MICROBIOLOGY COURSE MATERIAL

Semester - V

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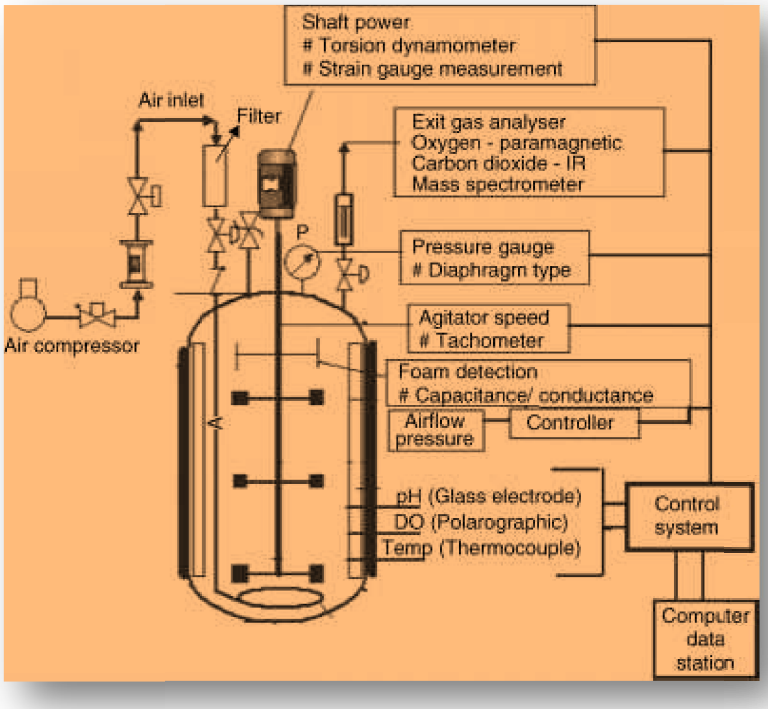
CC12: UNIT 3: (PART C): MEASUREMENT & CONTROL OF FERMENTATION PARAMETERS

B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE)
SEMESTER – V
CC12: UNIT – 3: PART- C
MEASUREMENT & CONTROL OF FERMENTATAION PARAMETERS



OVERALL VIEW OF A FERMENTOR

FACTOR 1: pH

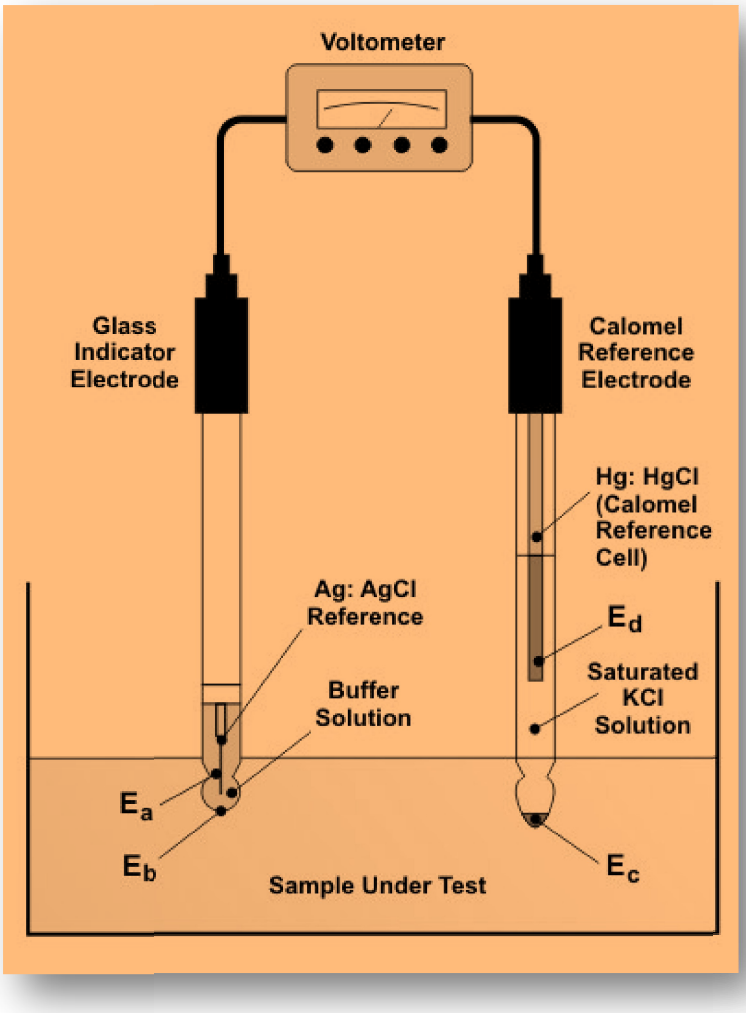
pH affects the shape of proteins. In the case of fermentation a collection of enzymes is responsible for the metabolic processes that occur. An enzyme is a protein which performs a metabolic process. For example sucrase is an enzyme which breaks sucrose down into fructose and glucose. They are kind of organic catalysts. Proteins are made of amino acids strung together in a long line. The amino acids bond up into a long polymer. Then cross linking occurs between the R groups on the amino acids. This makes the unique shape of the protein, because the cross-linking turns the protein into a 3-D shape.

Amino acids may be acidic amino acids, or basic amino acids, due to the R group on the amino acids. If the pH is increased, this affects the shape of proteins, by disrupting the bonds in the protein. In the case of fermentation, the rate increases when it gets more acidic i.e when the pH is lower. This is because the organisms (such as yeast) producing the enzymes to ferment glucose, have adapted to acidic conditions. This means that through natural selection the enzymes today have been selected because they work best in acidic conditions, which bend the protein into the correct shape to allow fermentation to occur. pH measurements can be an early sign of complications in the fermentation process, indicating the presence of undesirable acid producing bacteria or autolysis, the process of yeast cells dying and fragmenting.

❖ pH Sensor – Functional Principle:

The main purpose of a pH-meter is to ensure the pH level is maintained during fermentation, which allows the optimal catalytic conversion of sugar to alcohol. A classic pH-meter measures the acidity or alkalinity of a liquid. The pH meter involves the application of glass electrodes. Any pH-meter will need to be calibrated before being used to test for the acidity/alkalinity of a test solution and requires regular calibration so that the glass electrode generates an accurate pH reading. A standard calibration process should be carried out with two buffer solutions that cover a spectrum of pH values, usually between a pH value of 4 and 10. It is important to set the pH-meter to two control pH values that correspond to the two pH buffer values. The pH-meter is also adjusted to a control value for temperature. By calibrating the pH-meter for all three control values, the meter can achieve linear accuracy.

A standard pH-meter involves glass electrodes and reference electrodes (such as Ag/AgCl [silver/silver chloride]) that are important for measuring the pH of a test liquid. Electrical circuits containing a glass electrode, a reference electrode, and an instrument to measure the electrical potential between opposing electrical fields are the basic units to a pH-meter.



Diagrammatic View of a pH-meter inside a Fermentor

The main purpose of the glass electrode is vital to measuring the pH of a solution and carries an electrical current via a wire submerged in the test liquid. The wire and liquid are enclosed by a thin glass tube visible in the above figure. During a standard testing procedure, this electrode is exposed to an acidic solution that forces the positive ions from the acid solution to bind to the glass electrode. Whilst submerged, the electrodes need to maintain a neutral solution and so the electrons from the inner surface of the electrode move to the outer solution, but this will change the electrical potential of the testing solution. The purpose of the reference electrode is to

carry the electrical current to a meter that can measure the difference in electrical potential.

When brewing wine, it is important to measure total acidity. A graduated syringe is normally used to inject dilute sodium hydroxide (a neutralizer) to the wine solution together with phenolphthalein. The indicator used changes the wine to a pink color to ensure that the solution is no longer acidic. The problem with red wine is the dark color, which can obscure the color change and make it difficult to determine the complete neutralization of the wine solution. This is where the pH-meter becomes useful and this device can work to provide a neutral endpoint on a digital monitor.
There are currently a number of pH-meters on the market. Ocean Optics provides two types of pH sensors (In Situ Transmissive pH Sensors and Non-Intrusive Reflective pH Sensors) that deliver an accurate response time, are of low maintenance, and have a precise reading of the pH content.

❖ In Situ Transmissive pH Sensors:

This type of pH sensor uses a sol-gel solution combined with a colorimetric pH indicator dye that can reflect light through a read fiber, which will then provide an estimation of color change to a sample solution at a certain wavelength. pH sensors are typically susceptible to changes in salinity, though the In Situ Transmissive pH Sensor is built to avoid this problem. This type of pH sensor works well with organic solvents including acetone, alcohols, and aromatic compounds and thus would be of benefit to the beverage brewing industry.

❖ Non-Intrusive Reflective pH Sensors

These sensors are novel and have evolved pH sensor technology. The sensor uses an electroformed mesh material which adds a coating of metal on a non-metallic surface of the pH sensor; this creates a reflective ion permeable membrane allowing for pH

measurement through a clear wall to a cavity containing reflective probes. Again, this type of sensor is applicable to the food and beverage processing industry.

FACTOR 2: TEMPERATURE

We all know it's true, because it's printed on the side of yeast packages — fermentation temperatures matter. Some yeast works better in warm, some in cold, but did you ever ponder what happens to your brew when temperatures fluctuate from warm to cold and back again? Understanding what happens during fermentation when temperatures fluctuate better helps the brewer determine what needs to be done. The quality of the beer and vitality of the yeast both need to be examined. The pitching temperature of wort depends on the yeast strain — some ale strains routinely start fermenting around 70 °F (21 °C) and others start much warmer. Fermentation is exothermic, which means it will create its own heat. Having the ability to cool the fermentation once it starts to take off is an imperative. The reality is if you aren't keeping your fermenters cool, there may be a limit to what you can expect from your brewing efforts. However, since yeast growth and fermentations are exothermic and therefore generate heat, the temperature within the fermenter can be as much as 8 °F (4 °C) higher than outside of the fermenter during the early days of fermentation. So beers that are fermenting in refrigerators set at 65 °F (18 °C) are most likely fermenting at about 72 °F (22 °C).

The only time external temperature fluctuations may legitimately be a factor is during the first 12 hours of fermentation. If temperatures do swing drastically in these initial hours, the fermentation may become sluggish and a good deal of your yeast may drop out of suspension. The only way this could be happening would be a major "environmental" change, like putting the fermenter in a very cold ice bath or refrigerator. This assumes that an adequate pitch of viable yeast was made and the wort was properly oxygenated.

There are a variety of methods of cooling down wort. If you just need to get the temperature down a few degrees, try applying cool towels around the carboy. If you

are looking for more of a shift, immerse about half the height of the carboy into an ice bath to cool it. Temperature will also affect the rate of growth of the yeast. If the temperature is too high, yeast growth will be too vigorous; producing an excessive demand on nutrients and your beer will be depleted in these nutrients. This can have an effect on subsequent conditioning. In addition to this, and probably more importantly, a higher growth temperature will change the yeasts metabolism, producing a different range of by-products, which can have a major effect on flavor. If the temperature is too cool, the fermentation will be sluggish, resulting in an opportunity for the growth of contaminants, such as wild yeast and bacteria.
In terms of fermentation, lager yeasts are routinely fermented between 40–54 °F (4–12 °C) while ale yeast is used from 55–70 °F (13–21 °C). The optimal fermenting temperatures of yeast vary considerably. Some ale yeasts for example, do not perform well below 65 °F (18 °C). The Narragansett (Chico) strain is notorious for this, as well as certain Belgian and wheat beer strains. Common symptoms of fermenting too cold are stuck fermentations, poor attenuation (high finishing gravities) and off-flavors especially diacetyl.

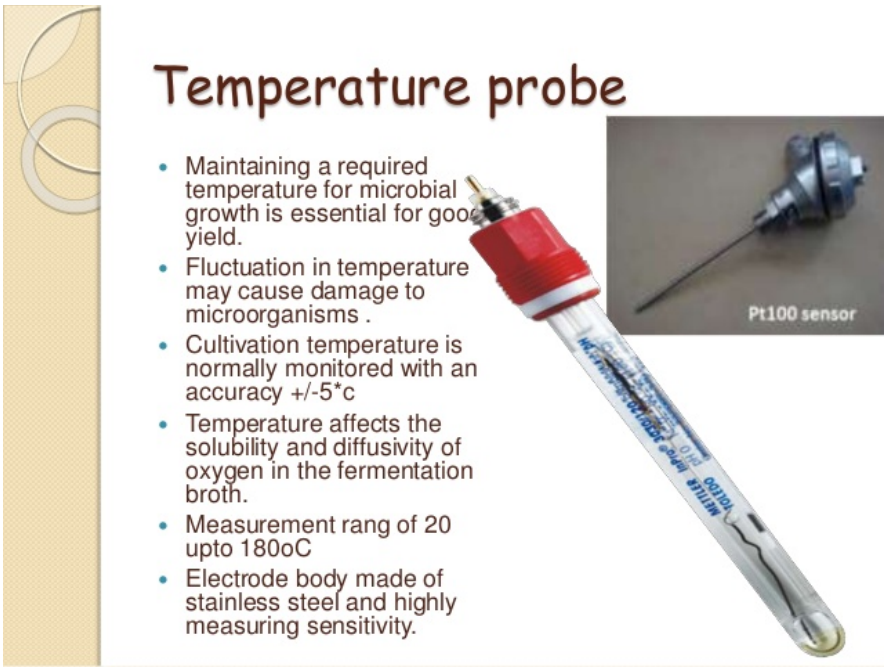
If you want to ferment cold, it may be necessary to acclimatize your starter to a lower temperature to prevent them from cold shocking. This can be done by slowly lowering the temperature of the starter the day before. Monitoring temperature and responding appropriately to shifts throughout the brew cycle, particularly during the fermentation period will make or break your beer. A typical bi-metal meat thermometer will suffice, but many floating and digital models are also available. Whatever thermometer you get, calibrate it to 32 °F (0 °C) degrees in 50/50 ice and water, and you're ready to go.

Yeast lives and dies according to the temperature, so be aware. Most strains of brewer's yeast can survive temperatures in excess of 110 °F (43 °C), but it's not a good idea to let your brew get anywhere close to that extreme. Unless your yeast strain is geared for warmer temperatures, pitching should be commenced around 70

°F (21 °C), with plenty of oxygen incorporated. Cold water fed garden hose and a wort chiller should get you close to this temperature.

A little clear thinking can lessen the fluctuating fermentation temperatures common in home brewing. Never under any circumstances leave fermenting beer where the sun can get to it. UV light can skunk a hoppy beer while it's still fermenting. A dark basement or closet that stays within a reasonable temperature range is a decent place. Home refrigerators always have some temperature fluctuation, but a small standing thermometer can give you a good idea what's going on in there. Any working refrigerator has less temperature fluctuation than the floor.

FERMOMETER

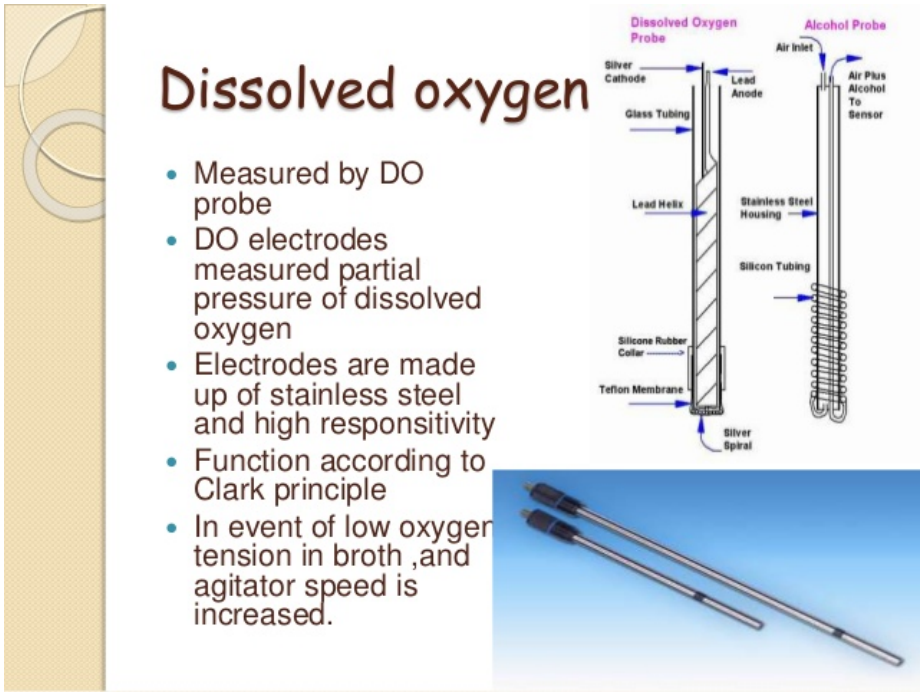


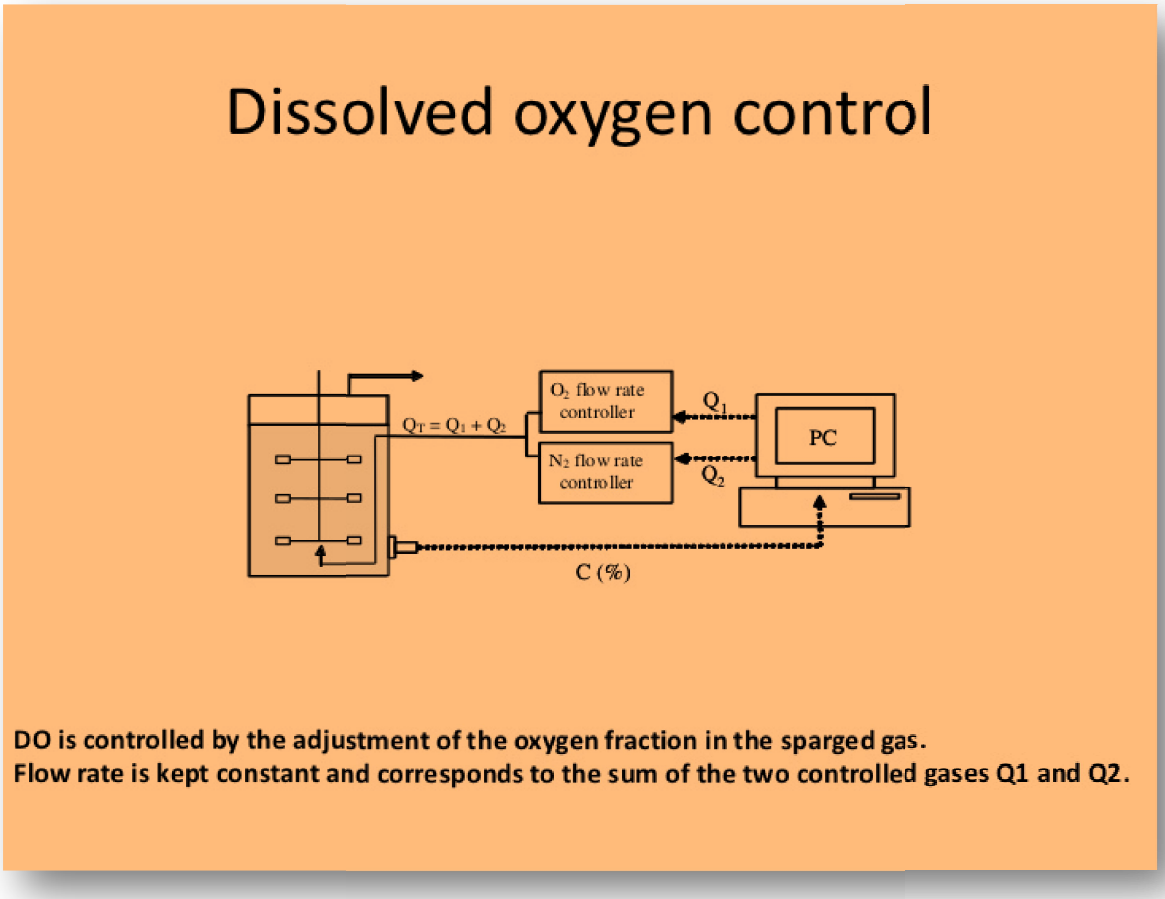
FACTOR-3: DISSOLVED OXYGEN

Fermentation is a crucial process in many industries, ranging from beer-brewing to wastewater treatment. The process of fermentation can be broken up into 2 main stages: Aerobic or Primary Fermentation, which takes place in the presence of oxygen, and Anaerobic or Secondary Fermentation, which takes place in the absence of oxygen. 70% of the fermentation process is aerobic and does not typically last for more than seven days. Using oxygen, the yeast or bacteria convert glucose into carbon dioxide, water and energy, where most of the energy is devoted to generation of new cells. During this period of time, the yeast can multiply up to 100 – 200 folds. Control of dissolved oxygen is crucial at this stage as it determines the successful growth of the culture. Although adequate oxygen must be injected to ensure sufficient bacterial growth, when in excess, it can lead to a reduction in alcohol producing activities and over-production of vicinal di-ketones—compounds that produce undesirable tastes. The remaining 30% of fermentation takes place in the absence of oxygen and occurs over a period of 2 to 3 weeks. Without oxygen, bacteria cannot respire aerobically to produce energy. Growth ceases and the rate of activities slows down. The bacteria switch to anaerobic respiration, when glucose is broken down chemically with enzymes to produce energy. Alcohol and carbon dioxide are produced as a side product in the process of anaerobic respiration. In comparison, anaerobic respiration produces significantly less energy than aerobic respiration. However, the amount of energy generated is still sufficient to sustain the bacteria's life processes. Apart from oxygen, temperature control is also critical throughout the process of fermentation. It casts a strong influence on the product's taste by causing an alteration to the ester, production of higher alcohol and acetaldehyde level, as well as increase in amino acid uptake. For reliable control of your dissolved oxygen levels and temperature, the alpha DO2000 pg features the amperometric mode of measurement that provides exceptional accurate readings, even at negligible flow rates. With a temperature range of up to 125°C, the controller is ideal even for a wide range of fermentation applications, from steam sterilization to antibiotics production.

Dissolved oxygen measurement is essential in fermentation and cell culture for ensuring conditions remaining optimal for cells. As explained, low dissolved oxygen levels in fermenters/bioreactors can impact growth rate, nutrients uptake, cellular morphology and metabolite synthesis; leading to reduced yield and lower end-product quality. High levels cause the formation of reactive oxygen species that can oxidize components in the medium and result in cell mutations. Further, on large fermenters that require powerful compressors to inject air, excessive sparging is a costly waste of energy. Maintaining dissolved oxygen in the required range is therefore critical for process optimization.

Accurate oxygen control is only possible if measurements from dissolved oxygen sensors installed in fermenters/bioreactors are reliable. Air bubbles that collect or cross over a sensor's measurement tip create noise on the sensor signal. Erroneous measurement peaks or troughs can lead to inappropriate adjustments to sparging or supplement feeding, compounding issues around fermentation/cell culture control strategies.





FACTOR-4: FOAMING

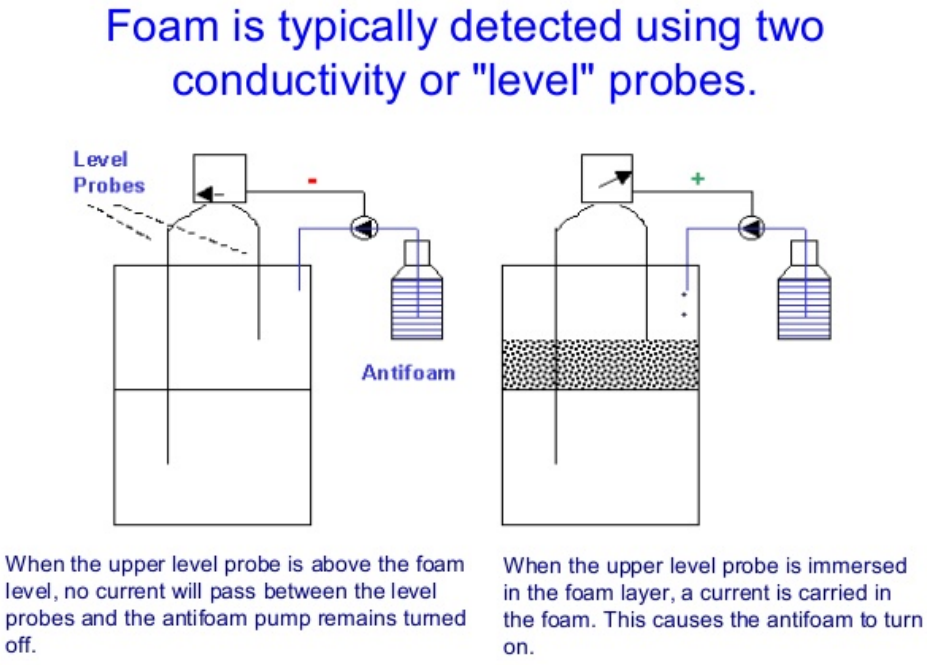
Foam in fermentation processes is defined as the entrapment of many air bubbles or other gases in culture medium or fermentation broth. A general definition of foam in fermentation process, determines foam to occur when gas holdup in a gas-liquid dispersion is greater than 60%. Two main types of foam are common in fermentations which are classified as unstable foams, which are short-lived, transitory, containing a wide range of bubble sizes and stable foams, which are usually smaller and uniform in size, long-lasting, rigid. Fermentation is often accompanied by foam formation because of the high foaming tendency of solutions containing biomaterials such as proteins. Foam never occurs in pure liquids even upon gas introduction and stirring due to the lack of surface active components. Conditions that affect the degree of foaming during fermentation include gas introduction, medium composition, cell

growth, metabolite formation, surface-active substance formation and indirectly, vessel geometry. The fermentation processes that produce excessive foam are of mainly cell cultures and aerobic fermentations which depend on the aeration. A little amount of foam doesn't cause any problem but in excess leads to problems while handling fermentation process. Excessive foam formation in fermentation process remains as a major technological challenge and require large attention and investigation. The foam in a fermentor moves upwards because of its low density compared to the liquid medium. If produced in excess, the foam touches the lid of fermentor and tries to come out through the exhaust outlet filter. In this case the wet and sticky media or the subsequent fouling of microorganisms can block the exhaust filter and cause the pressure to rise drastically within the fermentor. High pressures in the vessel can lead to slow cultivations or reduced productivity. If the filters are completely blocked, the elevated pressure in the vessel can cause the filters either to burst or trigger the opening of high pressure valve of the fermentor and discharge of the fermentor content into the area surrounding the fermentor, which can represent potential health hazard and certainly results in a large cleaning task. Sometimes foam builds up so rapidly that within a few seconds the entire liquid contents turn to foam and may overflow before any remedial action can be taken specifically in an aerobic fermentation. The presence of small bubbles from foam also can interfere with electrode sensors and cause false readings. Microorganisms trapped in foam experience oxygen and nutrient limitations. Removal of cells from medium due to foam formation can cause autolysis which releases microbial proteins that enhance foam stability. In general a process can run quite effectively with a certain amount of foam but there are certain cases where there should not be any foam present in the fermentation process. It is very important that foam should be detected and controlled to avoid such difficulties in fermentation.

❖ Detection of Foam:

Manual observation of foam by the operator is the basic method of foam detection. In order to avoid the difficulties with the manual observation, foam detection sensors

were developed that are inserted into the fermentor at a height. There are several types of foam sensors to detect the presence and in some cases, depth of foam. Conductive probes based on conduction of electrical charge i.e., DC voltage between the probe and vessel by ions present in the liquid are sensitive to biofilm which causes a permanent short circuit due to the collection of material across the probe's insulation. Capacitance probes have less biofilm interference than conductive probes since the entire capacitance probe is covered with insulation. Capacitance is sensed as the foam becomes dielectric. Both conductance and capacitance foam sensors are able to measure liquid level, possessing a user-adjustable detection level rather than any fixed point. Less common are admittance probes, which use high-frequency signals with wide band measuring bridges and amplifiers able to measure foam even with a biofilm present. Another alternative for foam detection is sonar level detection based on the presence of a foam layer in some cases interfering with sonar feedback. All antifoams tend to reduce the ability of gas to transfer into the liquid phase. For these reasons, antifoaming agents have to be added in a well controlled way and in small doses which is difficult to achieve without accurate sensing or detection.



❖ Foam controlling methods

In general foam controlling methods falls into three categories, physical, mechanical and chemical methods.

- Physical methods are designed to prevent foam by using ultrasound, thermal or electrical treatment.
- Mechanical methods are designed to break the foam by mechanical devices like a centrifugal foam breaker, external foam breaker and nozzle.
- Chemical methods are designed to break the foam by adding chemical agents usually called as either antifoam or defoamer.

Strictly speaking antifoam is added before the start of the fermentation and defoamer during the fermentation but the terms are frequently used indiscriminately. Since the physical methods were not recommended for fermentations, one has to depend on either mechanical or chemical methods. Both chemical and mechanical methods offer advantages and disadvantages for defoaming in fermentations. Both methods should be evaluated to determine which is most efficient and cost effective for specific fermentation process.

❖ Chemical method of foam controlling

It is recommended for most of the fermentations. Chemical methods of foam controlling are designed to break the foam by adding chemical agents usually called as either antifoam or defoamer into the fermentor in different ways. The general way of adding antifoam is by manual addition by the operator or by automatic method of addition using a tube and pump. The efficiency of chemical foam controlling methods depends on parameters like detection of foam, selection of suitable defoaming agent and methods of adding defoamer into the fermentor. Straight pipe entry of antifoam or defoamer into the fermentor is the earliest method reported. It was later observed that the antifoam distribution devices like spray distributor, diverter bars, wick

device, disc feeder and blow pot can improve defoaming efficiency over straight pipe entry in a fermentor. All automatic methods of adding antifoaming agents on demand are basically same as that a fermentor vessel is fitted with a sensor probe and when the foam reaches the circuit that was in connection with the sensor is closed allowing the antifoaming agent to enter the fermentor. Once the foam is broken then the circuit is opened allowing the solenoid valve to stop the entry of antifoaming agent. So it was designed to add the antifoam in shots to minimise the antifoam consumption. Despite of many advantages, chemical methods of foam controlling have few problems which are described below.

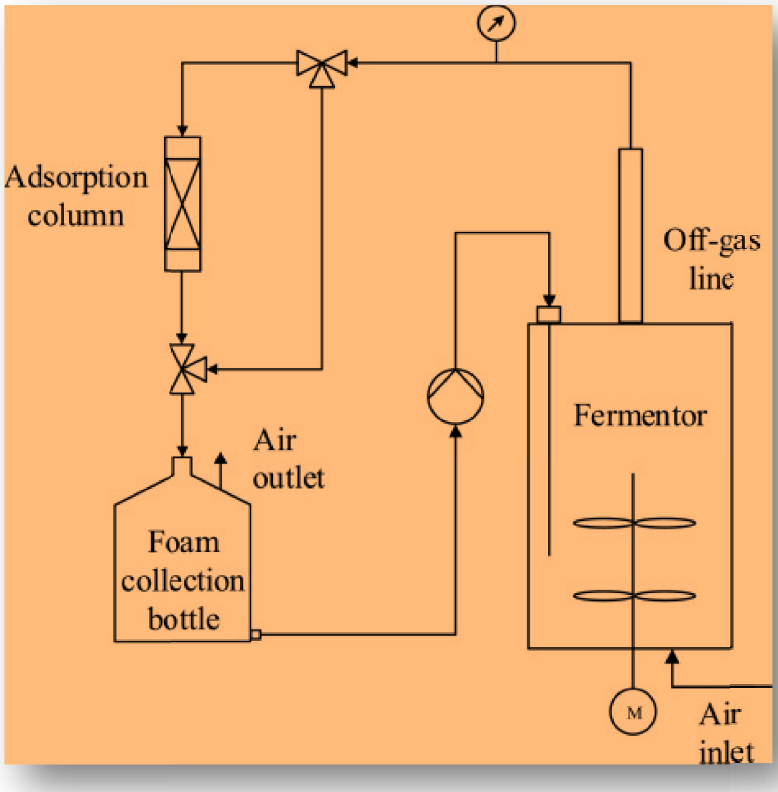
❖ Problems with the chemical methods of foam controlling

Foam sensing is a difficult task: Manual observation of foam by the operator is tedious because of the requirement of continuous monitoring for the presence of foam and especially in case of large fermentors. No operator can be expected to watch the fermentor continuously for hours or days. Often the foam may retain on the viewing glass which leads to false finding of foam by the operator and triggering of unnecessary addition of antifoam by the operator which further causes the disruption in the process.

The automatic foam detection methods which depend on the use of a probe are prone to fouling by condensation and surface growth as the foam leaves sticky deposits over the probe which obviously leads to the false readings and causes overdosing of antifoam. The overdosing of antifoam causes unfavorable conditions to the actively growing culture and may lead to poor yield of the product of interest. The foam sensing probe requires proper calibration otherwise leads to false detection of foam which triggers unnecessary addition of antifoam. The foam sensing probe may fail at any time during the fermentation thereby disrupting the process. So far these kinds of experiences have convinced that the sensing of foam cannot be done reliably. All

sensor probes are somewhat unreliable because they give spurious signals when they become fouled.

Defoamer addition is often a problem. The defoamer can be added into the fermentor through a pump and tubing which may sometimes leads to the operational problems like failure of the antifoam addition pump and damage of the antifoam supply tubing in the pump head by not supplying the required defoamer on demand and leads to the poor foam controlling. Ready to use antifoaming agents (for example: hyclone antifoam-pre sterilized, from Thermo Scientific) also have to be added to the fementor using a tube and pump which may cause operational troubles.



Mechanism showing Foam Detection in a Fermentor

- **FACTORS-5: AERATION**

The purpose of aeration in fermentation is to supply oxygen to and, at the same time, to remove carbon dioxide from microbial cells suspended in the culture broth. The

rate of aeration often controls the rates of cell growth and product formation. Mixing in the gas and liquid phases affects the aeration characteristics of a fermentor. Various types of aerobic fermentors could be classified into three major types:

(1) sparged mechanically stirred fermentor,

(2) bubble column fermentor, and

(3) loop fermentor.

In the sparged stirred fermentor, gas, usually air, is sparged into the broth, which is mechanically agitated. Fermentors of this type are still used most widely for various aerobic fermentations. The bubble column is a cylindrical vessel containing a liquid through which gas is bubbled; this can be operated continuously with either counter- or co-current flows of liquid or gas. In the loop fermentor, liquid is re-circulated by the difference of the average densities of the broth between the gassed and un-gassed sections or by means of a pump or fluid jet. All of these three major types and combinations thereof have various modifications. The main function of aeration is to supply enough oxygen to the microbes in submerge culture technique for proper metabolism, while agitation provides proper mixing of the nutrient so that each and every organisms get proper nutrients. Each fermentation process requires unique type of aeration and agitation system. The parts of the fermenter involved in aeration and agitation are:

A. The agitator (impeller).
B. The aeration system (sparger).

- **The agitator (impeller)-** The main aim of the agitator is to provide homogenous environment all over the fermenter. It is also used for mixing of different phases, oxygen and heat transport.

- **The aeration system (sparger)-** A sparger is a tool used for introducing air into the fermentation medium.

- **Three basic types of sparger:**
  1) The porous sparger,
  2) The orifice sparger (a perforated pipe) and
  3) The nozzle sparger (an open or partially closed pipe).

- **Porous Sparger-** The porous sparger is mainly used for laboratory scale non agitated fermenter. It is made up of sintered glass, ceramics or metal.

- **Orifice Sparger-** In small stirred fermenters the perforated pipes were arranged below the impeller in the form of crosses or rings (ring sparger), approximately three-quarters of the impeller diameter.

- **Nozzle Sparger-** Most modern mechanically stirred fermenter designs from laboratory to industrial scale have a single open or partially closed pipe as a sparger to provide the stream of air bubbles. Ideally the pipe should be positioned centrally below the impeller and as far away as possible from it to ensure that the impeller is not flooded by the air stream. The single-nozzle sparger causes a lower pressure loss than any other sparger and normally does not get blocked. .

- **Need For Aeration and Agitation**

The majorities of fermentation processes are aerobic and, therefore, require the provision of oxygen. If the stoichiometry of respiration is considered, then the oxidation of glucose may be represented as:

$$C_6H_{12}O_6 + 6O_2 = 6H_2O + 6CO_2$$

Thus, 192 grams of oxygen are required for the complete oxidation of 180 grams of glucose. However, both components must be in solution before they are available to a microorganism and oxygen is approximately 6000 times less soluble in water than is

glucose (a fermentation medium saturated with oxygen contains approximately 7.6 mg dm^{-3} of oxygen at 30°C). Thus, it is not possible to provide a microbial culture with all the oxygen it will need for the complete oxidation of the glucose (or any other carbon source) in one addition. Therefore, a microbial culture must be supplied with oxygen during growth at a rate sufficient to satisfy the organisms' demand. The aeration and agitation of the fermentation medium, provides necessary oxygen to the industrial fermentation process. However, the productivity of many fermentations is limited by oxygen availability and, therefore, it is important to consider the factors which affect a fermenter's efficiency in supplying microbial cells with oxygen.

❖ The Oxygen requirements of Industrial Fermentations

Although a consideration of the stoichiometry of respiration gives an appreciation of the problem of oxygen supply, it gives no indication of an organism's true oxygen demand as it does not take into account the carbon that is converted into biomass and products. It has been studied that a culture's demand for oxygen is very much dependent on the source of carbon in the medium. Thus, the more reduced the carbon source the greater will be the oxygen demand. However, it is inadequate to base the provision of oxygen for fermentation simply on an estimation of overall demand, because the metabolism of the culture is affected by the concentration of dissolved oxygen in the broth. It may be seen that the specific oxygen uptake rate increases with increase in the dissolved oxygen concentration up to a certain point (referred to as C_{crit}) above which no further increase in oxygen uptake rate occurs. Thus, maximum biomass production may be achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level. If the dissolved oxygen concentration were to fall below the critical level then the cells may be metabolically disturbed. However, it must be remembered that it is frequently

the objective of the fermentation technologist to produce a product of the micro-organism rather than the organism itself and that metabolic disturbance of the cell by oxygen starvation may be advantageous to the formation of certain products.

Equally, provision of a dissolved oxygen concentration greater than the critical level may have no influence on biomass production, but may stimulate product formation. Thus, the aeration conditions necessary for the optimum production of a product may be different from those favoring biomass productions. The oxygen demand of fermentation largely depends on the concentration of the biomass and its respiratory activity, which is related to the growth rate. By limiting the initial concentration of the medium, the biomass in the vessel may be kept at a reasonable level and by supplying some nutrient component as a feed, the rate of growth and hence the respiratory rate, may be controlled.

- **Oxygen Supply-** Oxygen is normally supplied to microbial culture in the form of air, this being the cheapest available source of the gas. The method for provision of a culture with a supply of air varies with the scale of the process:

1. **Laboratory scale**- Cultures may be aerated by means of the shake-flask method. Flask are shaken on a platform contained in a controlled environment chamber

2. **Pilot and Industrial Scale**- Air is provided to the cultures by specific types of fermenter (Bubble fermenter) represents the transfer of oxygen from air to the cell, during fermentation, as occurring in a number of steps:
   (i) The transfer of oxygen from an air bubble into solution.
   (ii) The transfer of the dissolved oxygen through the fermentation medium to the microbial cell.
   (iii) The uptake of the dissolved oxygen by the cell.

The rate of oxygen transfer from air bubble to the liquid phase may be given by the equation:

$$\mathbf{dC_L / dt = K_{La} (C^* - C_l)}$$

Where C_L is the concentration of dissolved oxygen in the fermentation broth (mmole dm^{-3}), t is time (hours), dC_L/dt is the change in oxygen concentration over a time period, i.e. the oxygen transfer rate (mmole O_2 dm^{-3} h^{-1}), K_L is the mass transfer coefficient (cm h^{-1}), a is the gas/liquid interface area per liquid volume (cm^2 cm^{-3}), C* is the saturated dissolved oxygen concentration (mmoles dm^{-3}).

K_L may be considered as the sum of the reciprocals of the resistances to the transfer of oxygen from gas to liquid and (C^*-C_L) may be considered as the 'driving force' across the resistances. It is extremely difficult to measure both K_L and 'a' in a fermentation and, therefore, the two terms are generally combined in the term K_{La}, the volumetric mass transfer coefficient, the units of which are reciprocal time (h^{-1}). The volumetric mass-transfer coefficient (K_La) is used as a measure of the aeration capacity of a fermenter. The aeration capacity of the system will be more if K_{La}, is higher. The K_{La} value will depend upon the design and operating conditions of the fermenter and will be affected by such variables as aeration rate, agitation rate and impeller design. These variables affect '**K_L**' by reducing the resistances to transfer and affect 'a' by changing the number, size and residence time of air bubbles.

Determination of KLa Values

1. **The sulphite oxidation technique**

$$Na_2SO_3 + 0.5O_2 = Na_2SO_4$$

The rate of reaction is such that as oxygen enters solution it is immediately consumed in the oxidation of sulphite, so that the sulphite oxidation rate is equivalent to the oxygen transfer rate.

2. **Gassing-out techniques**

a. **The Static Method of Gassing Out**

The technique was first described by Wise (1951) where the concentration of oxygen in the solution is decreased by passing nitrogen gas into the liquid. This will remove all the oxygen from the solution. The aeration and agitation of deoxygenated liquid increase the dissolved oxygen which is monitored using some form of dissolved oxygen probe. This technique has the advantage over the sulphite oxidation method in that it is very rapid (normally taking up 15 minutes) and may utilize the fermentation medium, to which may be added dead cells or mycelium at a concentration equal to that produced during the fermentation.

b. **The Dynamic Method of Gassing Out**

The procedure involves stopping the supply of air to the fermentation which results in a linear decline in the dissolved oxygen concentration due to the respiration of the culture. The aeration and agitation of deoxygenated liquid increase the dissolved oxygen which is monitored using some form of dissolved oxygen probe. The dynamic gassing out method has the advantage over the previous methods of determining the KLa during an actual fermentation and may be used to determine KLa values at different stages in the process. The technique is also rapid and only requires the use of a dissolved oxygen probe, of the membrane type.

C. **The oxygen-balance technique**

The oxygen balance technique is used for the determination of transportation of amount of oxygen into the fermentation medium in a given period of time. It is also used for the measurement of K_La of a fermenter.

- **The procedure involves measuring the following parameters:**

1) The amount of medium in the fermenter (dm^3)
2) The rate of flow of air (incoming and outgoing air), dm^3min^{-1}
3) The total pressure at inlet and outlet (atm)
4) The temperature of the gases of the inlet and outlet,(K)
5) The partial pressure of oxygen of the inlet and outlet

The oxygen balance technique appears to be the simplest method for the assessment of K_La and has the benefit of measuring aeration efficiency during fermentation. The sulphite oxidation and static gassing out techniques have the disadvantage of being carried out using either a salt solution or an un-inoculated, sterile fermentation medium.

- **The factors affecting K_La values in fermentation vessels are:**

1. The air flow rate employed
2. The degree of agitation
3. The rheology properties of the medium
4. The presence of antifoam agents

1. The effect of air-flow rate on K_La

The rate of air flow in fermentation media in agitated and non-agitated fermenter will be different.

- **Mechanically Agitated Reactors-** The air-flow rate of 0.5-1.5 volumes of air per volume of medium per minute is to be

maintained constant on scale-up. If the impeller is unable to disperse the incoming air then extremely low oxygen transfer rates may be achieved. Thus proper flow rate should be maintained by agitator.

- **Non-Mechanically Agitated Reactors-** Bubble columns and air-lift reactors are not mechanically agitated. Mixing and aeration is dependent on the air passage.

- **Bubble columns-** Bubble column reactor cannot be used for highly viscous medium. Pattern of gas bubbles in a bubble column reactor is dependent on the gas superficial velocity. Gas velocity should be 1-4 cm per second for uniform bubbles throughout medium which will provide proper mixing. If gas velocity is higher or lower than uniform bubbles will not be produced, thus when bubbles coalesce produces differences in fluid density which will disturb air flow rate.

- **Air lift reactors-** In this fermenter, medium circulation is also accomplished with bubble formation. K_{La} obtained in air-lift reactor will be less than bubble fermenter due to shorter contact time between bubble and medium.

2. **The degree of agitation**
   - Agitation is playing a vital role in the oxygen transfer rate in agitated fermenter.
   - Agitation increases the area available for oxygen transfer by dispersing air into the medium.
   - It increases the contact time for bubbles in the medium.
   - It prevents coalesces of air bubbles.
   - It decreases thickness of liquid film at gas-liquid interface.

3. **Medium Rheology (Medium Flow characteristics)**

Mostly the product of fermentation process is not interfering with medium flow rate or viscosity. But certain bacterial strain producing polysaccharide which can increase the viscosity and hence affect the medium rheology. Thus bacterial polysaccharide will decrease the oxygen transfer rate and bulk mixing.

4. **Antifoam agent**

Antifoam agents collapse the foam and thus increase the oxygen transfer rate of the fermentation medium. Thus K_La value decreases due to use of antifoam agent.

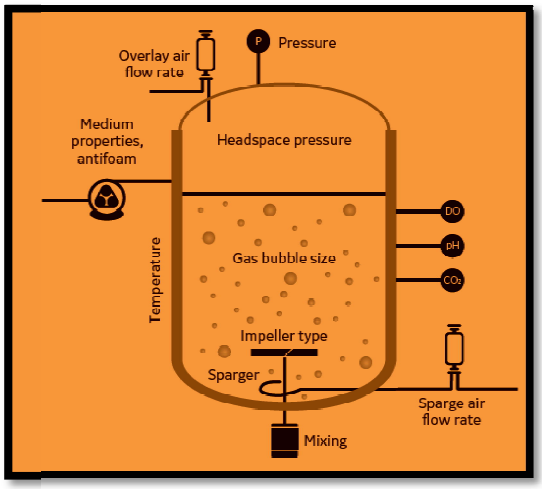


Diagram showing Mechanism of Foam Measurement and Control in a Fermenter