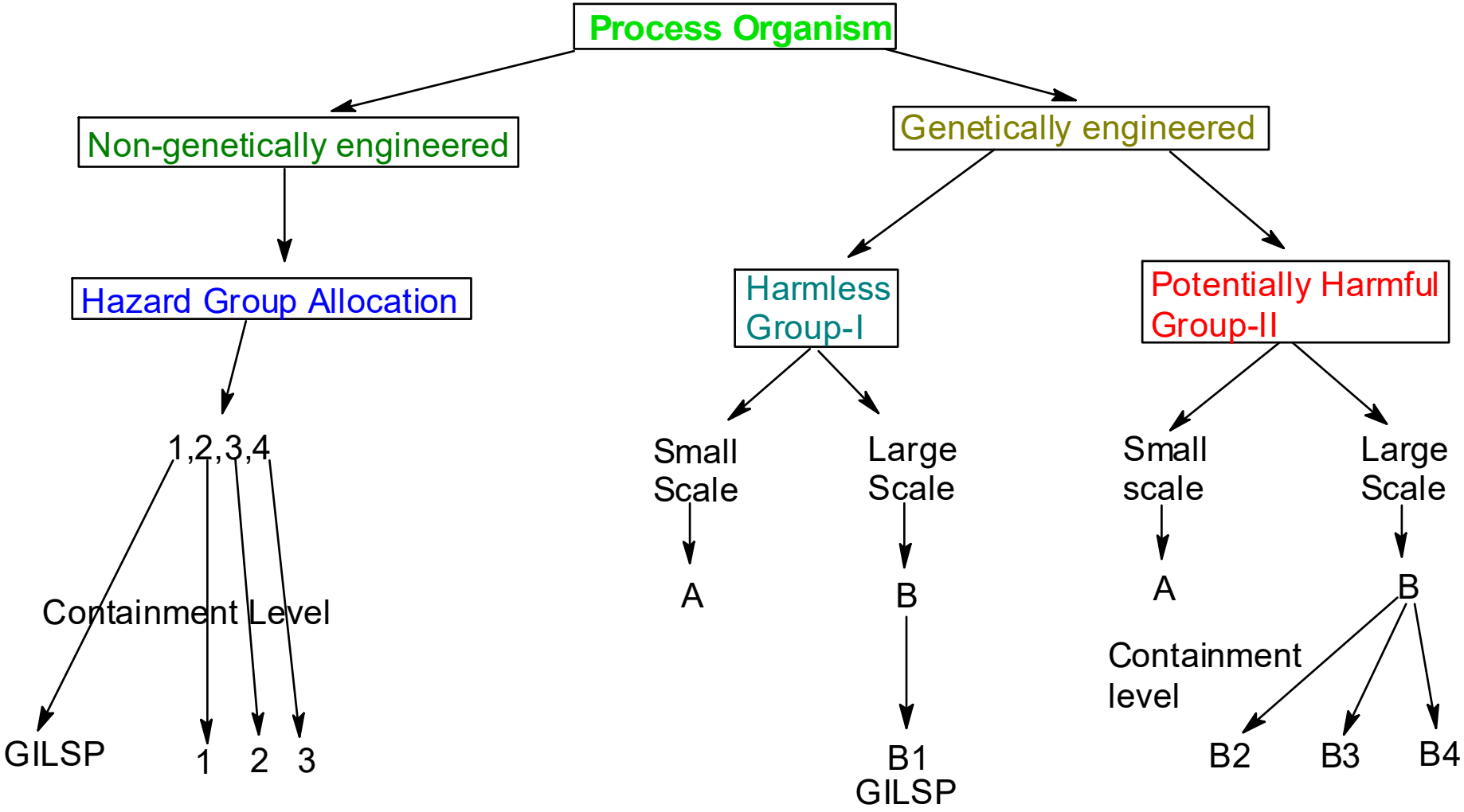
- **Basic functions of a Fermenter for microbial and animal cell culture**
  - The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product.
  - Points to be considered in designing and constructing a fermenter:
    1. **The vessel should be capable of being operated aseptically for a number of days and should be reliable in long term operations and meet the requirements of container regulations**
    2. **Adequate aeration and agitation should be provided to meet the metabolic requirements of the micro-organism. However, mixing should not cause any damage to the organism.**
    3. Power consumption should be as low as possible
    4. A system of temperature should be provided
    5. A system of pH control should be provided
    6. Sampling facilities should be provided
    7. Evaporation losses from the fermenter should not be excessive
    8. The vessel should be designed to require the minimal use of labour in operation, harvesting, cleaning and maintenance.

9. Ideally the vessel should be suitable for a wide range of processes, but this may be restricted because of containment regulations.
10. The vessel should be constructed to ensure smooth internal surfaces, using welds instead of flange joints whenever possible.
11. The vessel should be of similar geometry to both smaller and larger vessels in pilot plant or plant to facilitate scale-up.
12. The cheapest material which enable satisfactory results to be achieved should be used.
13. There should be adequate service provisions for individual plants

- The most commonly used fermenters are based on stirred upright cylinders having single or multiple impellers with sparger aeration.
- A very small percentage of the different types of fermenters have been satisfactory for industrial aerobic fermentations

- **Utilities or service provisions for a fermentation plant:**
  1. Compressed air
  2. Sterile compressed air(1.5 - 3 atm)
  3. Chilled water (12-15^{o}C)
  4. Cold water (4^{o}C)
  5. Hot water
  6. High Pressure Steam
  7. Steam condensate
  8. Electricity
  9. Stand-by-generator
  10. Drainage of effluents
  11. Motors
  12. Storage facilities for media components
  13. Control and monitoring equipment for fermenters
  14. Maintenance facilities
  15. Extraction and recovery equipment
  16. Accessibility for delivery of material
  17. Appropriate containment facilities

- **Aseptic operation and containment:**
  - Aseptic operation involves protection against contamination.
  - Containment involves prevention of escape of viable cells from the fermenter or downstream equipment.
  - To establish the appropriate level of containment risk assessment is to be carried out. This is done through categorization of process microorganisms and designation of its appropriate level of containment at research or industry sites say as per the European Federation of Biotechnology (see fig)
    - In the non-genetically engineered organisms placed in Hazard Grp-I require only **Good Industrial Large Scale Practice**(GILSP). The processes in this category need to be operated aseptically but no containment is necessary including prevention of organisms, whereas those placed in Hazard Grp-4 stringent level-3 requirements are to be met.
    - Genetically engineered organisms are classified as either harmless (Grp-I) or potentially harmful(Grp-II). The process is further classified as small scale (A) or large scale (B). Large scale fall in two categories IB or IIB. IB processes require containment level B1 and are subject to GILSP. Whereas IIB processes are further assessed to determine the most suitable containment level, ranging from B2 to B4 corresponding to levels 1 to 3 for non-genetically engineered organisms.


Process Organism
Non-genetically engineered
Genetically engineered
Hazard Group Allocation
Harmless
Group-I
Potentially Harmful
Group-II
1,2,3,4
Small
Scale
Large
Scale
Small
scale
Large
Scale
Containment Level
A
B
A
B
Containment
level
GILSP
1
2
3
B1
GILSP
B2
B3
B4

- Most micro-organisms used in industrial processes are in the lowest hazard group and require only GILSP barring a few especially those used in viral vaccine production

- **Body Construction:**
- **Construction material:**
  - In fermentations with strict aseptic requirements it is important to select materials that can withstand repeated steam sterilization cycles. On a small scale (1-30dm^3) glass and stainless steel can be used.
  - Advantages of using Glass:
    - Smooth surfaces
    - Non-toxic
    - Corrosion proof
    - Easy to examine the interiors
  - Two basic types of fermenter are used:
    - A glass vessel with a round or flat bottom and a top flanged carrying plate. This type have to be sterilized by autoclaving. The largest practical diameter for glass fermenters is 60cm
    - A glass cylinder with stainless steel top and bottom plates. These fermenters may be sterilized insitu but 30cm diameter is the upper size limit to safely withstand working pressures. Vessels with two SS plates cost twice as that one with a single plate.

- Pilot and industrial scale are normally constructed of SS or at least have a SS cladding to limit corrosion. As per American Iron and Steel Institute(AISI) steels containing >4% chromium are classified as SS and those <4% as steel alloys.
- The corrosion resistance of SS is due to a thin film of hydrous oxide film on the surface of the metal. The composition of this film varies with different steel alloys and is stabilised with chromium which is considered to be continuous, non-porous, insoluble and self healing.
- Increasing the chromium content enhances resistance to corrosion, but only steel containing at least 10-13% chromium develop an effective film.
- The inclusion of nickel in high percent chromium steels enhances their resistance and improves their engineering properties.
- The presence of **Molybdenum** improves the resistance of stainless steel to solutions of halogen salts and pitting by chloride ions in brine or sea water.
- Corrosion resistance can be improved by tungsten, silicon and other elements
- Mild steel coated with glass or phenolic epoxy materials have also been used

- AISI grade 316 steel is commonly used in fermenter construction. This contains:
  - 18% chromium
  - 10% nickel
  - 2-2.5% molybdenum
- AISI grade 304 is extensively used in brewing equipment and it contains
  - 18.5% chromium
  - 10% nickel
- The thickness of the construction material will increase with scale. At 300000 to 400000 dm^3 capacity, 7mm plate may be used for the side of the vessel and 10mm plate for the top ad bottom, which should be hemispherical to withstand pressures

- **Temperature Control:**
  - Heat is generated in the reactor by:
    - Microbial action
    - Mechanical agitation
  - Heat may have to be given or removed depending upon the process. This is done using cooling through jacket and/or internal coils.
  - It is impossible to specify accurately the necessary cooling surface of a fermenter since the temperature of the cooling water, the sterilization process, the cultivation temperature, the type of micro-organism and the energy supplied by stirring can vary considerably in different processes.
  - A cooling area of 50-70m^2 may be taken as average for 55000 dm^3 fermenter and with a coolant temperature of 14^oC the fermenter may be cooled from 120 to 30^oC in 2.5 – 4hrs without stirring. The consumption of cooling water in this size of vessel during a bacterial fermentation ranges from 200 to 2000dm^3 h^{-1} while fungi might require 2000 to 10000dm^3 h^{-1} due to lower optimum temp for growth.

- To accurately estimate heating and cooling requirements for a specific process it is important to consider all the contributing factors. The over all energy balance of a fermenter during normal operation can be estimated through the following equation:

$$Q_{met} + Q_{ag} + Q_{gas} = Q_{acc} + Q_{exch} + Q_{evap} + Q_{sen}$$

  - Where Q_{met} = rate of heat generation due to microbial metabolism
  - Q_{ag} = rate of heat generation due to mechanical agitation
  - Q_{gas} = rate of heat generation due to aeration power input
  - Q_{acc} = Rate of heat accumulation by the system
  - Q_{exch} = rate of heat transfer to the surroundings and/or heat exchanger
  - Q_{evap} = rate of heat loss by evaporation
  - Q_{sen} = Rate of enthalpy gain by the flow streams

- The equation can be rewritten as:

$$Q_{exch} = Q_{met} + Q_{ag} + Q_{gas} - Q_{acc} - Q_{evap} - Q_{sen}$$

- The cooling requirements (jackets and / or pipes) to remove the excess heat from a fermenter may be determined by the formula:

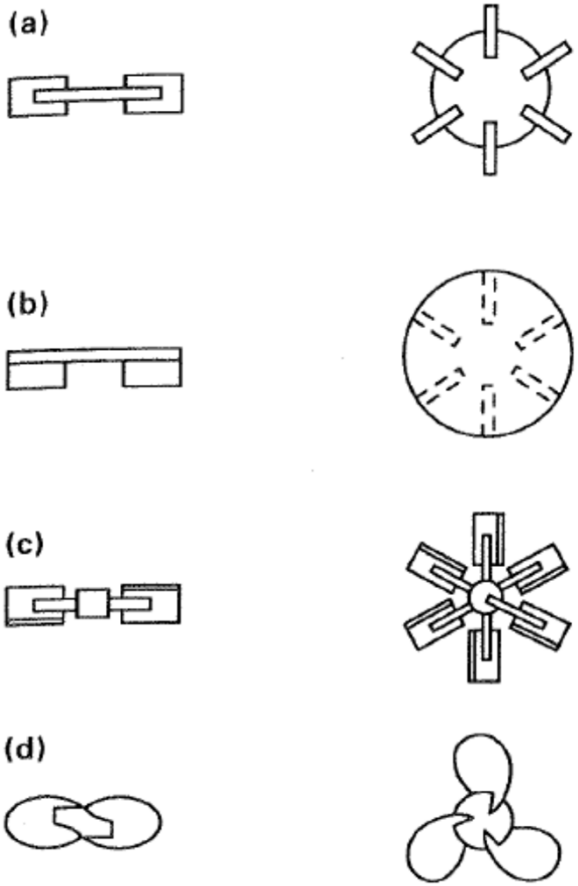
$$Q_{exch} = U \cdot A \cdot \Delta T$$

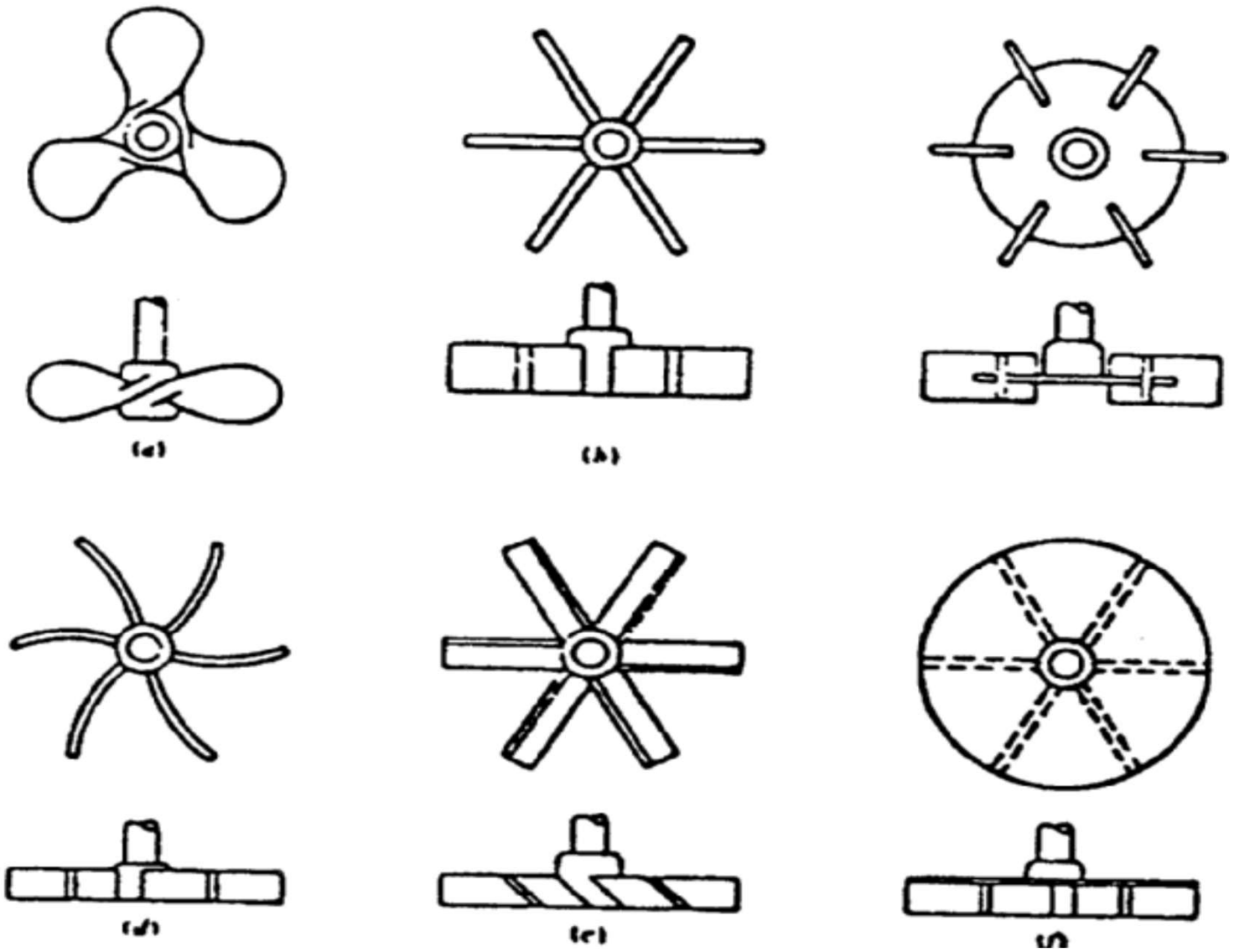
where A = the heat transfer surface available, m^2,
Q = the heat transferred, W,
U = the overall heat transfer coefficient, W/m^2K, Watts per squared meter kelvin
ΔT = the temperature difference between the heating or cooling agent and the mass itself, K.

- **Aeration and Agitation**
- Aeration is done to provide microorganism with sufficient oxygen to carry out metabolic requirements.
- The type of aeration-agitation system used depends on the characteristics of the fermentation process under consideration
- The bubble aerators without mechanical agitators have the advantage of lower equipment and power costs. In this process broths of low viscosity and low total solids are used.
- Mechanical agitation is usually required in fungal and actinomycete (a heterogenous gram positive bacteria) fermentation.
- The structural components of the fermenter involved in aeration and agitation are:
  - **The agitator(impeller)**
  - **Stirrer glands and bearing**
  - **Baffles**
  - **The aeration system(sparger)**

- **Agitator**
  - Agitator is required to achieve:
    - Bulk fluid and gas-phase mixing
    - Air dispersion
    - Oxygen transfer
    - Heat transfer
    - Suspension of solid particles
    - Maintaining a uniform environment in the vessel

  - **Types of Agitators:**
  - Agitators may be classified as
    - (a)Disc turbine
    - (b)Vaned discs,
    - (c)Open turbines of variable pitch
    - (d)Propellors



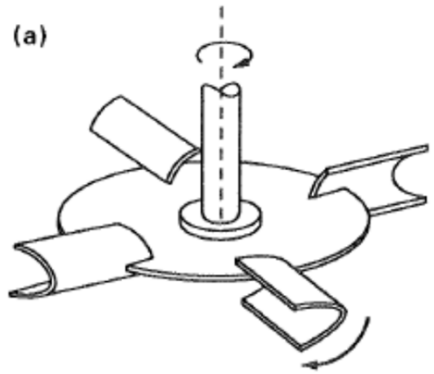


Different Impeller Types. (a) Marine-type propellers; (b) Flat-blade turbine, $W_i = D_i/5$. © Disk flat-blade turbine, $W_i = D_i/5$, $D_i = 2D_t/3$, $L_i = D_i/4$; (d) Curved-blade turbine, $Wi = D_i/3$; (e) Pitched-blade turbine, $W_i = D_i/8$; and (f) Shrouded turbine, $W_i = D_i/8$.

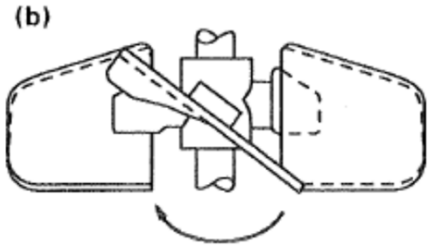
D_t = tank diameter,
D_i = impeller diameter
W_i = impeller blade height

- **Disc turbine**: It consists of a disc with a series of rectangular vanes set in a vertical plane around the circumference. **Disc turbine** is the most suitable in a fermenter since it can break up a fast air stream without itself becoming flooded in air bubbles.
- **Vaned Disc**: It has a series of rectangular vanes attached vertically to the underside.
    - Air from the sparger hits the underside of the disc and is displaced towards the vanes where the air bubbles are broken up into smaller bubbles.
- **Variable Pitch open turbine & Marine propeller:** The vanes are attached to a boss on the agitator shaft. The air bubbles do not hit any surface before dispersion by the vanes or blades

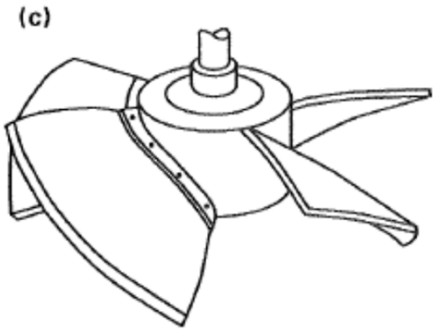
NEW AGITATORS



Scaba Agitator



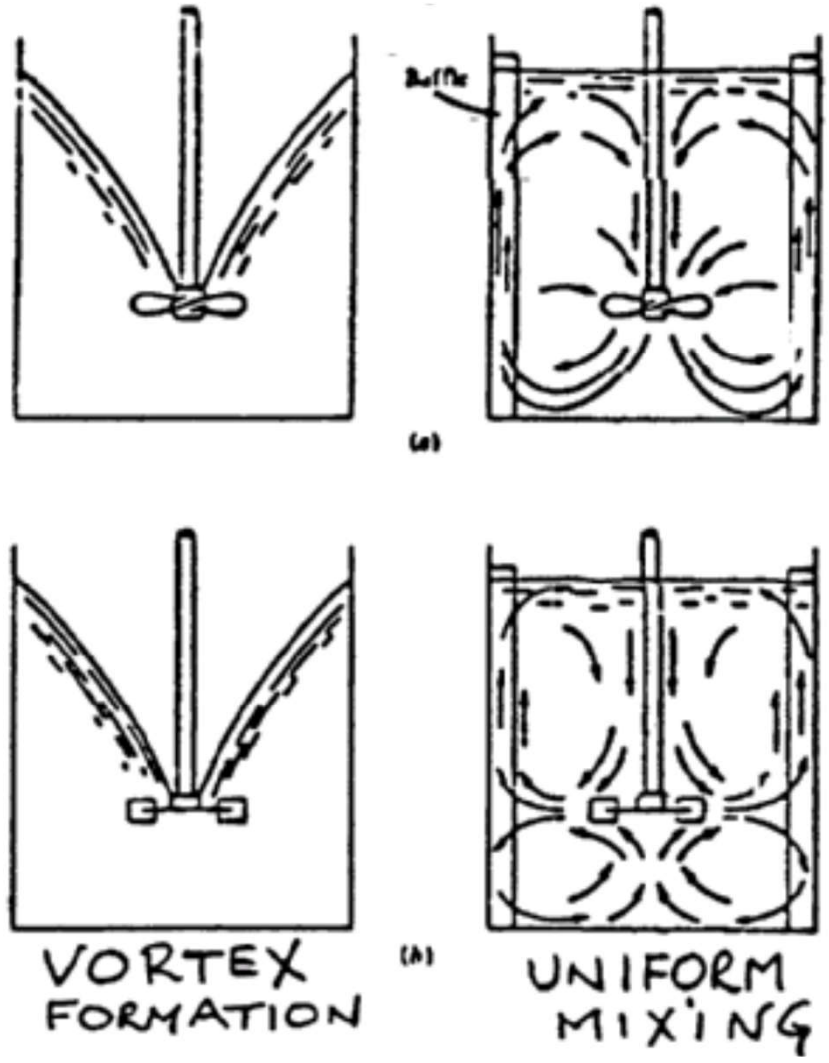
Lightnin A315 Agitator



Prochem Maxflo T agitator

- **Scaba 6SRTG agitator**
  - Developed to handle problems associated with efficient blending in *high viscosity fermentations*.
  - At a given power input can handle a high air flow rate before flooding.
  - Radial-flow agitation is better for bulk blending than a Rushton turbine, but does not give good top to bottom blending in a large fermenter which leads to lower concentrations of oxygen in broth away from the agitators and higher concentrations of nutrients, acid or alkali or anti-foam near to the feed points.
- **Prochem Maxflo Agitator**
  - Consists of 4-6 hydrofoil blades set at a critical angle on the central hollow hub.
  - A hydrodynamic thrust is created during rotation increasing the downward pumping capacity of the blades.
  - This design minimizes the drag forces(is a **force**acting opposite to the relative motion of any object moving with respect to a surrounding fluid.) associated with the rotation of the agitator such that the energy losses due to drag are low resulting in low power number

- **BAFFLES**
  - Baffles are metal strips that prevent vortex formation around the walls of the vessel.
  - These metal strips attached radially to the wall for every 1/10 th of vessel diameter.
  - Usually 4 baffles are present but when the vessel diameter is over $3dm^3$ around 6-8 baffles are used.
  - There should be enough gap between wall and baffle so that scouring action(removing loose particle from interior surfaces and flushing out) around vessel is facilitated. This movement minimizes microbial growth on baffles and fermentation walls. If needed cooling coils may be attached to baffles.


(a)
(b)
VORTEX FORMATION
UNIFORM MIXING


(c)

- **STIRRER GLANDS AND BEARINGS**
- The entry point of stirrer into fermenter may be from top to bottom or sides. Mostly used from bottom so that that leaves more space for entry ports on top. There are four types of stirrer glands and bearings.
- 1) Stuffing box
  - a. Sealed by several layers of packing rings of asbestos or cotton yarn-pressed against the shaft by a gland follower
  - b. At high speeds- packing wears – pressure should be applied to ensure tightness
  - c. Difficult to sterilize- satisfactory heat penetration
  - d. Sufficient for GILSP(good industrial large scale practice) containment
- 2) Mechanical seal
  - a. 2 parts; i) stationary in the bearing housing, ii) other rotates on the shaft.
  - b. Two parts pressed together by springs or expanding bellows
  - c. Steam condensate use to lubricate and cool seals
  - d. safe for containment
  - e. double mechanical seal for level 2
  - f. at level 2 and 3, the condensate is piped to a kill tank
  - g. Disinfectants flushed through the seal
  - h. steam condensate outlet monitoring indicates any seal failure

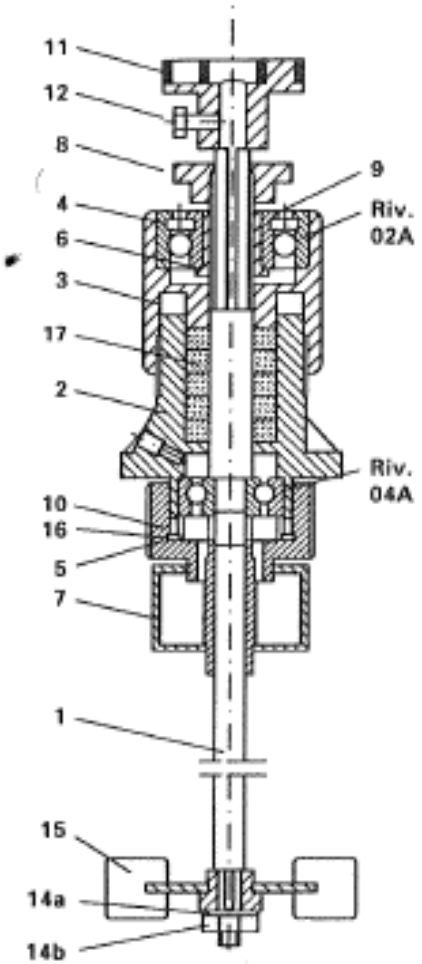


FIG. 7.13. Packed-gland stirrer seal (Chain *et al.*, 1954) (Components: 1, agitator shaft; 2, stuffing box; 3, upper cap; 4, lock ring; 5, lower cap; 6, chuck; 7, greasecup; 8, lock ring; 9, lock nut; 10, distance ring; 11, half coupling; 12, half coupling; 14a, washer; 14b, nut; 15, impeller; 16, shim; 17, packing rings).

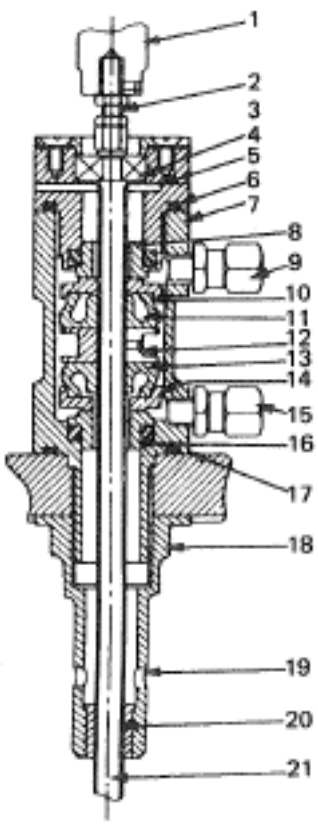


FIG. 7.14. Mechanical seal assembly (Elsworth *et al.*, 1958). (Components; 1, flexible coupling; 2, stirrer shaft; 3, bearing housing; 4, ball journal fit on mating parts; 5, two slots for gland leaks, only one shown; 6, 'O'-ring seal; 7, seal body; 8, stationary counter-face sealed to body with square-section gasket; 9, exit port for condensate, fitted with unequal stud coupling; 10, rotating counter-face; 11, bellows; 12, shaft muff; 13, as 11; 14, as 10; 15, entry port for condensate, as 9; 16, as 8; 17, as 6; 18, shaft bush support; 19, leak holes; 20, Ferobestos bush; 21, ground shaft).

- 3) Magnetic drives (some animal cell cultures)
  - a. shaft does not pierce the vessel
  - b. two magnets- one driving, held in bearing in housing on outside of head plate and one driven, placed on one end of impeller shaft held in bearing in suitable housing
  - c. ceramic magnets –magnetic power cross 16mm gap
  - d. 300 – 2000 rpm rotation possible
- 4) Simple bush seals
  - Disadvantage of double seals are more difficult to assemble, difficult to detect failure of seal from normal and dead spaces and seals leading to contamination. Hence simple bush seal is preferred in some cases.

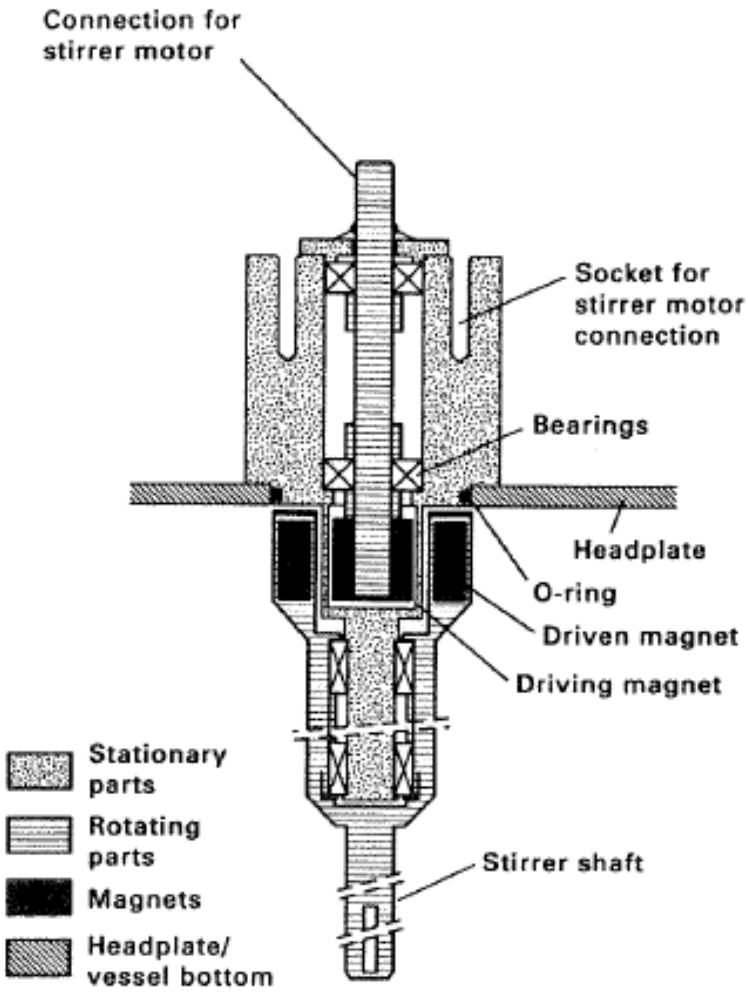


FIG. 7.16. Diagram of magnetically coupled top stirrer assembly

- **AERATION SYSTEM (SPARGER)**
- Sparger is a device for introducing air into fermenter. Aeration provides sufficient oxygen for organism in the fermenter. Fine bubble aerators must be used. Large bubbles will have less surface area than smaller bubbles which will facilitate oxygen transfer to a greater extent. Agitation is not required when aeration provides enough agitation which is the case Air lift fermenter. But this is possible with only for medium with low viscosity and low total solids. For aeration to provide agitation the vessel height/diameter ratio (aspect ratio) should be 5:1. Air supply to sparger should be supplied through filter. There are three types of sparger viz. porous sparger, orifice sparger and nozzle sparger.
- 1. **Porous sparger**: made of sintered glass, ceramics or metal. It is used only in lab scale-non agitated vessel. The size of the bubble formed is 10-100 times larger than pore size. There is a pressure drop across the sparger and the holes tend to be blocked by growth which is the limitation of porous sparger.

- 2. **Orifice sparger**: used in small stirred fermenter. It is a perforated pipe kept below the impeller in the form of crosses or rings. The size should be ~ ¾ of impeller diameter. Air holes drilled on the under surfaces of the tubes and the holes should be atleast 6mm diameter. This type of sparger is used mostly with agitation. It is also used with out agitation in some cases like yeast manufacture, effluent treatment and production of SCP.
- 3. **Nozzle sparger**: Mostly used in large scale. It is single open/partially closed pipe positioned centrally below the impeller. When air is passed through this pipe there is lower pressure loss and does not get blocked.
- 4. **Combined sparger agitator**: This is air supply via hallow agitator shaft. The air is emitted through holes in the disc between the blades of agitator. The design gives good aeration in baffled vessels.

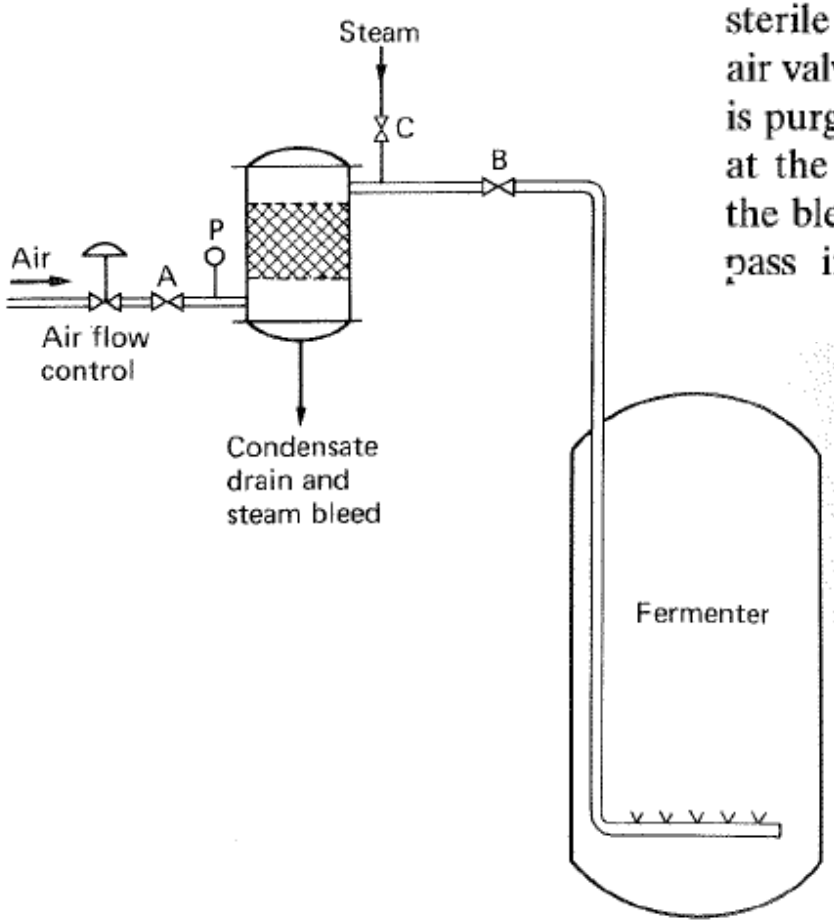
The achievement and maintenance of aseptic conditions

- The following operations have to be performed according to specifications to maintain aseptic conditions and containment during fermentation:
  1. Sterilization of the fermenter
  2. Sterilization of the air supply and the exhaust gas
  3. Aeration and Agitation
  4. The addition of inoculum and other supplements
  5. Sampling
  6. Foam control
  7. Monitoring and control of various parameters

- On a small scale <10dm^3 the biohazard risk can be controlled by a combination of containment cabinets and work practices.
- When the volume of culture is > 10dm^{3,} GILSP is required for non-pathogenic and non-toxigenic agents
- For level 1, B2 or higher containment levels the following points need to be considered when designing a fermenter
  - All vessels containing live organism should be suitable for steam sterilization and have sterile vent filters
  - Exhaust gas should pass through sterile vent filters
  - Seal on flange joint shall be fitted with a single ‘O’ ring at the lower levels of containment
  - For containment level B3 or B3/4 double ‘O’ ring or double ‘O’ ring with a steam barrier
  - Suitable seals should be provided at the entry ports for sensor probes, inoculum, sampling, medium addition, acid , alkali and antifoam
  - Rotating shafts into a closed system should be sealed with a double acting mechanical seal with steam or condensates between the seals
  - During operation a steam barrier should be maintained in all fixed piping leading to the ‘contained’ vessels
  - Appropriate pressure release facilities to be provided

- **Sterilization of fermenter**
  - Fermenter to be designed so that it can be steam sterilized under pressure
  - The medium may be sterilized in the vessel or separately, and added to the vessel aseptically
  - If the medium is sterilized *in situ* then its temperature must be raised before injection of the steam to prevent the formation of large condensates.
  - Every point of entry and exit to the fermenter is a potential source of contamination and hence steam should be introduced at such points
  - All pipes should be constructed simply and should slope towards drainage points.
  - Each drainage point should be fitted with a steam trap
  - Fermenter should be free of crevices (narrow opening) of 0.05mm depth especially when using animal cells
  - Welded joints should be used wherever possible

- **Sterilization of air supply**
  - Air is sterilized through two processes: Heat and Filtration
  - Sterilization through heat for full system is too costly
  - Glass wool, Glass fibre, mineral slag wool have been used for filtration. These have been replaced with cartridge type filters in fermenters.
  - The filter also needs to be sterilised in association with the fermenter

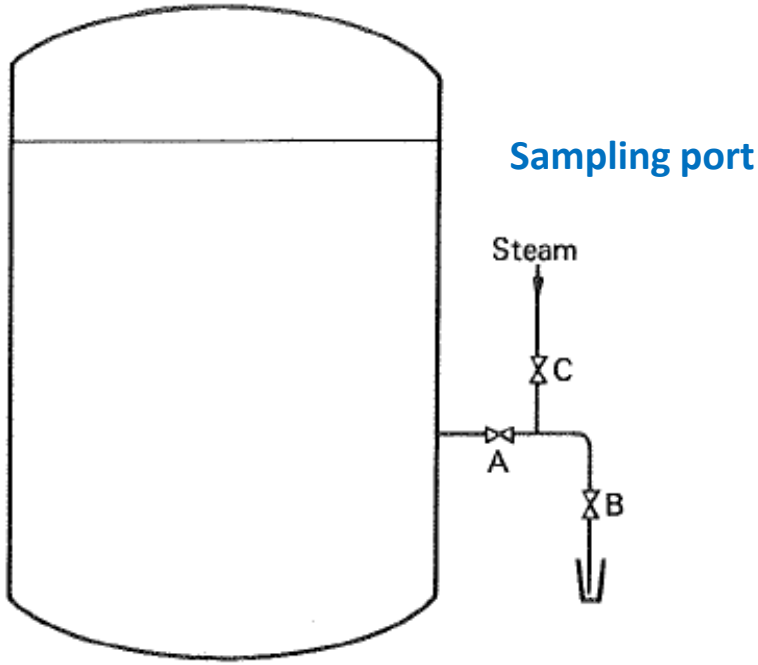


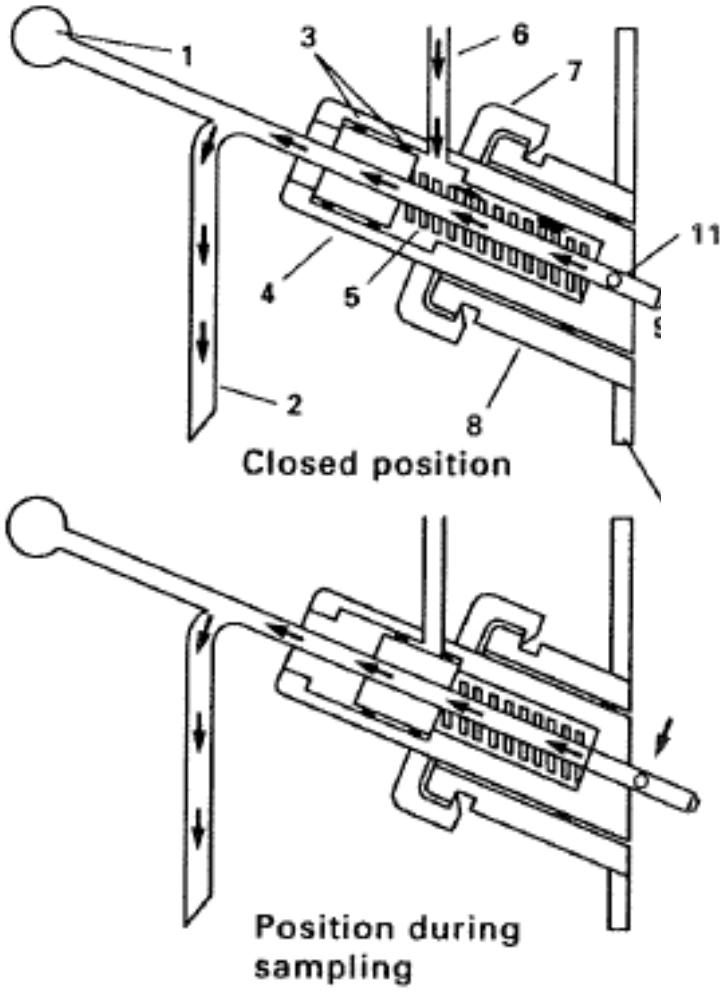
During sterilization the main non-sterile air-inlet valve A is shut, and initially the sterile air valve B is closed. Steam is applied at valve C and air is purged downwards through the filter to a bleed valve at the base. When the steam is issuing freely through the bleed valve, the valve B is opened to allow steam to pass into the fermenter as well as the filter.

- **Sterilization of the exhaust gas from the fermenter**
  - Sterilisation of the exhaust is achieved by passing through a 0.2micron filter put on the outlet pipe.
  - For satisfactory operation a cyclone separator and a coalescer (A **coalescer** is a technological device performing coalescence. They are primarily used to separate emulsions into their components via various processes; operating in reverse to an emulsifier.)is included upstream of two filters in series
- **Addition of inoculum, nutrients and other supplements**
  - When operating under GILSP both the addition vessel and the fermenter is placed under positive pressure and the addition port is equipped with a steam supply.
  - For containment levels 1 and B2, the addition is carried out in such a way that the release of microorganism is restricted. This is done by aseptic piercing of membranes or connections with steam locks
  - At containment level 2 & B3/4 no microorganism must be released during inoculation or other additions. This is done by tight screwing and clamping and making all pipelines steam sterilizable

- **Sampling:**
  - Sampling points are fitted to large fermenters so as to maintain sterility.
  - A sterile barrier must be maintained between the fermenter contents and the exterior when the sample port is not being used and it must be sterilizable after use.

In normal operation valves A, B and C are closed and a barrier is formed by submerging the end of the sampling port in 40% formalin or a suitable substitute. A sample is obtained by removing the container of formalin and closing valve A. Valves B and C are then opened until the piping has been sterilized by steam. Valves B and C are then partially closed to allow a slow stream of steam and condensate out of the sampling port. Valve A is then opened slightly to cool the piping. The broth is discarded. Valve C is then closed and a sample is collected. Valve A is then closed and the piping is resterilized and left in the out of use arrangement.





Sterilizable sampling system for Category 1 (Werner, 1992). Key: (1) handle for opening and closing; (2) piping for sample or condensate, respectively; (3) 'O'-ring; (4) housing; (5) spring; (6) steam inlet; (7) union nut; (8) welding socket; (9) product; (10) wall of the bioreactor; (11) port of sampling tube.

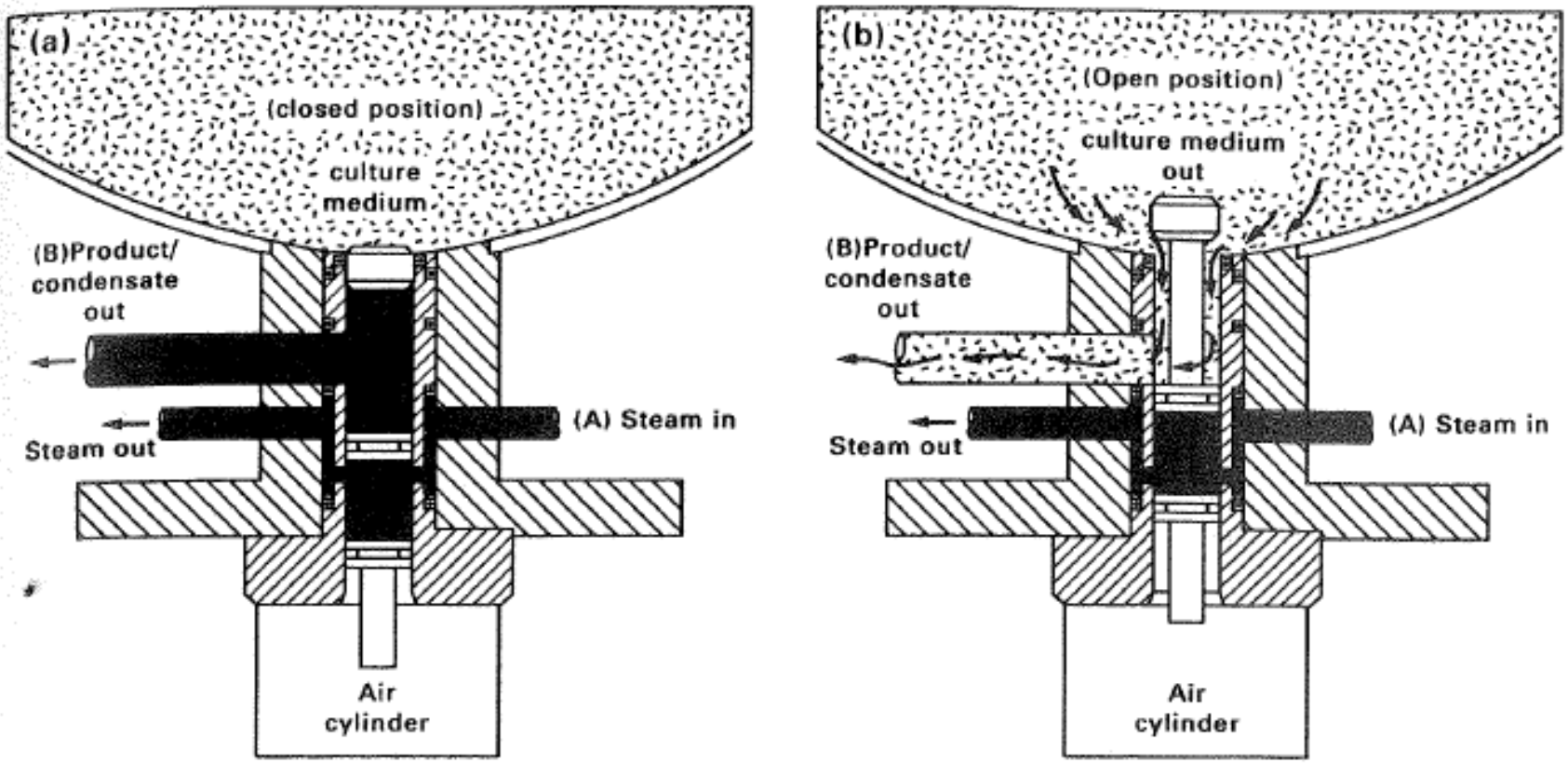


FIG. 7.26. Resterilizable harvest-sampling valve for Level 2 containment (New Brunswick, Hatfield, England). This spool-type valve is connected to the bottom of the fermenter vessel in the closed position (a), pressurized steam circulates throughout the entire valve body and through the product condensate line (B) via a steam inlet line (A). Aseptic withdrawal of samples is achieved with the valve in the open position (b). To prevent possible contamination when the plunger is raised, steam is circulated to the lower valve area. Action of the plunger is controlled by an air cyclinder.

- **Feed Ports:**
  - Addition of nutrients, acid /alkali to fermenters are normally made via silicon tubes which are autoclaved and pumped by a peristaltic pump(a series of wave like movement-a diaphragm pump) after aseptic connections
  - In large fermenters, the nutrient reservoir and associated piping are usually an integrated part which can be sterilized with the vessel
- **Sensor Probes:**
  - Double ‘O’ ring seals have been used to provide aseptic seals for glass electrodes in stainless steel housing in fermenters using GILSP. System is suitable for level 1 & B2 type of containment
  - For containment levels 2 & B3/4 probes are fitted with triple ‘O’ rings seals
  - The use of pre-inserted backup probes is recommended as a means for dealing with probe failure rather than using a retractable electrode housing during a fermentation cycle because of danger of leakage of broth

- **Foam control:**
  - It is important to minimize foam in any fermentation process
  - With excessive foam there is a danger of filters getting wet resulting in contamination
  - Siphoning may also result due to foam resulting in loss of part or entire contents of the fermenter.
  - Antifoam agents, mechanical foam breakers are used to overcome problems associated with foam.
  - Commonly used **agents** are insoluble oils, polydimethylsiloxanes and other silicones, certain alcohols, stearates and glycols
- **Valves and Steam Traps**
  - Valves attached to fermenters are used to control the flow of gas and liquid.
  - The valves may be
    - Simple ON/Off valve which are either fully open or closed
    - Valves which provide coarse control of flow rates
    - Adjustable valves which may be used for accurate control of flow rates
    - Safety valve which allow unidirectional flow

- **Gate Valve**:
  - This is a general purpose valve used in steam or water line for fully closing and opening. This is not used as a controlling valve
  - A sliding disc is moved in or out of the flow path by turning the stem of the valve
  - The flow path is such that the pressure drop is minimal.
  - Unsuitable for aseptic conditions
- **Globe Valves**:
  - A horizontal disc or plug is raised or lowered in its seating to control the rate of flow.
  - This type of valve is very commonly used for regulating the flow of water or steam since it may be adjusted rapidly
  - There is high pressure drop in the flow path.
  - Unsuitable for aseptic conditions

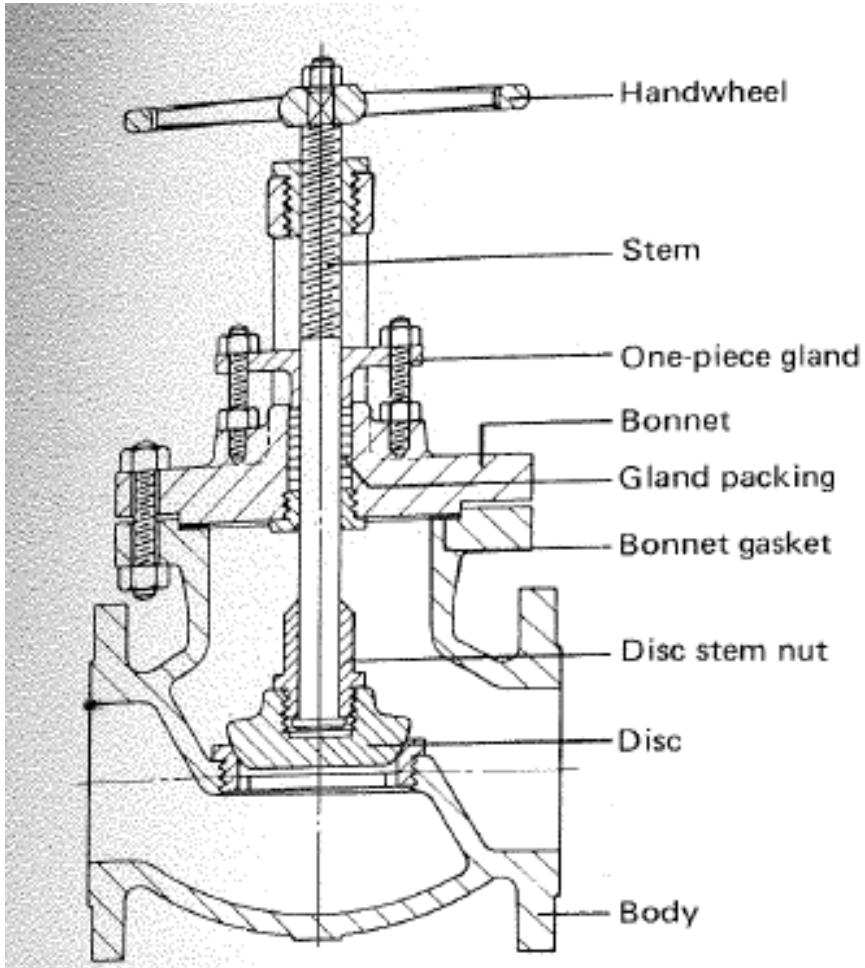
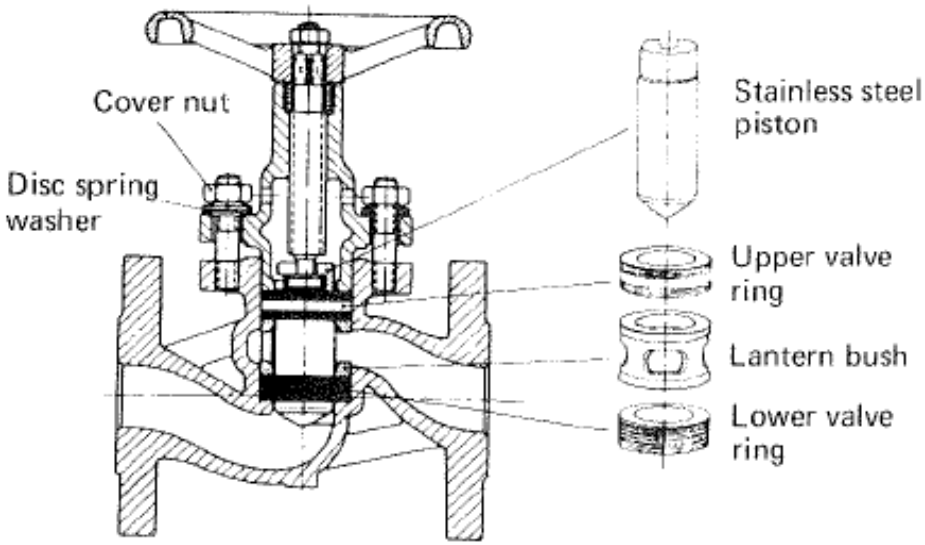


FIG. 7.29. Globe valve with outside screw and conventional disc

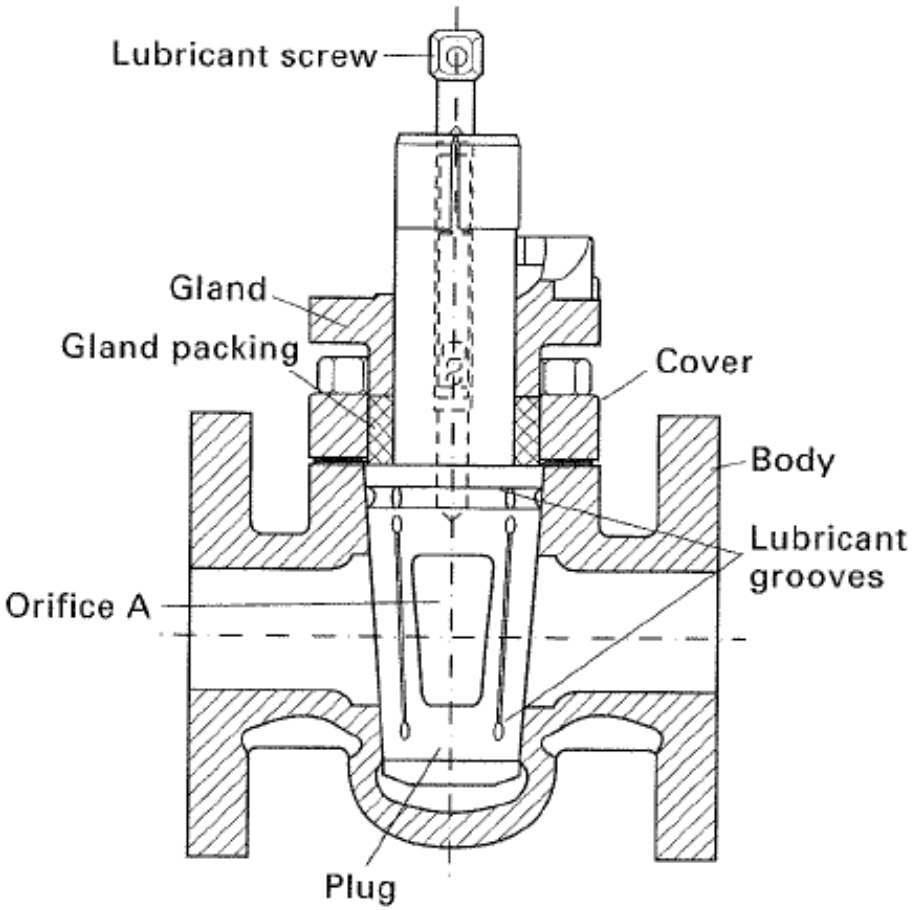


Piston valve

- **Piston valve:**
  - It is similar to globe valve except that the flow is controlled by a piston passing between two packing rings
  - The design is efficient for aseptic operations
  - The pressure drop is similar to globe valves
  - Steam is passed to sterilize the part open valve
- **Needle Valve:**
  - This is also similar to globe valve except that the disc is replaced with a tapered plug or a needle fitting into tapered valve seat
  - It has limited aseptic applications
  - The valve can give a fine controlled steam or liquid flow.
- **Plug valve:**
  - It provides a good flow control
  - In this valve there is a parallel or tapered plug sitting in a housing through which an orifice has been machined
  - This type of valve has a tendency to leak or sieze up, but can be controlled with the use of lubricants

- **Ball Valve:**
  - The valve element is a stainless steel valve through which an orifice is machined.
  - The ball is sealed between two wiping surfaces which wipe the surface and prevent the deposition of the mater at this point.
  - The orifice tin the ball is of the same diameter of the pipeline giving excellent flow path.
  - The valve is suitable for aseptic operations and can be operated at high temperatures & pressures.
- **Butterfly valve:**
  - It consists of a disc which rotates about a shaft in a housing. The disc closes against the seal to stop flow of the liquid
  - It is used in large diameter pipes operating under low pressure where absolute closure is not essential.
  - It is unsuitable for aseptic operations

Plug valve



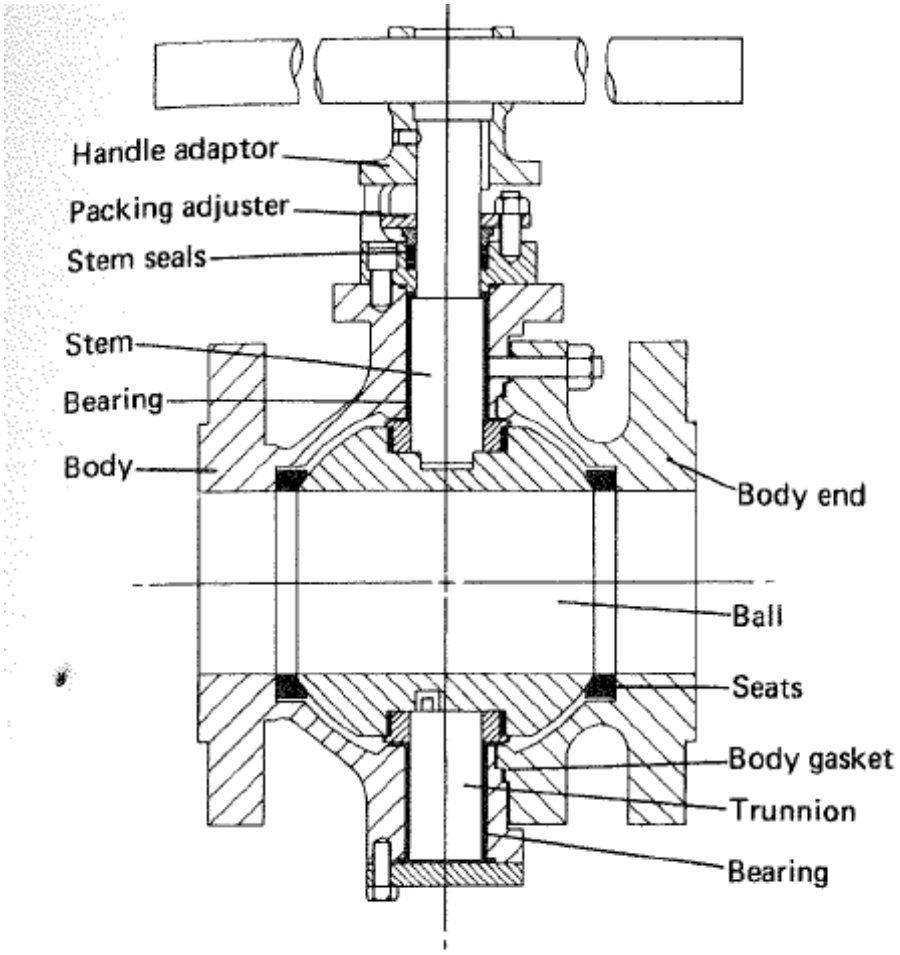


FIG. 7.33. Sectional view of end-entry ball valve (British Valve

- **Pinch Valves:**
  - In this valve, a flexible sleeve is closed by a pair of pinch bars.
  - The flow rate can be controlled 10 – 95% of rated flow capacity.
  - It is suitable for aseptic operations with fermentation broths as there are no dead spaces in the valve structure, and the closing mechanism is isolated from the contents of the piping.
- **Diaphragm Valve:**
  - The valve makes use of the flexible closure with or without a weir.
  - Suitable for aseptic operations provided the diaphragm material can withstand the sterilization operations
  - The valve can be sued for on/off flow regulation, and for steam services with in pressure limits
  - Diaphragm failure is the primary fault of the valve.
  - EPDM – Ethylene Propylene Diene Modified is the preferred material.
  - A diaphragm valve with a steam seal on the clean side is considered a potentially safer valve

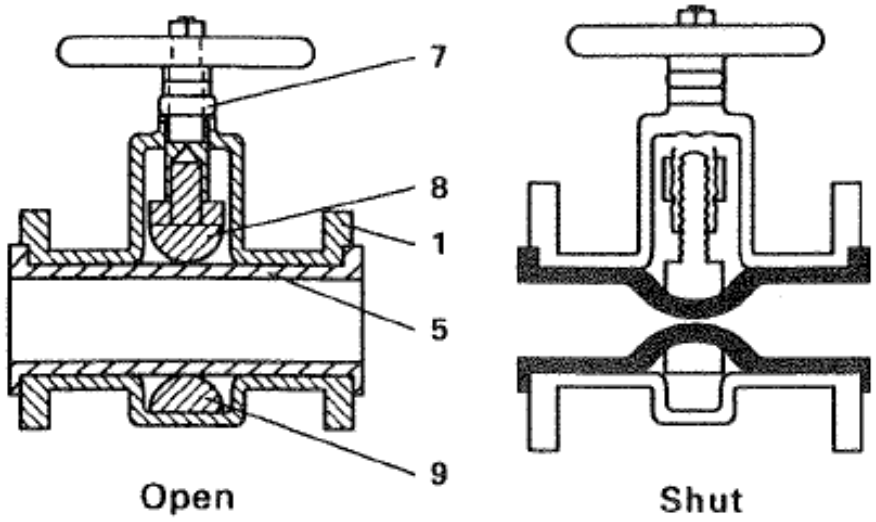


FIG. 7.35. Sectional view of pinch valve in open and shut position: (1) body; (5) flexible tube; (7) spindle; (8) top pinch bar; (9) lower pinch bar (British Valve Manufacturers Association, 1966; Kemplay, 1980).

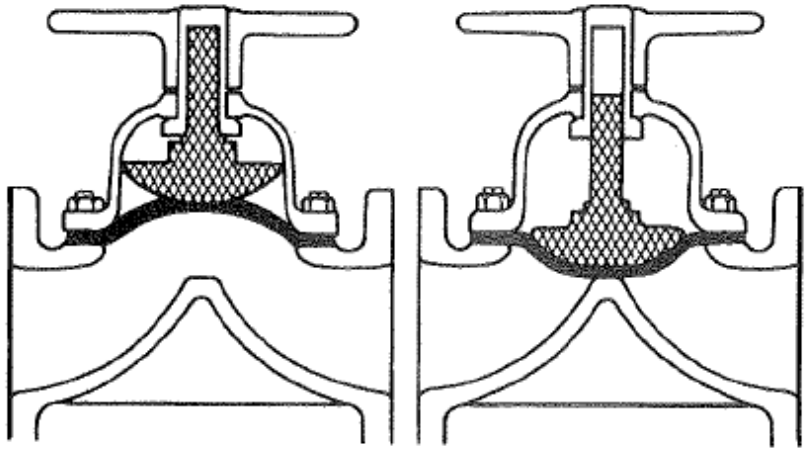


FIG. 7.36. Sectional views of weir-type diaphragm valves in open and closed positions (Thielsch, 1967).

Questions

- What is fermentation?
- With the help of a neat diagram show the basic features of a fermenter?
- What are the basic components of a fermentation process?
- List the major types of fermentations and briefly describe each one of them?
- What is the main function of a fermenter? List the main points in designing and constructing a fermenter?
- List utilities or service provisions for a typical fermentation plant?
- What is containment? Discuss how the containment levels are designated based on the process microorganism?
- With respect to fermentation, discuss:
  - material of construction
  - temperature control
- Write a note on agitation in a typical fermentation process?
- Write a note on aeration system(Sparger) in a typical fermentation process?
- Describe the major types of stirrer glands and bearings in a fermenter?
- Describe with the help of a diagram how air supplied to a fermenter is sterilized?
- Describe with the help of a diagram the working principle of a sampling port in a fermenter?
- Write a note on any two types of valves used in a fermenter?

- **TYPES OF FERMENTERS**
- The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product. Few of the bioreactor types are discussed below:
- **STIRRED TANK FERMENTER**
- Stirred tank reactor is the choice for many (more than 70%) though it is not the best. Stirred tank reactor's have the following functions:
  - homogenization,
  - suspension of solids,
  - dispersion of gas-liquid mixtures,
  - aeration of liquid and
  - heat exchange.
- The Stirred tank reactor is provided with a baffle and a rotating stirrer is attached either at the top or at the bottom of the bioreactor. The typical decision variables are: type, size, location and the number of impellers; sparger size and location. These determine the hydrodynamic pattern in the reactor, which in turn influence mixing times, mass and heat transfer coefficients, shear rates etc. The conventional fermentation is carried out in a batch mode. Since stirred tank reactors are commonly used for batch processes with slight modifications, these reactors are simple in design and easier to operate.

- Many of the industrial bioprocesses even today are being carried out in batch reactors though significant developments have taken place in the recent years in reactor design, the industry, still prefers stirred tanks because in case of contamination or any other substandard product formation the loss is minimal. The batch stirred tanks generally suffer due to their low volumetric productivity. The downtimes are quite large and unsteady state fermentation imposes stress to the microbial cultures due to nutritional limitations. The fed batch mode adopted in the recent years eliminates this limitation. The Stirred tank reactor's offer excellent mixing and reasonably good mass transfer rates. The cost of operation is lower and the reactors can be used with a variety of microbial species. Since stirred tank reactor is commonly used in chemical industry the mixing concepts are well developed. Stirred tank reactor with immobilized cells is not favored generally due to attrition problems; however by separating the zone of mixing from the zone of cell culturing one can successfully operate the system.

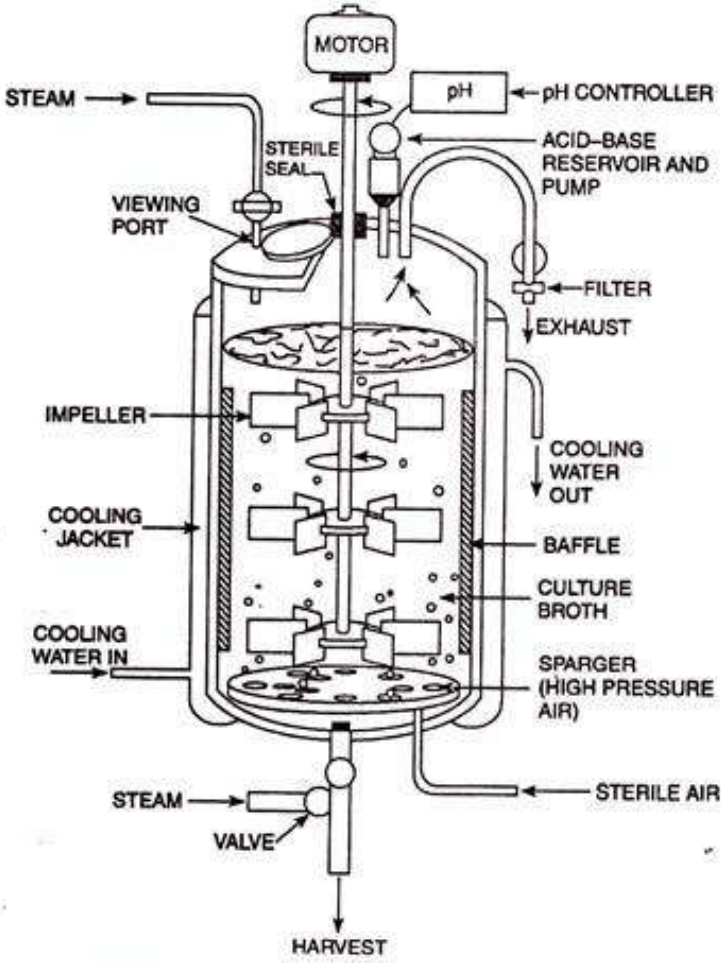


FIG. 39.1. An industrial aerobic fermentor (internal view)