

Biochemical Engineering Lab, BT-311

PRACTICAL - 1

**Overview: UNDERSTANDING THE DIFFERENT COMPONENTS OF A
BIOREACTOR AND GENERAL OVERVIEW OF LABORATORY
INSTRUMENTS**

EQUIPMENT 1

Bioreactor

Introduction:

A Bioreactor is defined as a closed system used for growing microorganisms (bacteria, fungi, algae), plant cells and animal cells in a culture medium where the growth of the same can be enhanced in a sterile condition by controlling environmental conditions e.g., pH, Temperature, Dissolved oxygen etc.

Principle:

The fermentor apparatus is designed to contain all culture within the confines of the bioreactor vessel, and to allow sampling without exposing the contents of the tank to the open atmosphere which would allow any extraneous organisms to enter. In addition, pH, temperature and DO are all controlled at user-specified set-points, so that the production of by-products will not have adverse effects on the culture environment. The vessel is to be adequately stirred and aerated so as to prevent any stagnant or oxygen-poor zones.

Main components of a bioreactor:

1. Glass vessel (and static seal)
2. DO Probe
3. pH Probe
4. Temperature Probe
5. Sampling tube
6. Agitator
7. Impeller
8. Sparger
9. Motor to run the agitator
10. Condenser
11. Head plate with ports
12. Cooling water inlet
13. Cooling water outlet
14. Baffle

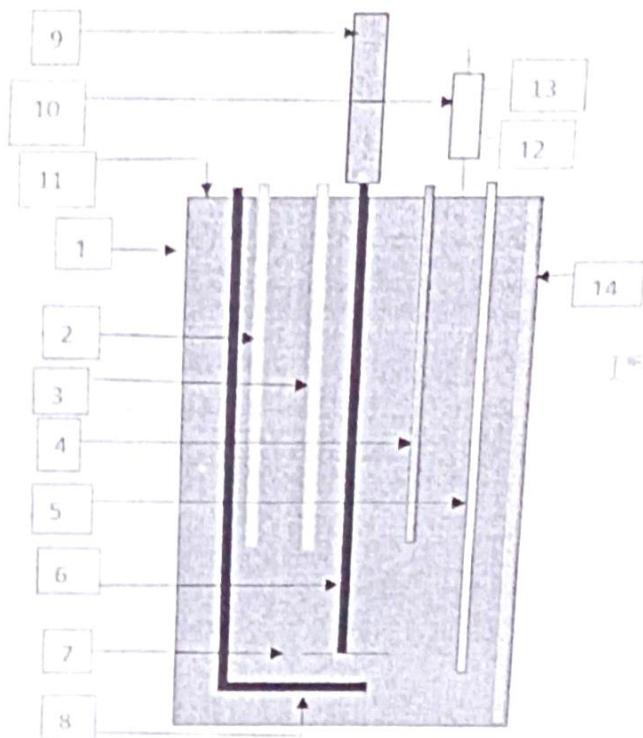


Fig.1 Schematic diagram of a typical Bioreactor

Glass vessel

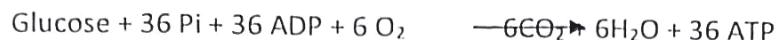
It is the main component of a bioreactor where an organism is grown and an aseptic culture medium, with all the probes like temperature, pH, DO etc, is maintained at a desired temp., pH, DO etc for the particular organism being grown.

Static Seal

Sealing between the top plate and vessel is an important criterion to maintain airtight condition and aseptic environment. Sealing is done between three types of surfaces like glass-glass, glass-metal and metal-metal. There are three types of sealing which includes gasket, lipseal and 'O' ring type sealing.

DO Probe

The concentration of dissolved oxygen in bioreactors is normally measured using a dissolved oxygen (DO) electrode. Oxygen has a very low solubility in water, only about 7 PPM at standard temperature and pressure. Hence, oxygen is usually the limiting nutrient for organism growth, unless a feed strategy is designed to limit the growth rate via a different nutrient of choice. Above a critical O₂ concentration, growth rate is independent of DO concentration. Oxygen limitations usually result in the production of by-products because of incomplete substrate oxidation to CO₂. Molecular O₂ consumed by most organisms via respiration can only be used as a terminal electron acceptor and therefore, must be converted to CO₂.



Air is fed to the vessel from an air compressor, through the DO controller and into the vessel through a tube with fine holes in it (the sparger). The mixing energy of the impeller causes the air bubbles to dissolve somewhat into the culture broth.

The DO probe must be calibrated prior to the fermentation run. Because DO probes are often unreliable, it is a good idea to calibrate the probe prior to autoclaving to ensure its proper function. Autoclaving the probe usually requires readjustment prior to inoculation.

pH Probe

The pH *i.e.*, concentration of the hydrogen ion in the media which is one of the crucial factors for the growth of the desired organism is measured by the use of a pH electrode.

All microorganisms that grow in culture are sensitive to the pH of the medium. The pH in the cytoplasm of the organism is normally a neutral value, since proteins depend on hydrogen bonding for their proper folding and function. Most, but not all culturable organisms grow best around neutral pH (between pH 5.5 to 8). High cell densities in the culture result in the rapid depletion of one or more nutrients, which often results in the production of organic acids: acetic acid, succinate, formate and lactate. Likewise, production of CO₂ results in the dissolution of CO₂ in the broth and the formation of bicarbonate. To maintain the pH in the vessel, base is added in response to a low offset from a pH set-point. The bases most often used for pH control are NH₄OH (which also contributes Nitrogen in a usable form to the bacteria), KOH, NaOH, or bicarbonate.

Like the DO probe, the pH probe should be calibrated both before and after autoclaving.

Temperature Probe

The temperature probe measures the prevailing temperature of the culture medium. This temperature probe is inserted into the culture vessel such that the temperature in the center of the vessel is measured. Temperature may also be an important parameter for optimization of the production of a desired product. Heat production is a direct result of metabolism, and in particular, of respiration. Hence, while it may be necessary to add heat to the bioreactor just after inoculation, once a critical culture density is reached, it is usually necessary to cool the reactor to maintain the optimum temperature for the organisms. Temperature control is easily achieved in most bioreactors with a

cooling water jacket or internal cooling coils, as is done in very large industrial bioreactors. A temperature probe (thermocouple) is inserted into the culture vessel such that the temperature in the center of the vessel is measured. The probe is connected to a control box which adjusts a heating (or cooling element) until there is no offset from the set-point.

Sampling tube

Through this tube, using a syringe of generally 5ml or 10ml size, culture of the organism taken for its further analysis w.r.t. its growth stage, product formation (if any) etc.

Agitator (for culture mixing)

In shake flasks cultures are mixed through shaking, whereas in a bioreactor cultures are stirred with an impeller. The impeller is mounted on a rotor shaft which is in turn connected to a motor. The agitator is required to achieve a number of mixing objectives like bulk fluid and gas phase mixing, air dispersion, oxygen transfer, heat transfer, suspension of solid particles and maintain homogenous environment throughout the vessel contents.

Agitators are classified as disc turbines, vaned discs, open turbines of variable pitch and marine propellers. The disc turbine consists of a disc with series of rectangular vanes set in a vertical plane around the circumference. The vaned disc has a series of rectangular vane.

Sparger (for aeration)

The oxygen demand of aerobic microbial cultures is high. Bioreactor system needs to be supplying enough air or oxygen to maintain DO at desired levels. In this case, fine bubble aerators should be used. Large bubbles will have less surface area than small bubbles which will facilitate oxygen transfer to a great extent. Bubbles of smaller size with maximized surface area, take more time to reach the surface, increasing the residence time of each bubble in the water, allowing a better oxygen transfer rate. To maximize the dissolved oxygen concentration in the microbial cultures air/oxygen are usually introduced to the culture medium through submerged gassing with the help of a sparger.

Three basic type of sparger being used and they are classified as porous sparger, orifice sparger and nozzle sparger. Porous sparger has been primarily used on lab-scale bioreactors where there is no greater mixing is required. The throughput of air from this sparger is low due to pressure drop across the sparger and there is also problem of fine holes becoming blocked by growth of the microbial culture. Orifice sparger is a perforated pipe kept below the impeller in the form of crosses or rings. Nozzle sparger consists of a single opening to provide stream of air bubbles. Ideally this sparger is placed centrally below the impeller and as far away as possible from it to ensure that the impeller is not flooded by the air stream.

Condenser

In the exhaust line a condenser is connected to remove the moisture from exhaust gas. As moisture containing gas will damage exhaust gas analyzer it is important to remove moisture.

Head plate with ports

Head plate is usually constructed with stainless steel and provides provision for various ports to install sensors for monitoring and control of fermentation. Most commonly used sensors in bioreactor are DO probe, pH probe and temperature probe. Cells receive oxygen in the form of dissolved oxygen through sparger. As DO is critical for an organism to survive continuous real-time monitoring is required. pH probe measures the acidity or alkalinity of the culture broth by measuring H⁺ ion concentration.

Baffles

Baffles are metal strips that prevent vortex formation around the walls of vessel. These metal strips are radially attached to the wall of vessel. Usually 4 baffles are present. There should be enough gap between wall and baffle to minimize microbial growth on the baffle and walls of the vessel.

The above mentioned are the major parts of a typical Bioreactor. However, depending upon various types of bioreactors, there could be more accessories which will serve to increase the efficiency of the system.

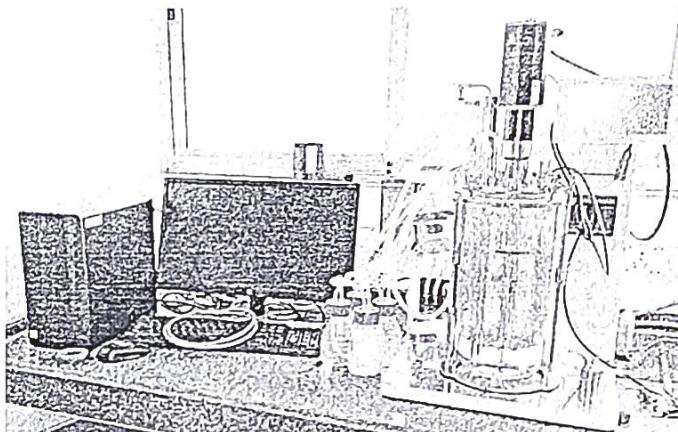


Fig. 2 Bioreactor set up

EQUIPMENT 2

Weighing balance/analytical balance

Introduction:

An analytical balance is a form of scale that measures mass to a high degree of precision. The readability range of a typical laboratory analytical balance lies between 0.1 mg to 220 g. However, the readability range may vary depending on the instrument model and brand.

Principle:

An analytical balance consists of a measuring pan, which is placed inside a transparent enclosure called the draft shield, so as to prevent the air currents in the room from affecting the balance's operation. Analytical balances work on the principle of "magnetic force restoration", which means that the force exerted by the object being weighed is lifted by an electromagnet. A detector measures the current required to oppose the downward motion of the weight in the magnetic field, and displays the weight of the sample.

Uses:

1. To weigh small amounts of chemicals
2. To weigh dry cell biomass



Fig.3: Analytical Balance

EQUIPMENT 3

Refrigerated Centrifuge

Introduction:

Centrifuge is used to separate materials of different densities, especially when the difference in their densities is so small that they cannot be separated by gravitational force. In this equipment temperature can be maintained from - 20 °C to + 40 °C.

Principle:

It uses centrifugal force, which causes the denser particles to settle to the bottom, and the low-density particles to rise to the top. A centrifuge typically has three basic parts: a rotor, a drive shaft and a motor. The rotor holds the tubes containing the liquids to be centrifuged. Rotor is mounted on the drive shaft, which connects to the motor. Motor provides power to run the rotor. Rotor comes in different sizes and can be chosen based on the volume of the tubes containing the sample to be centrifuged. The centrifuge allows the user to set the speed, time duration and temperature for centrifugation. The speed of the centrifuge can be set in terms of "revolutions per minute (RPM)" or "relative centrifugal force (RCF) or the g-number". The maximum RPM achievable in a typical laboratory centrifuge is 15,000 RPM. Depending upon the maximum RCF limit of the instrument, there are "high-speed centrifuges" and "ultracentrifuges", which can attain very high RCF values of up to 50,000 and 5 lacks, respectively.

Uses:

- I. Used for separation of cells from liquid medium
- II. To separate two immiscible liquids whose density difference is small.

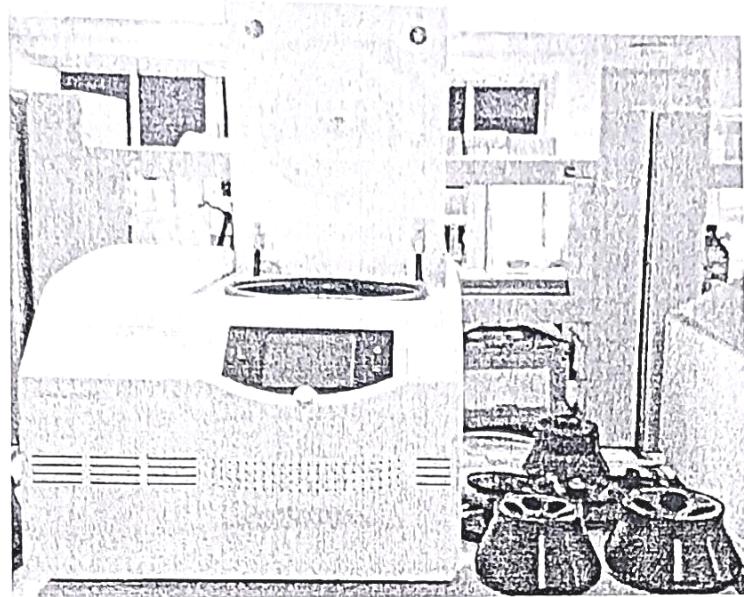


Fig.4: Refrigerated Centrifuge

EQUIPMENT 4

UV-visible spectrophotometer

Introduction:

UV-Vis Spectroscopy (or Spectrophotometry) is a quantitative technique used to measure how much a chemical substance absorbs light. This is done by measuring the intensity of light that passes through a sample with respect to the intensity of light through a reference sample or blank. This technique can be used for multiple sample types including liquids, solids, thin-films and glass. When incident light strikes matter it can either be absorbed, reflected, or transmitted. The absorbance of radiation in the UV-Vis range causes atomic excitation, which refers to the transition of molecules from a low-energy ground state to an excited state.

UV-Vis spectrophotometers are therefore able to determine the concentration of specific analytes in a microvolume by controlling the analysis wavelengths and the pathlength. It uses light in the visible and adjacent (near-UV and near-infrared (NIR)) ranges.

UV/Vis spectrophotometer is used in the quantitative determination of concentrations of the absorber in the solutions of transition metal ions and highly conjugated organic compounds.

Principle:

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. Spectroscopy is based on the interaction between light and matter. When the matter absorbs the light, it undergoes excitation and de-excitation, resulting in the production of a spectrum.

When matter absorbs ultraviolet radiation, the electrons present in it undergo excitation. This causes them to jump from a ground state (an energy state with a relatively small amount of energy associated

with it) to an excited state (an energy state with a relatively large amount of energy associated with it). It is important to note that the difference in the energies of the ground state and the excited state of the electron is always equal to the amount of ultraviolet radiation or visible radiation absorbed by it.

The statement of the Beer-Lambert law can be written as follows: When a beam of monochromatic light is made incident on a solution that contains a substance that absorbs the monochromatic light, the rate at which the intensity of the beam decreases along the thickness of the solution is directly proportional to the concentration of the absorbing substance in the solution and is also directly proportional to the intensity of the incident monochromatic radiation.

As per the Beer-Lambert law, the greater the number of absorbing molecules (that have the ability to absorb light of a specific wavelength), the greater the extent of absorption of the radiation.

The Beer-Lambert law states that: $A = \epsilon L c$

where,

- A is the amount of light absorbed for a particular wavelength by the sample
- ϵ is the molar extinction coefficient
- L is the distance covered by the light through the solution
- c is the concentration of the absorbing species

Following is an equation to solve for molar extinction coefficient:

$$\epsilon = A / (Lc)$$

But Beer-Lambert law is a combination of two different laws: Beer's law and Lambert law.

What is Beer's Law?

Beer's law was stated by August Beer which states that concentration and absorbance are directly proportional to each other.

What is Lambert Law?

Johann Heinrich Lambert stated Lambert law. It states that absorbance and path length are directly proportional.

Beer-Lambert Law Formula

$$I = I_0 e^{-\mu x}$$

Where,

- I is the intensity
- I_0 is the initial intensity
- x is the depth in meters
- μ is the coefficient of absorption

There are four basic components to a simple single beam UV/Vis spectrophotometer; a light source, a monochromator, a sample, and a detector.

Uses:

UV-Visible spectroscopy is widely used in the field of analytical chemistry, especially during the quantitative analysis of a specific analyte. For example, the quantitative analysis of transition metal ions can be achieved with the help of UV-Visible spectroscopy. Furthermore, the quantitative analysis of conjugated organic compounds can also be done with the help of UV-Visible spectroscopy. It can also be noted that this type of spectroscopy can also be carried out on solid and gaseous analytes in some conditions.

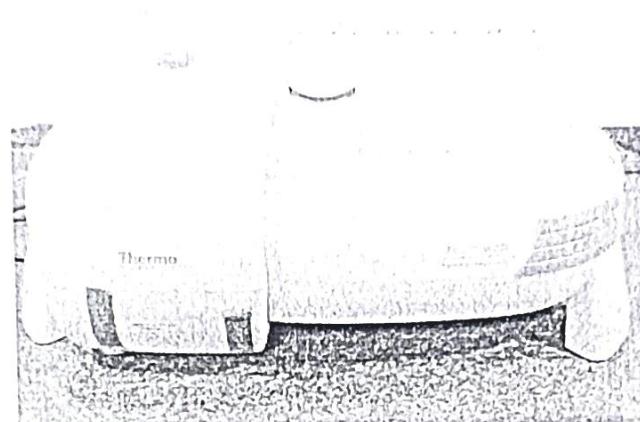


Fig.5: UV-Vis Spectrophotometer

EQUIPMENT 5

Horizontal Laminar Air flow hood

Introduction:

A laminar flow cabinet is defined as enclosed workbench which is used to create a contamination free work environment through installed HEPA (High Efficiency Particulate Air) filters that capture all the particles entering the cabinet. A laminar flow hood is used for work with substances which are not hazardous for the personnel health.

A laminar flow unit has an industry-wide usage and can be applied in quite a lot of industries such as medical, research, pharmacy, educational, and also in electronics, optics, micromechanics, plastic industries, etc. as all these need be carried out in a clean and sterile environment. A laminar hood is used for work with substances which are not hazardous for the personal health, as it does not provide personal health protection. The best usage of a clean bench is for working on certain specialized experiments in the labs that require a clean environment to design products that are nontoxic. It creates clean air for an individual environment inside a cabinet. It can also be tailor-made under the specific requirements of the laboratory.

Principle:

- The principle of laminar flow cabinet is based on the laminar flow of air through the cabinet.
- The device works by the use of inward flow of air through one or more HEPA filters to create a particulate-free environment.
- The air is taken through a filtration system and then exhausted across the work surface as a part of the laminar flow of the air.
- The air first passes through the filter pad or pre-filter that allows a streamline flow of air into the cabinet.
- Next, the blower or fan directs the air towards the HEPA filters.

- The HEPA filters then trap the bacteria, fungi and other particulate materials so that the air moving out of it is particulate-free air.
- Some of the effluent air then passes through perforation present at the bottom rear end of the cabinet, but most of it passes over the working bench while coming out of the cabinet towards the face of the operator.
- The laminar flow hood is enclosed on the sides, and constant positive air pressure is maintained to prevent the intrusion of contaminated external air into the cabinet.

The procedure to be followed while operating a laminar flow cabinet is given below:

1. Before running the laminar flow cabinet, the cabinet should be checked to ensure that nothing susceptible to UV rays is present inside the cabinet.
2. The glass shield of the hood is then closed, and the UV light is switched on. The UV light should be kept on for about 15-20 minutes to ensure the surface sterilization of the working bench.
3. The UV light is then switched off, and a time period of around 10 minutes is spared before the airflow is switched on.
4. About 5 minutes before the operation begins, the airflow is switched on (**always switch on the airflow before opening glass shield**).
5. The glass shield is then opened, and the fluorescent light is also switched on during the operation.
6. To ensure more protection, the working bench of the cabinet can be sterilized with other disinfectants like 70% ethanol.
7. Once the work is completed, the airflow and fluorescent lamp both are closed and the glass shield is also closed.

Precautions:

While operating the laminar airflow, the following things should be considered:

1. The laminar flow cabinet should be sterilized with the UV light before and after the operation.
2. The UV light and airflow should not be used at the same time.
3. No operations should be carried out when the UV light is switched on.
4. The operator should be dressed in lab coats and long gloves.
5. The working bench, glass shield, and other components present inside the cabinet should be sterilized before and after the completion of work with 70% ethanol.

Uses:

The following are some common uses of a laminar flow cabinet in the laboratory:

1. Laminar flow cabinets are used in laboratories for contamination sensitive processes like plant tissue culture.
2. Other laboratories processes like media plate preparation and culture of microorganisms can be performed inside the cabinet.
3. Operations of particle sensitive electronic devices are performed inside the cabinet.
4. In the pharmaceutical industries, drug preparation techniques are also performed inside the cabinet to ensure a particulate-free environment during the operations.
5. Laminar flow cabinets can be made tailor-made for some specialized works and can also be used for general lab techniques in the microbiological as well as the industrial sectors.

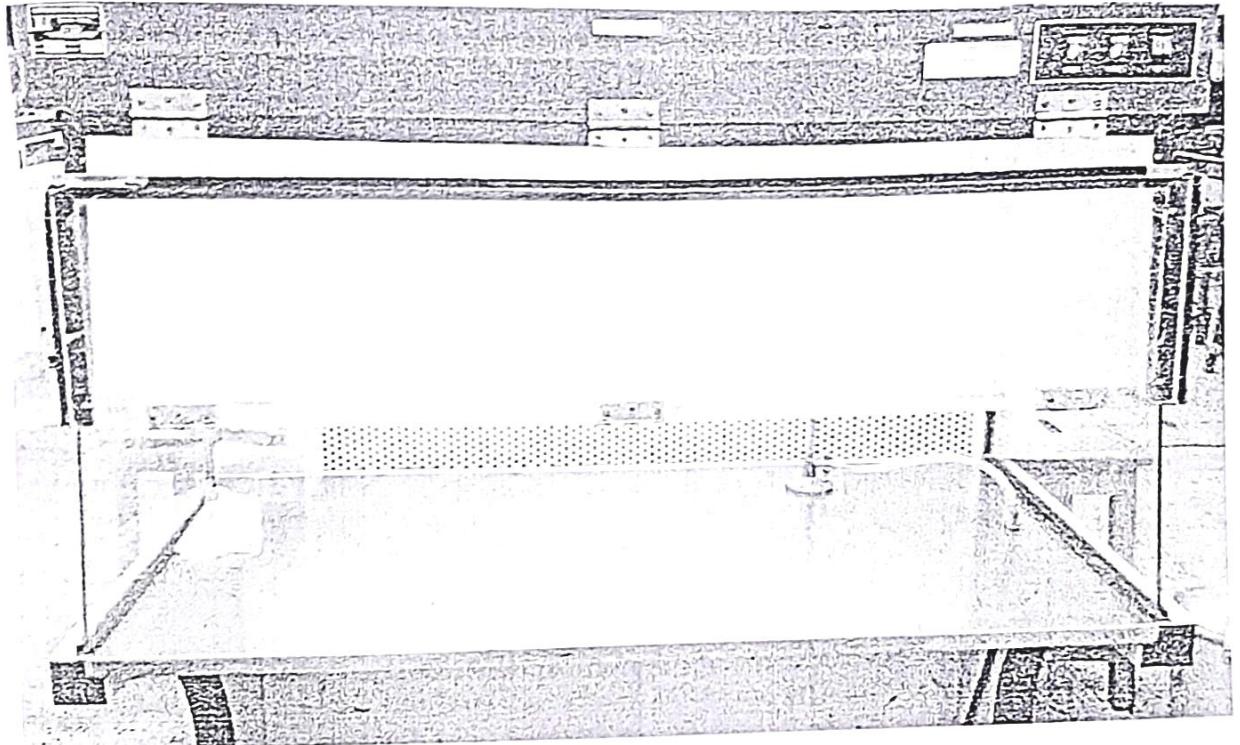


Fig.6: Horizontal Laminar Air-flow hood

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