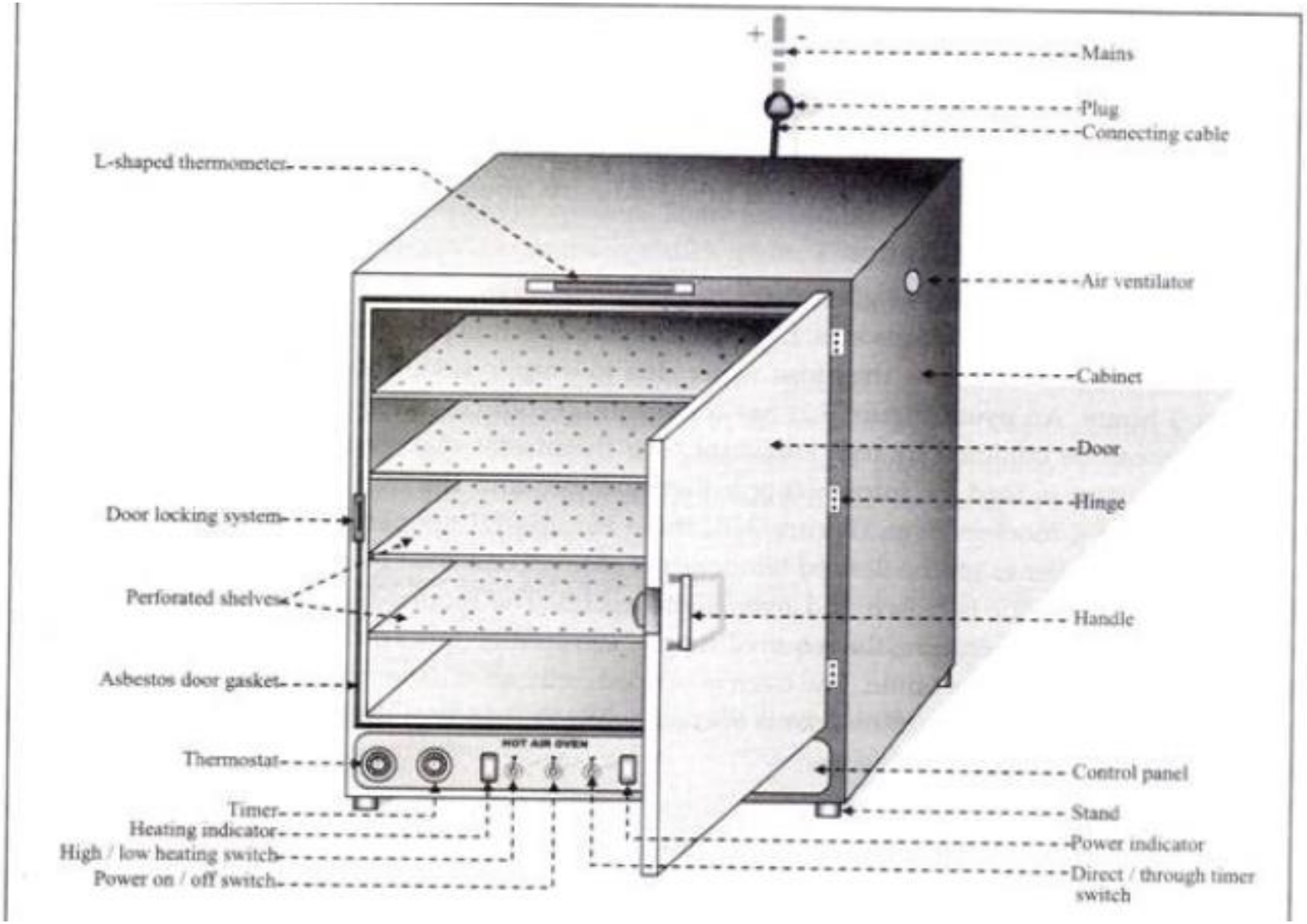
Industrial Microbiology

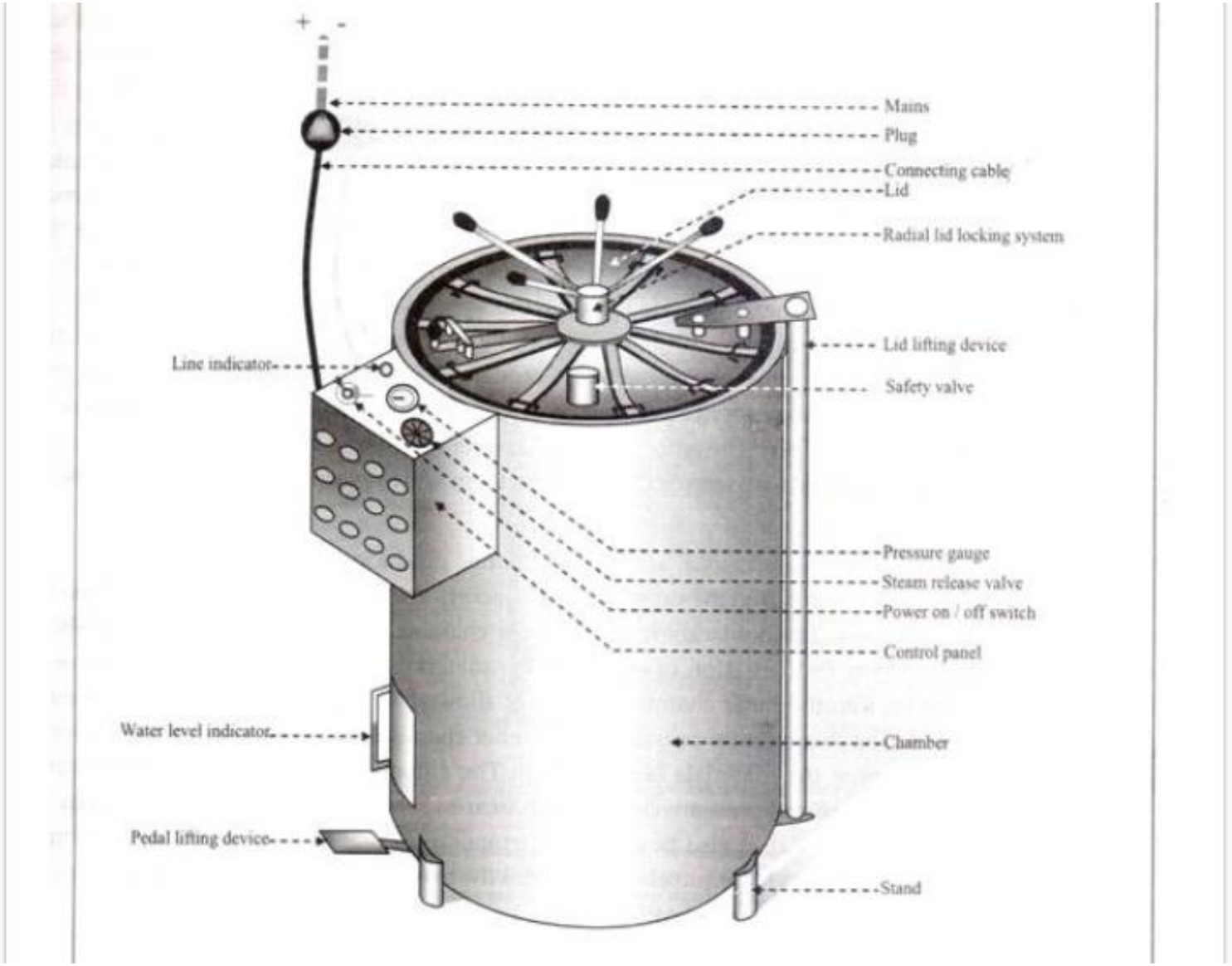
Instruments in microbiology laboratory

1. Hot air oven

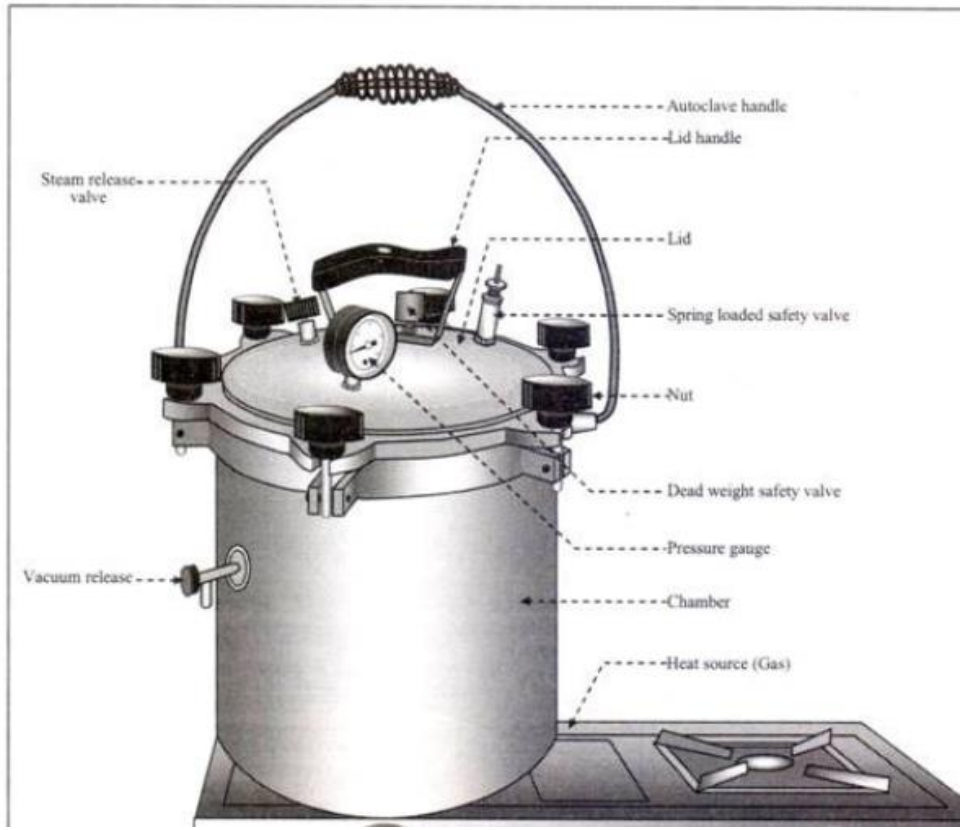


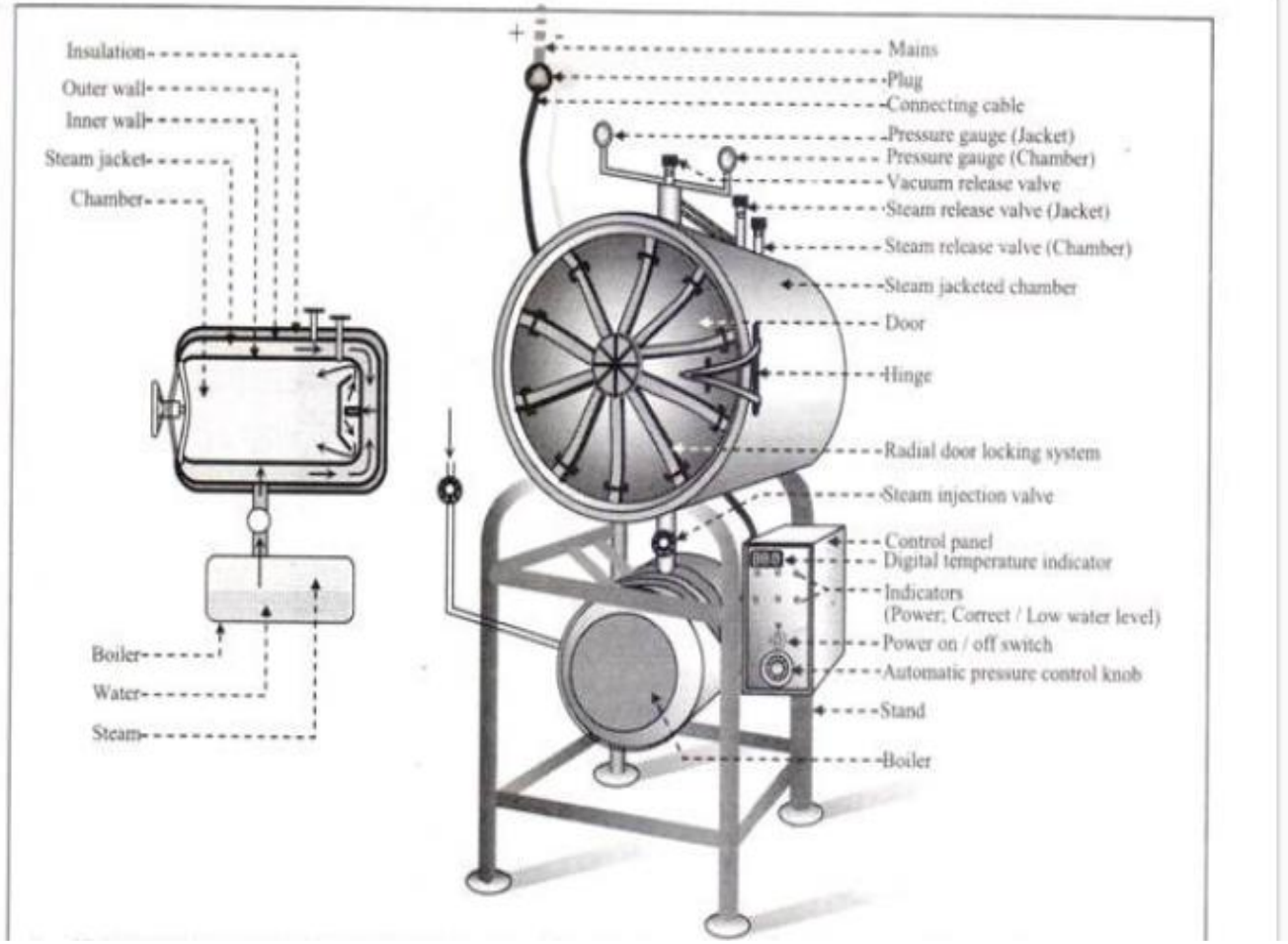
http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475

2. Autoclave



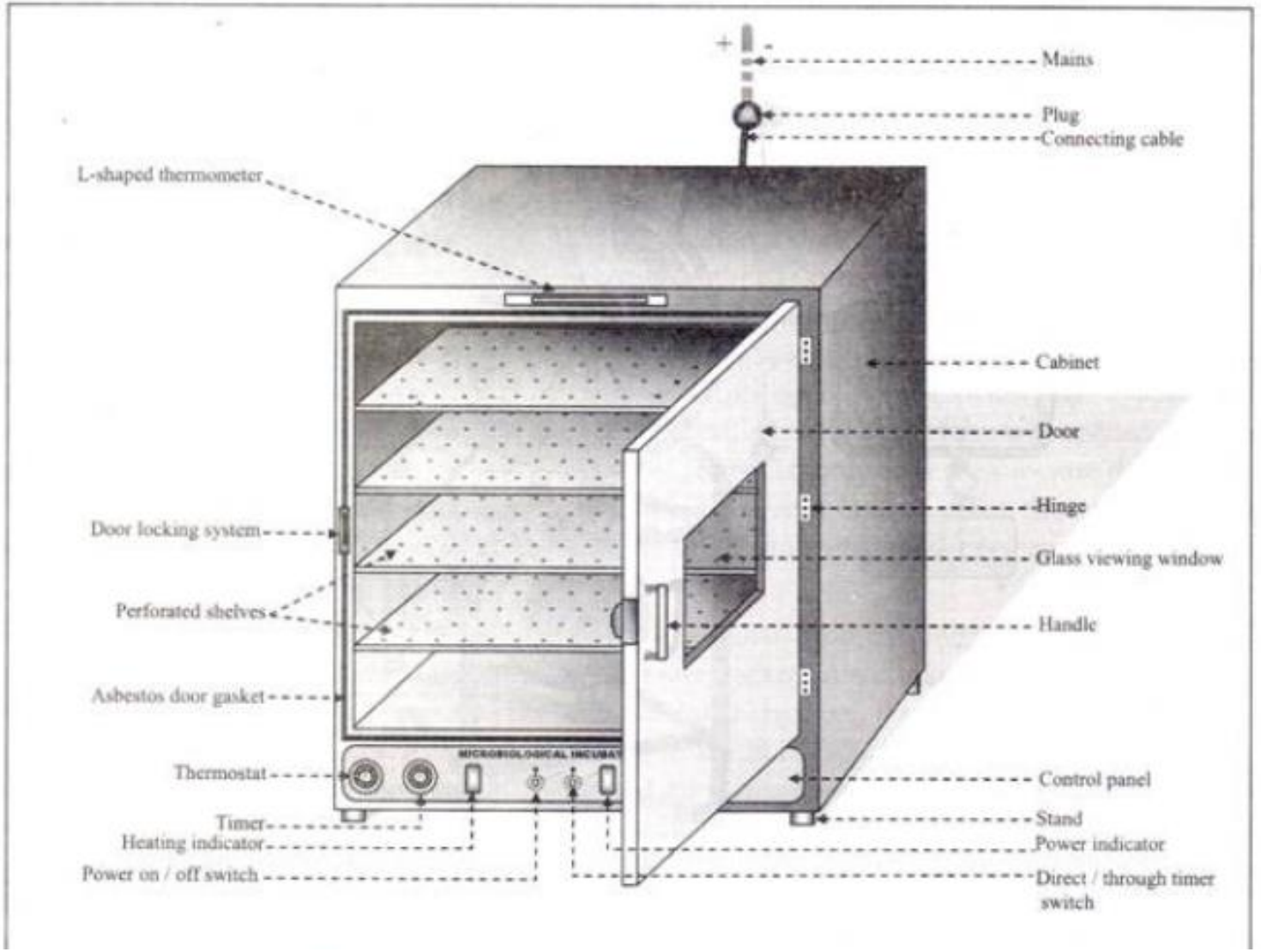
http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475





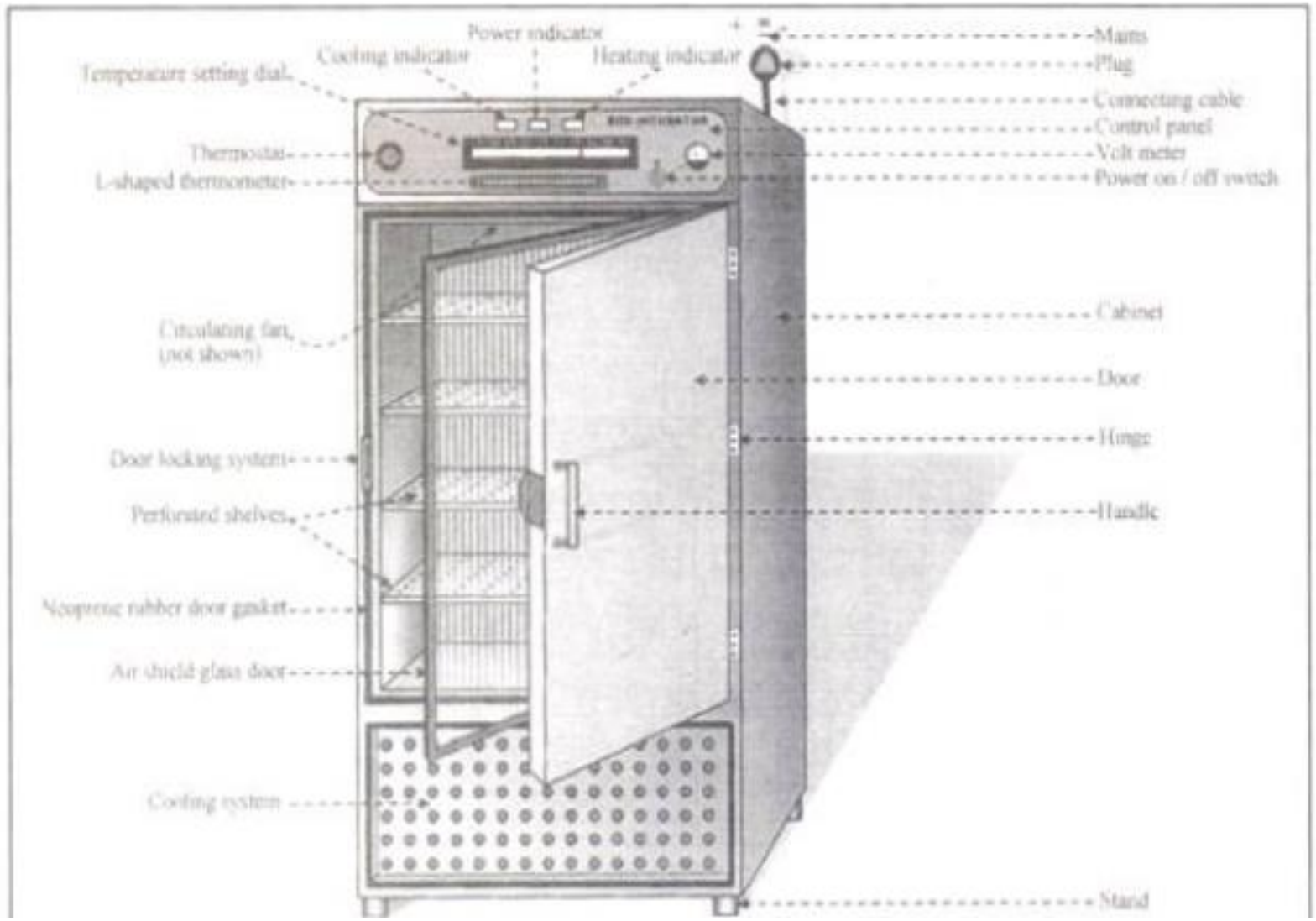
http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475

3. Incubator



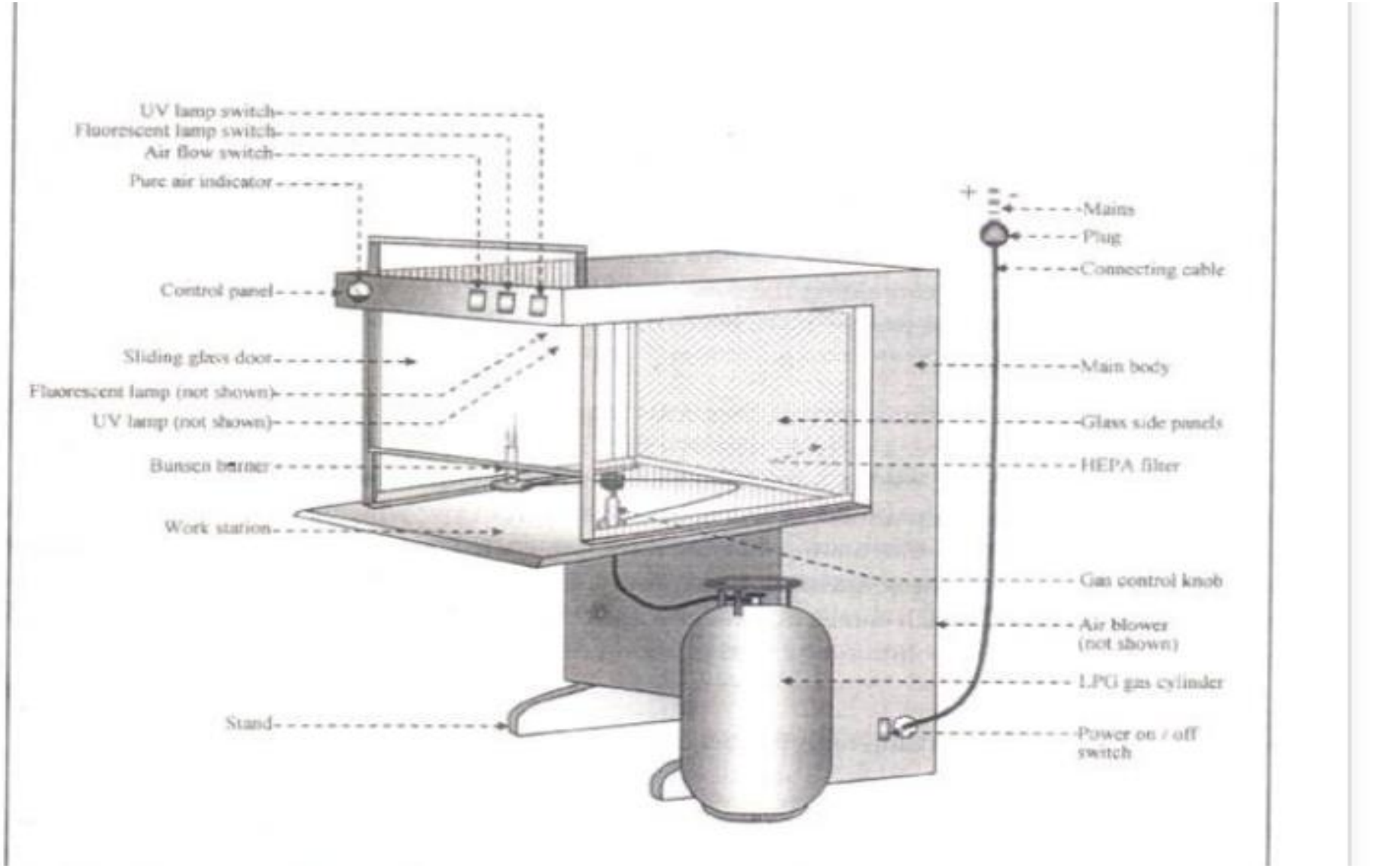
http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475

4. BOD incubator



http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475

5. Laminar air flow chamber



http://www.yourarticlelibrary.com/micro-biology/29-equipments-every-microbiology-laboratory-should-have/26475

6. Incubator shaker


SHAKER X

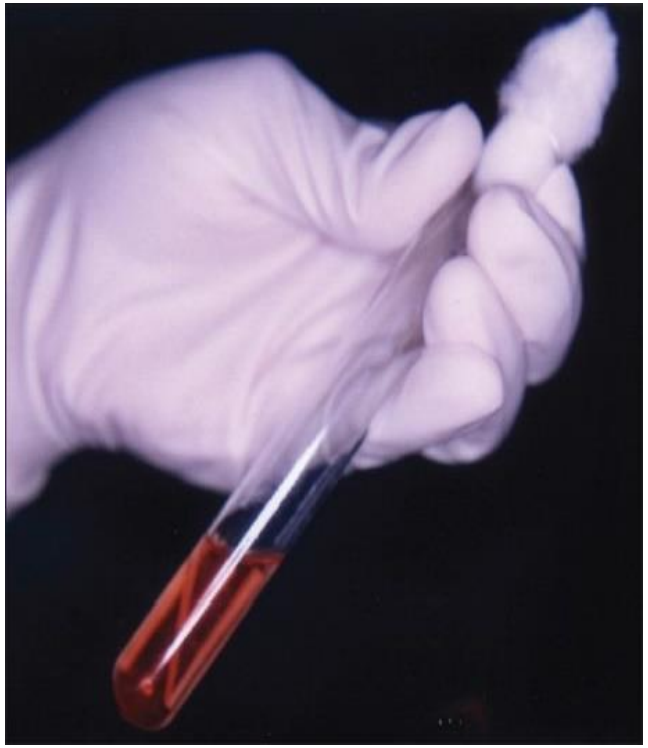
7. Fridge (refrigerator)

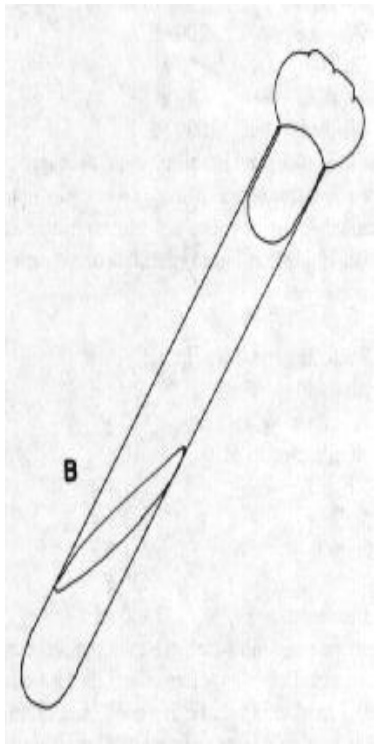
8. Deep fridge at -20 °C

9. Electronic analytical and top-pan balances

10. pH meter

11. Hot plate, water bath, colony counter, magnetic stirrer, sonicator.



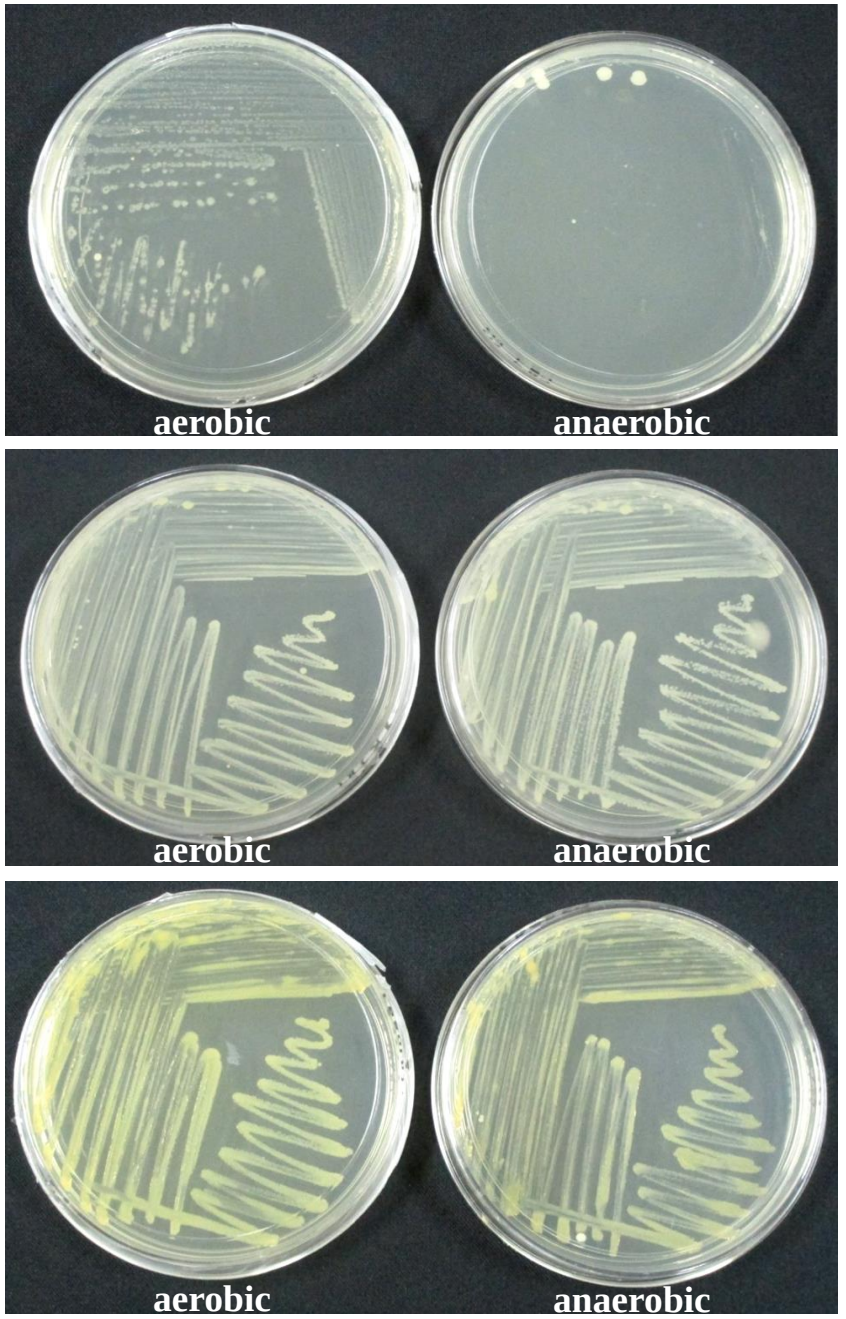


Culture tubes and cotton plugs

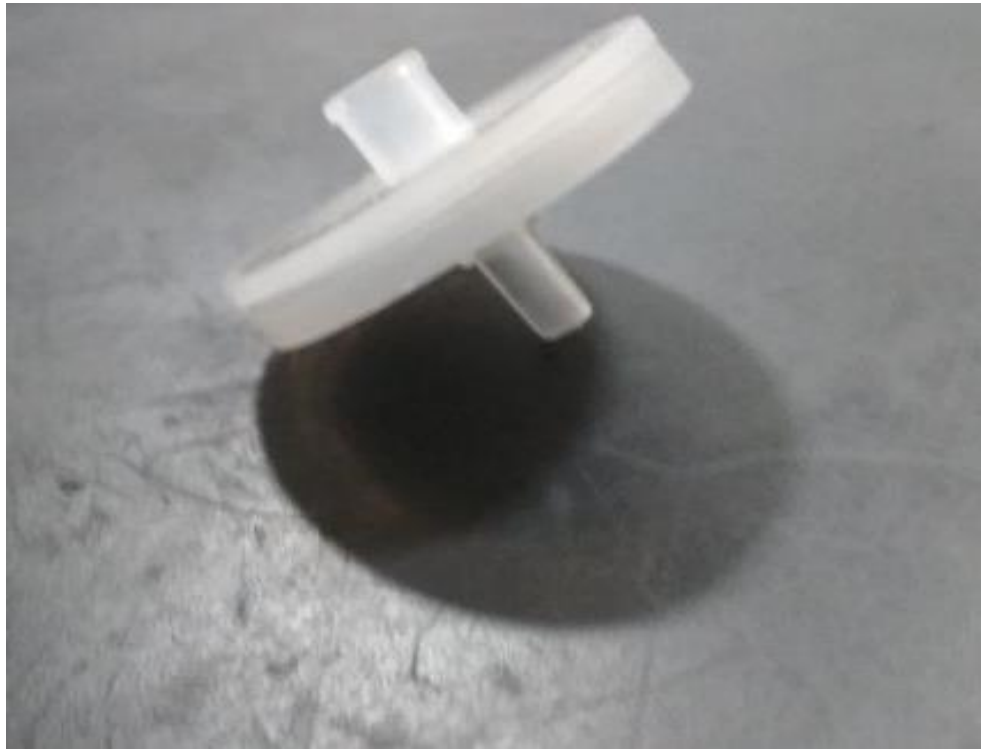
Inoculation needle and loop

Spreader

http://website.nbm-mnb.ca/mycologywebpages/Moulds/Cultivation.html



Bacteria growing in a culture tube and petriplates

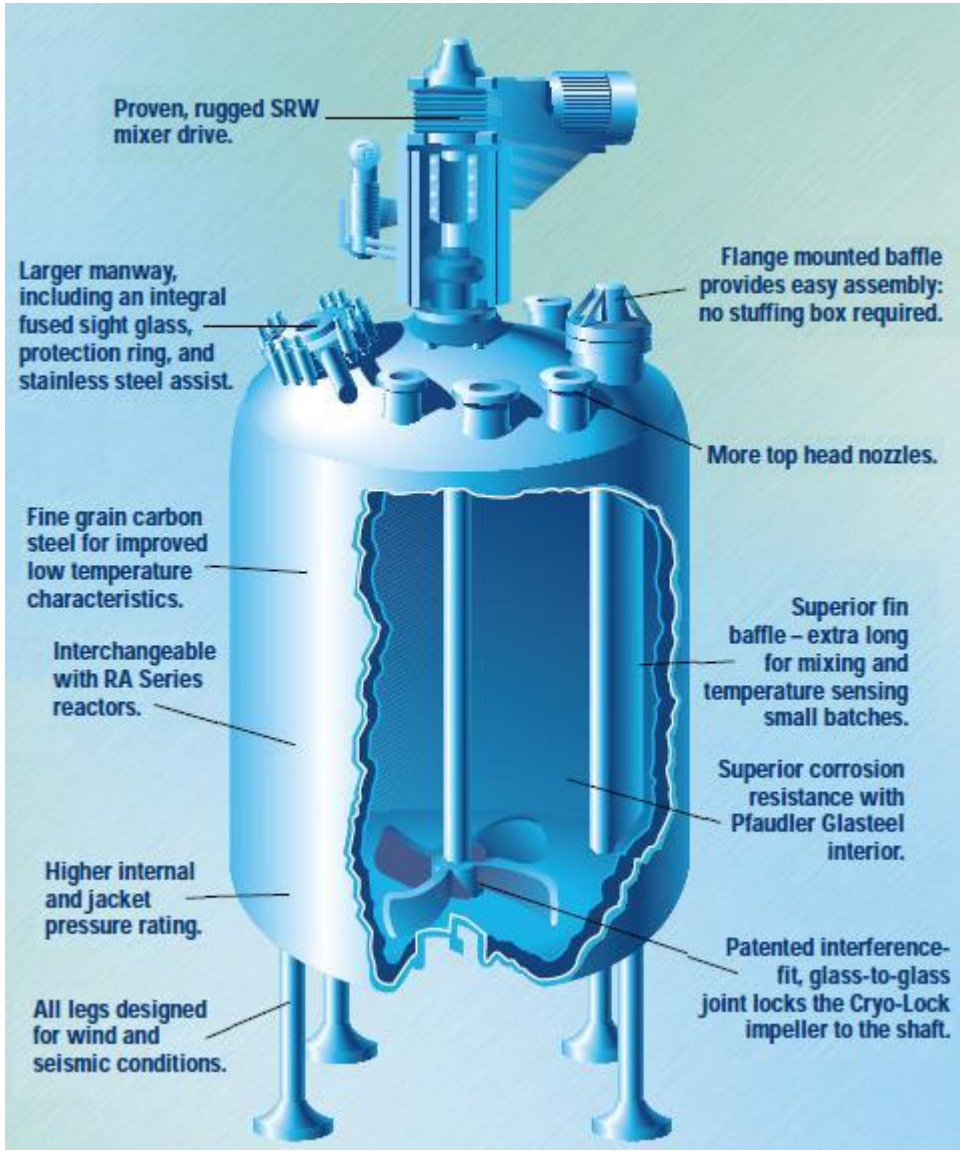


Membrane filter

Vortex

Distillation unit

Stirred tank fermenter



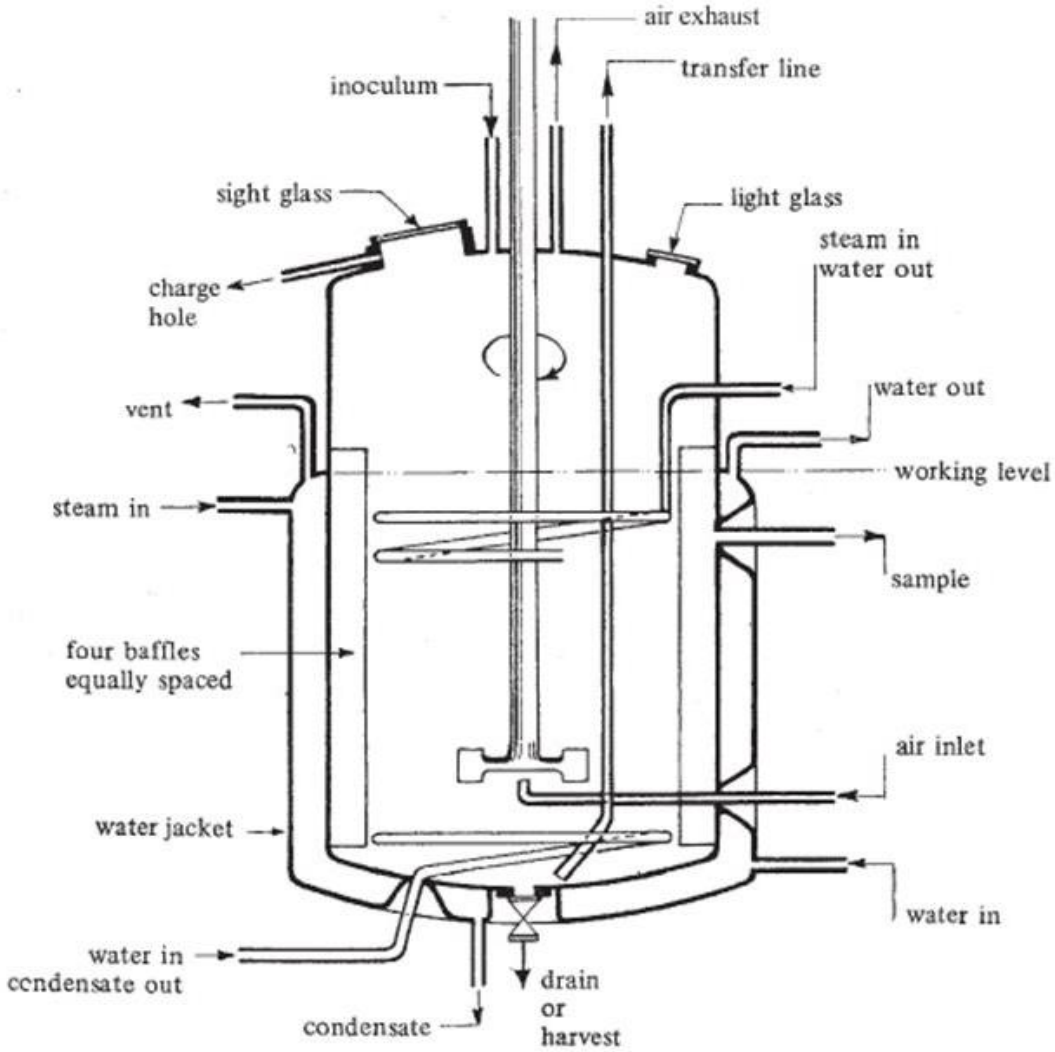
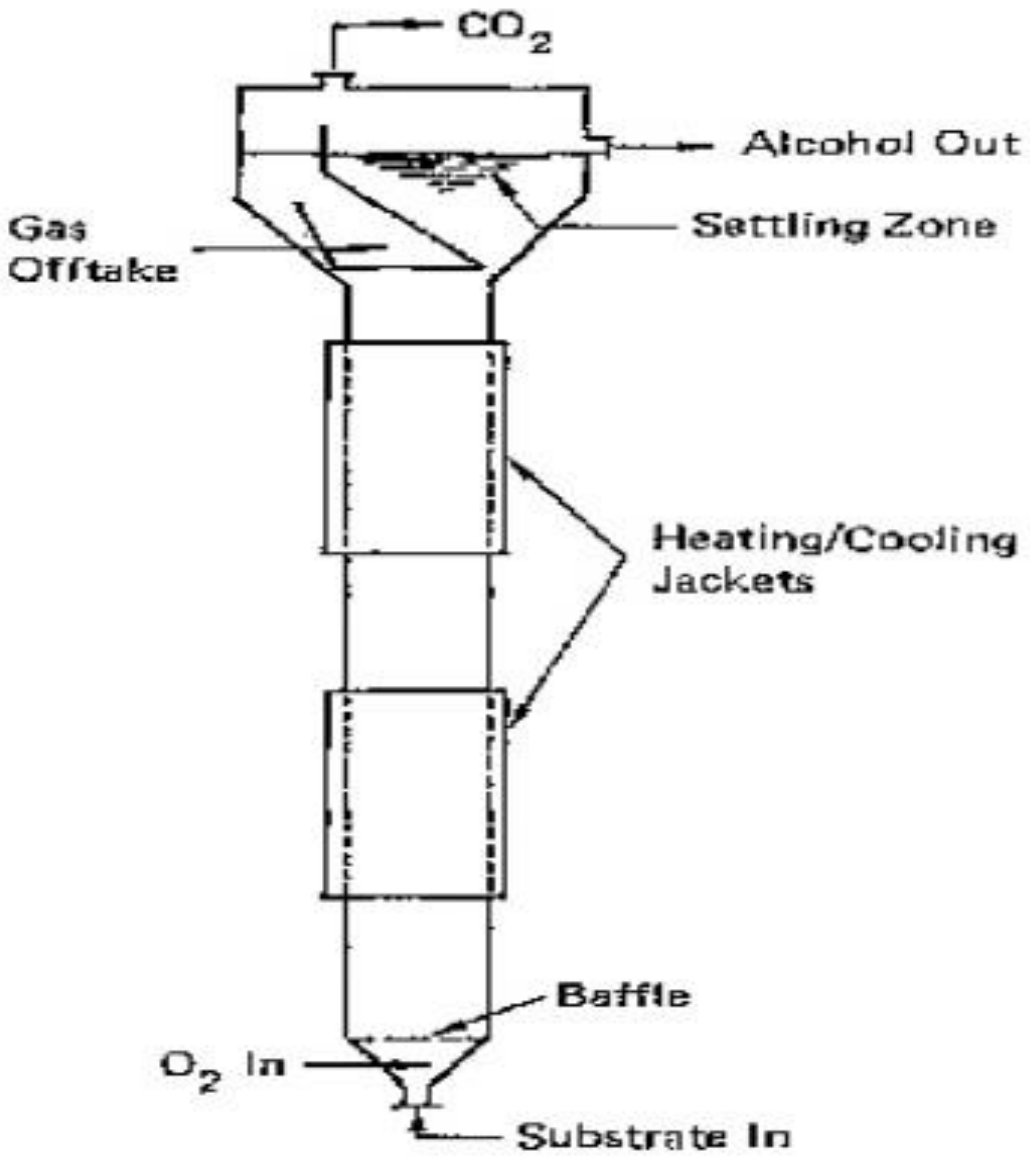


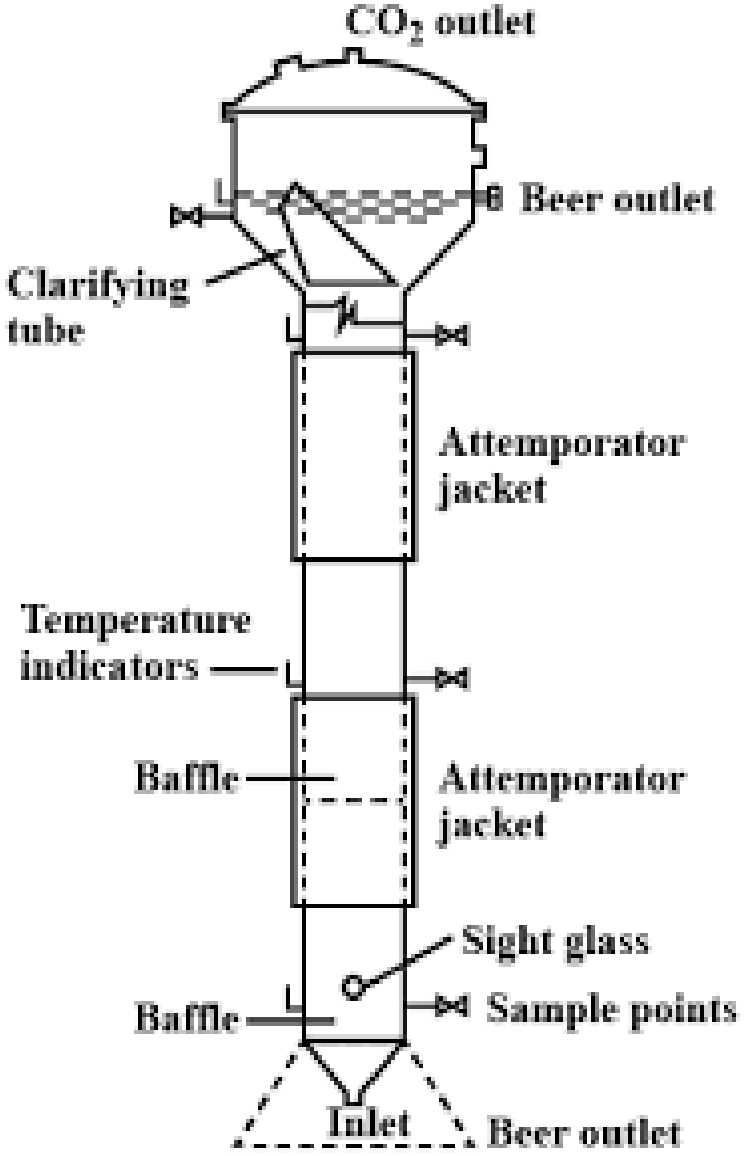
Fig. 9.1 Structure of a Typical Fermentor (Stirred Tank Batch Bioreactor)

http://encyclopedia.che.engin.umich.edu/Pages/Reactors/CSTR/CSTR.html; https://slideplayer.com/slide/5010700/

Tower fermenter

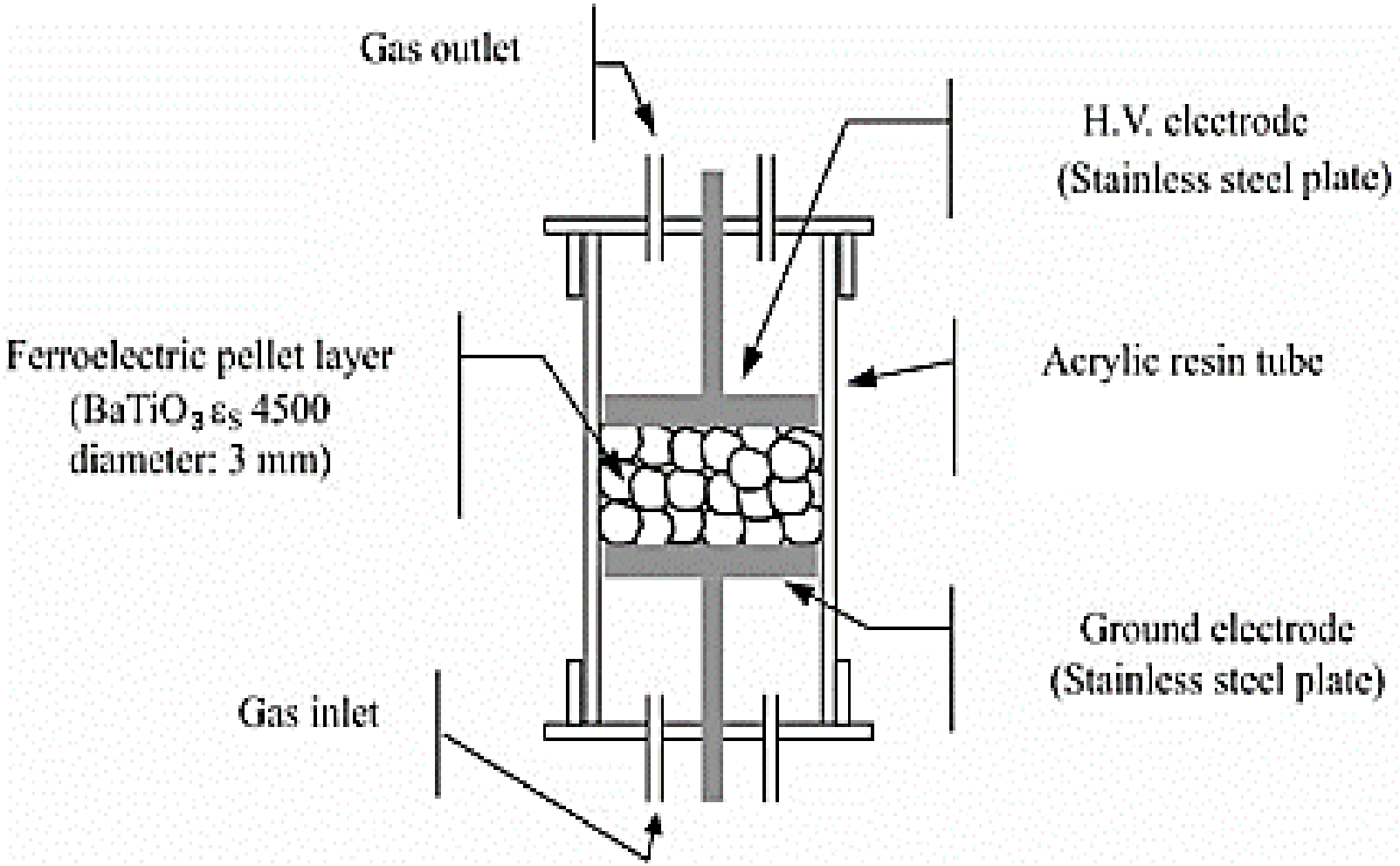


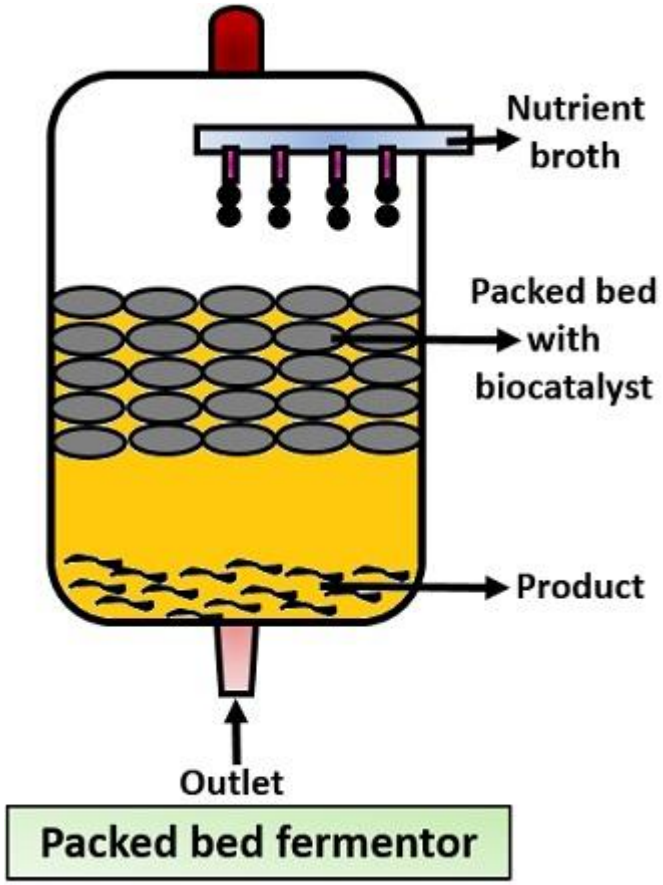
https://www.slideshare.net/DineshS50/tower-fermernter



https://images.app.goo.gl/S6SgYMGn5rWXKmQZ6

Fixed bed fermenter

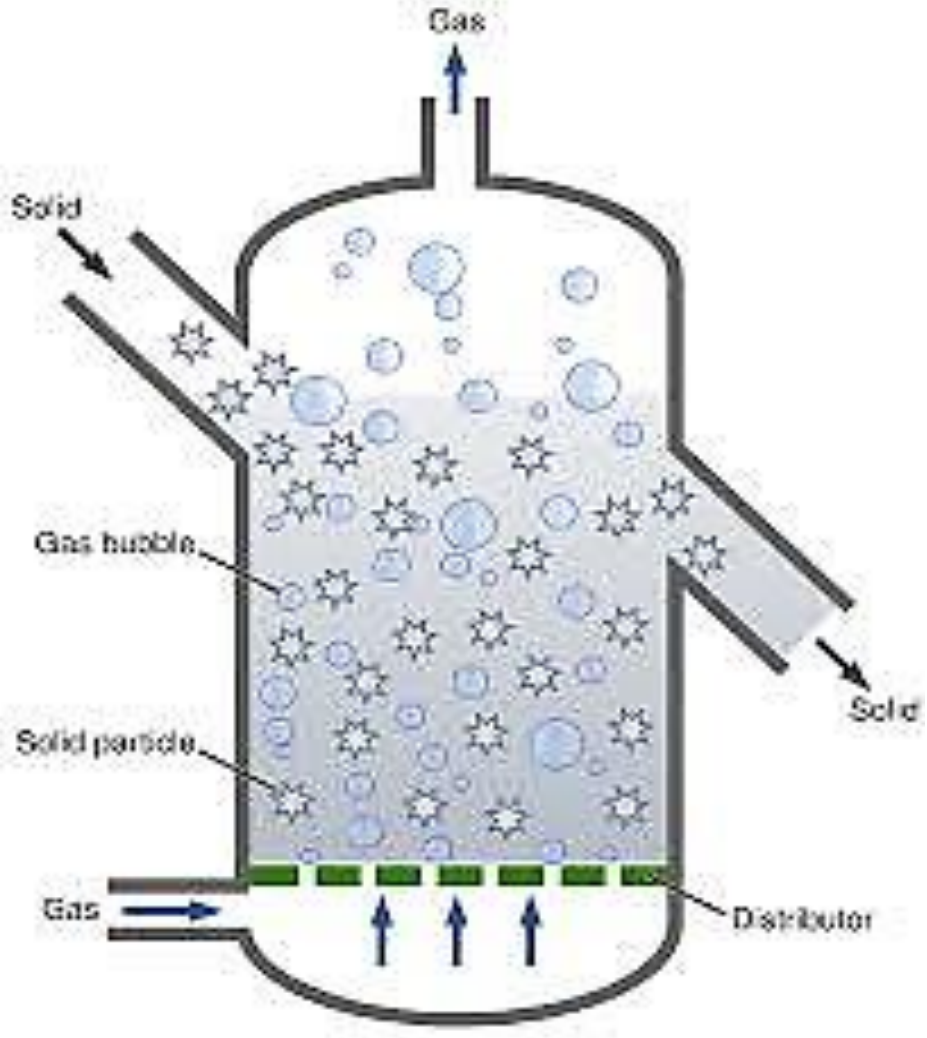


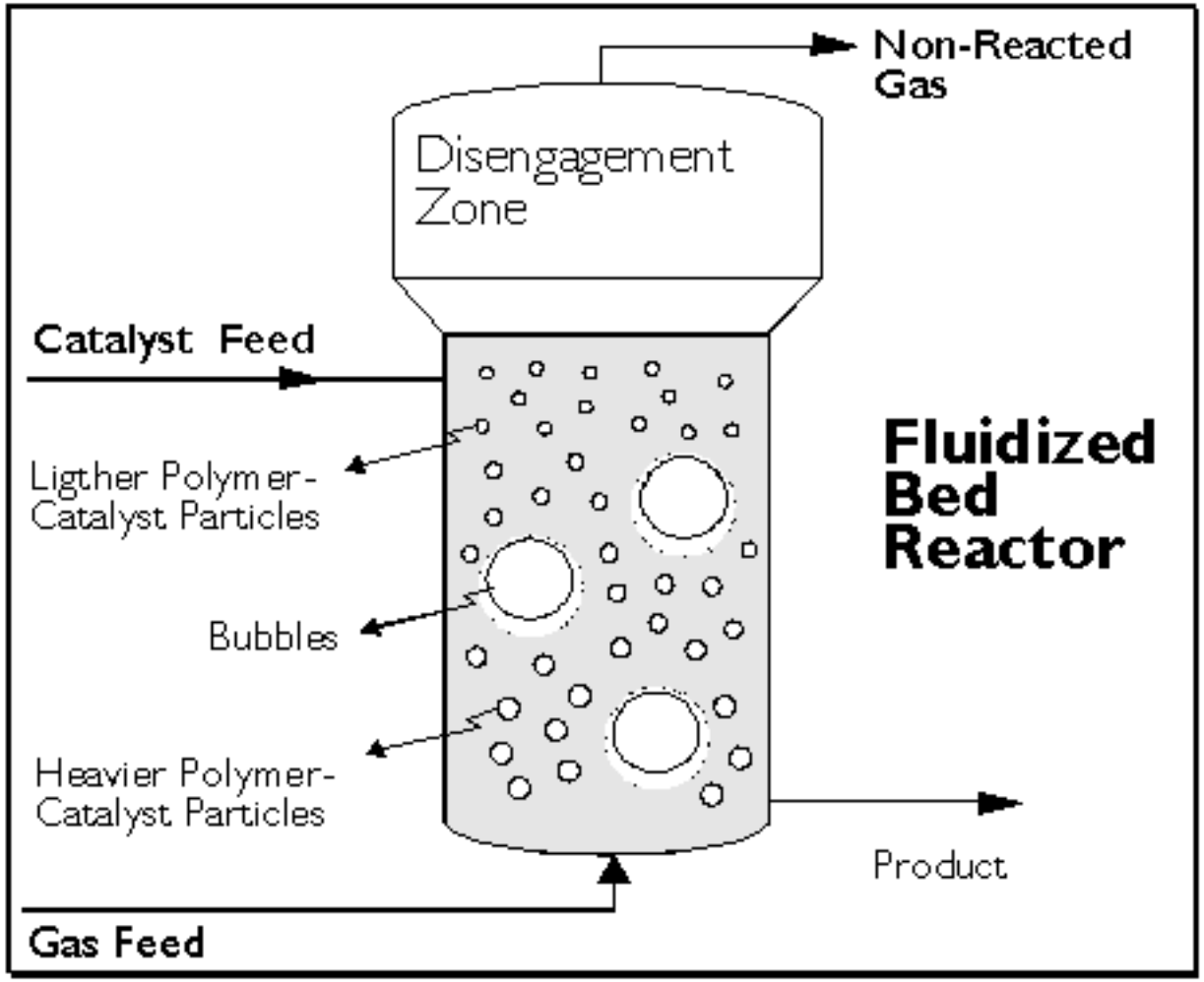


https://processdesign.mccormick.northwestern.edu/index.php/Reactors

https://images.app.goo.gl/H6mkkLCQsTZfMgTWA

Fluidized bed fermenter

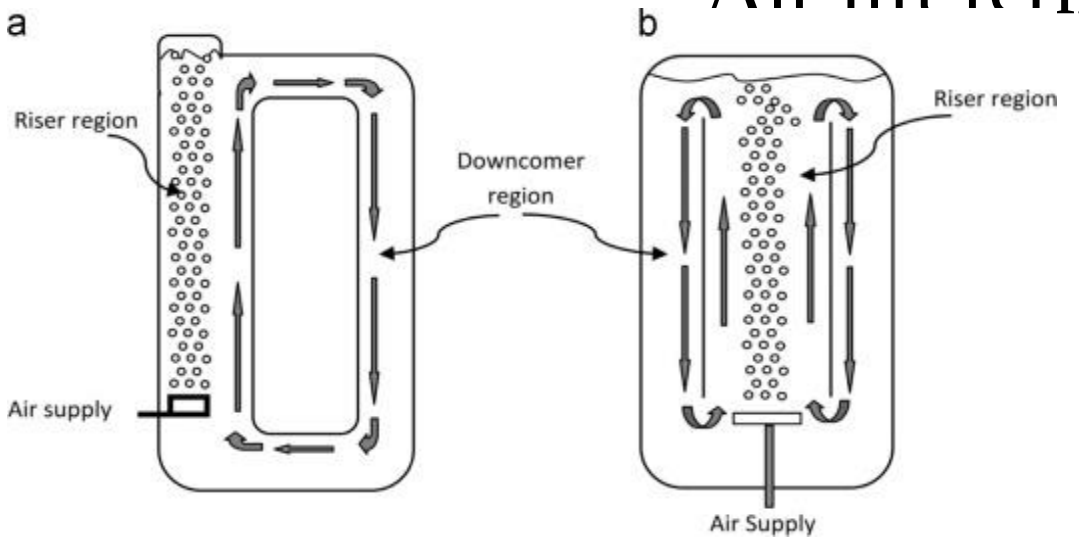




https://en.wikipedia.org/wiki/Fluidized_bed_reactor;

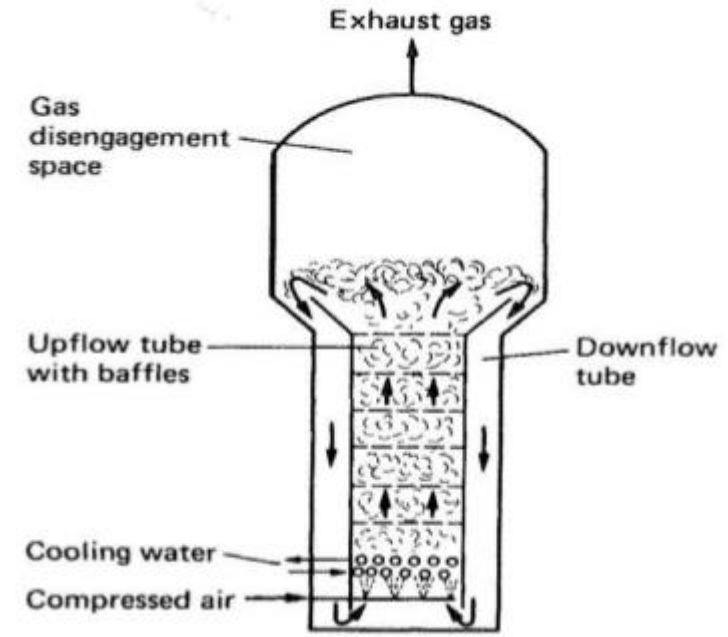
https://images.app.goo.gl/gBffwRHg4jojcQHS6

Air lift fermenter (ALF)

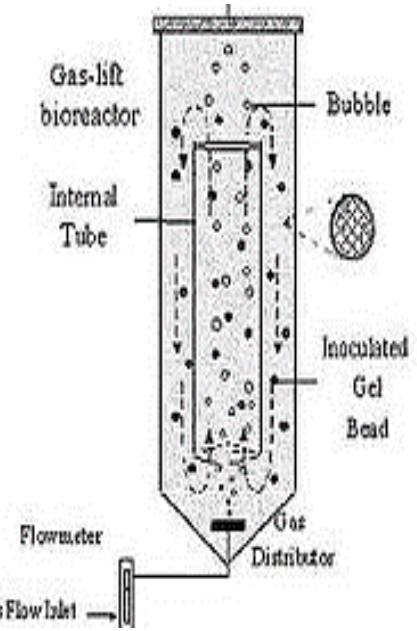


ALF with external loop

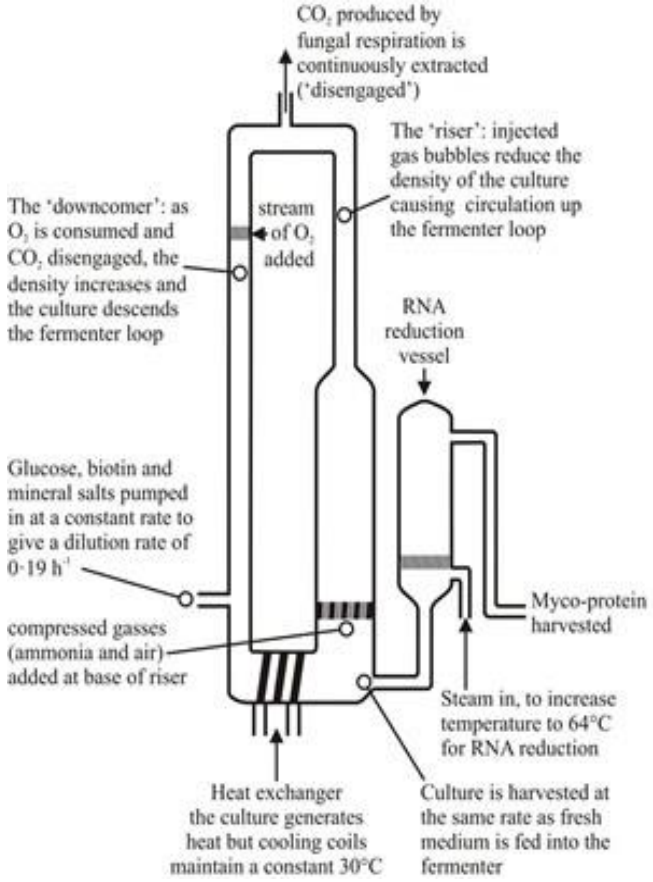
ALF with inner loop



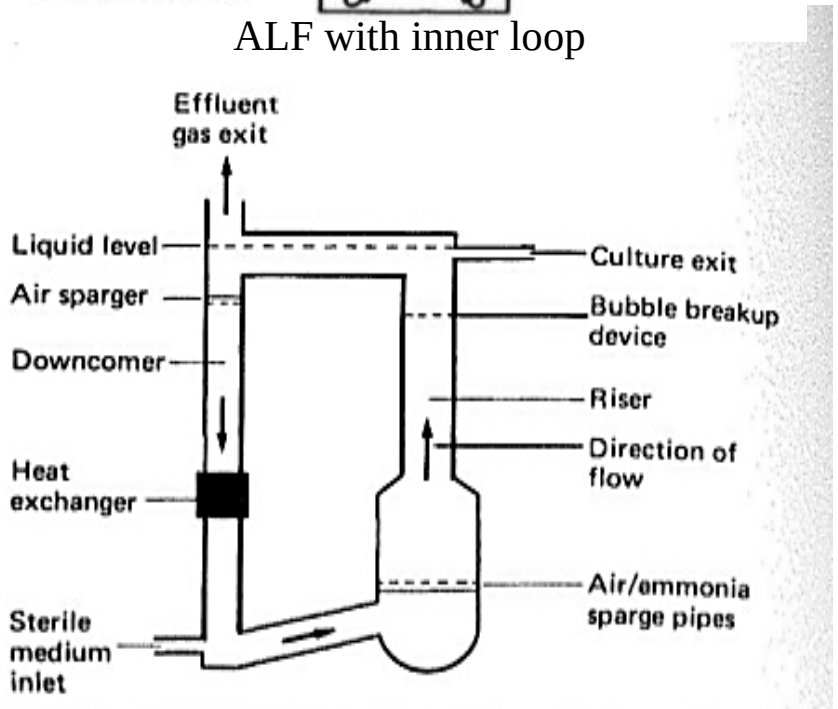
ALF with inner loop



ALF with inner loop



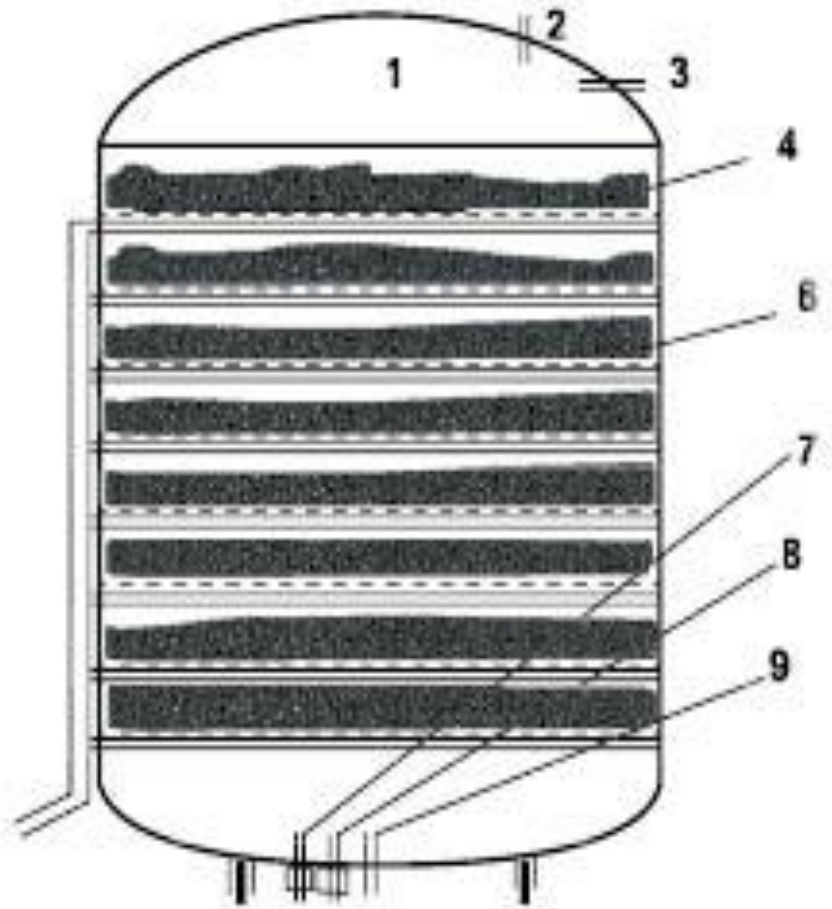
ALF with external loop



ALF with external loop

https://www.sciencedirect.com/science/article/pii/0009250915004406,
https://images.app.goo.gl/cAxc7yzfyqNn6Y1BA,
https://images.app.goo.gl/QLgeJuSgjwfUDWSy6,
https://images.app.goo.gl/t6Cf8G6Ukzk2ZebWA,
https://images.app.goo.gl/dwuDSaWhDbfSzAet6

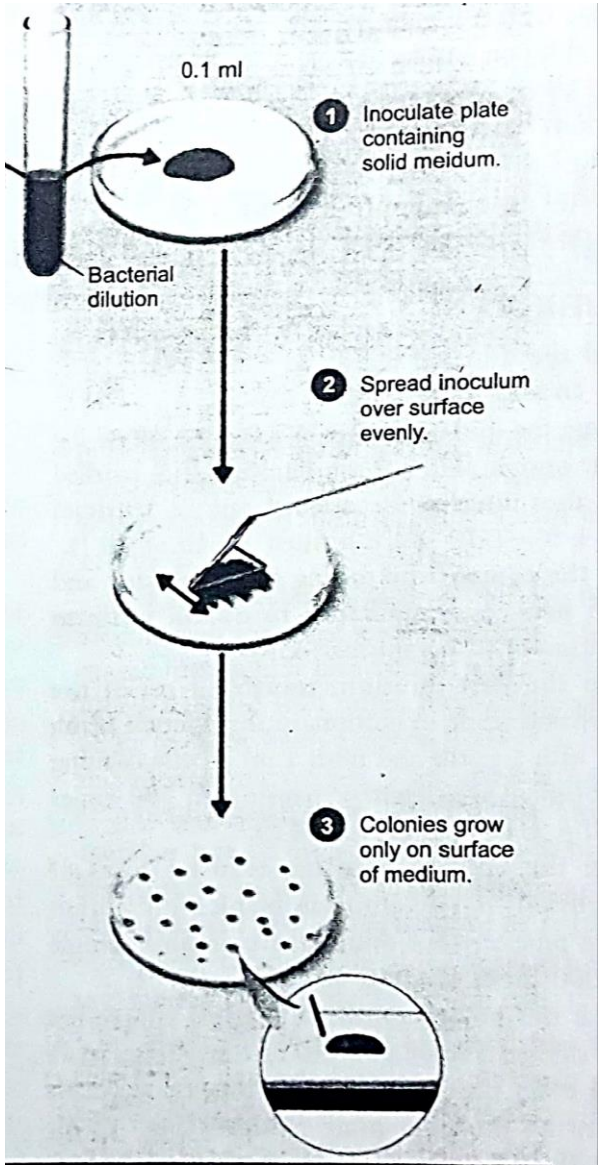
Solid state fermentation



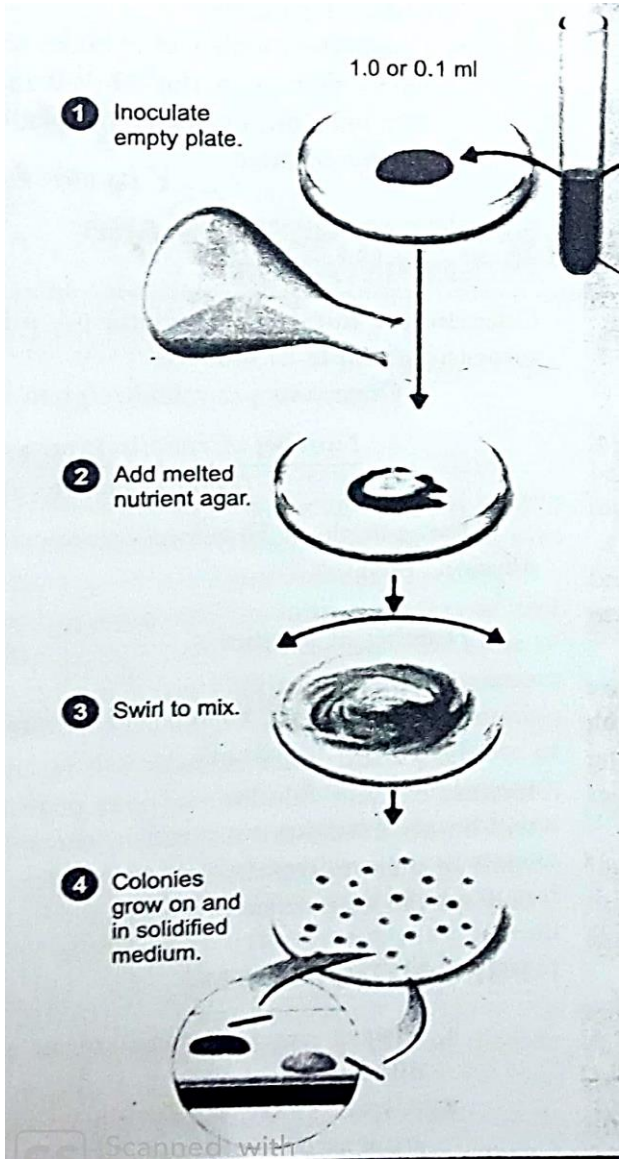
Schematic of fermenter typically used for solid-state fermentation

https://patents.google.com/patent/US6620614

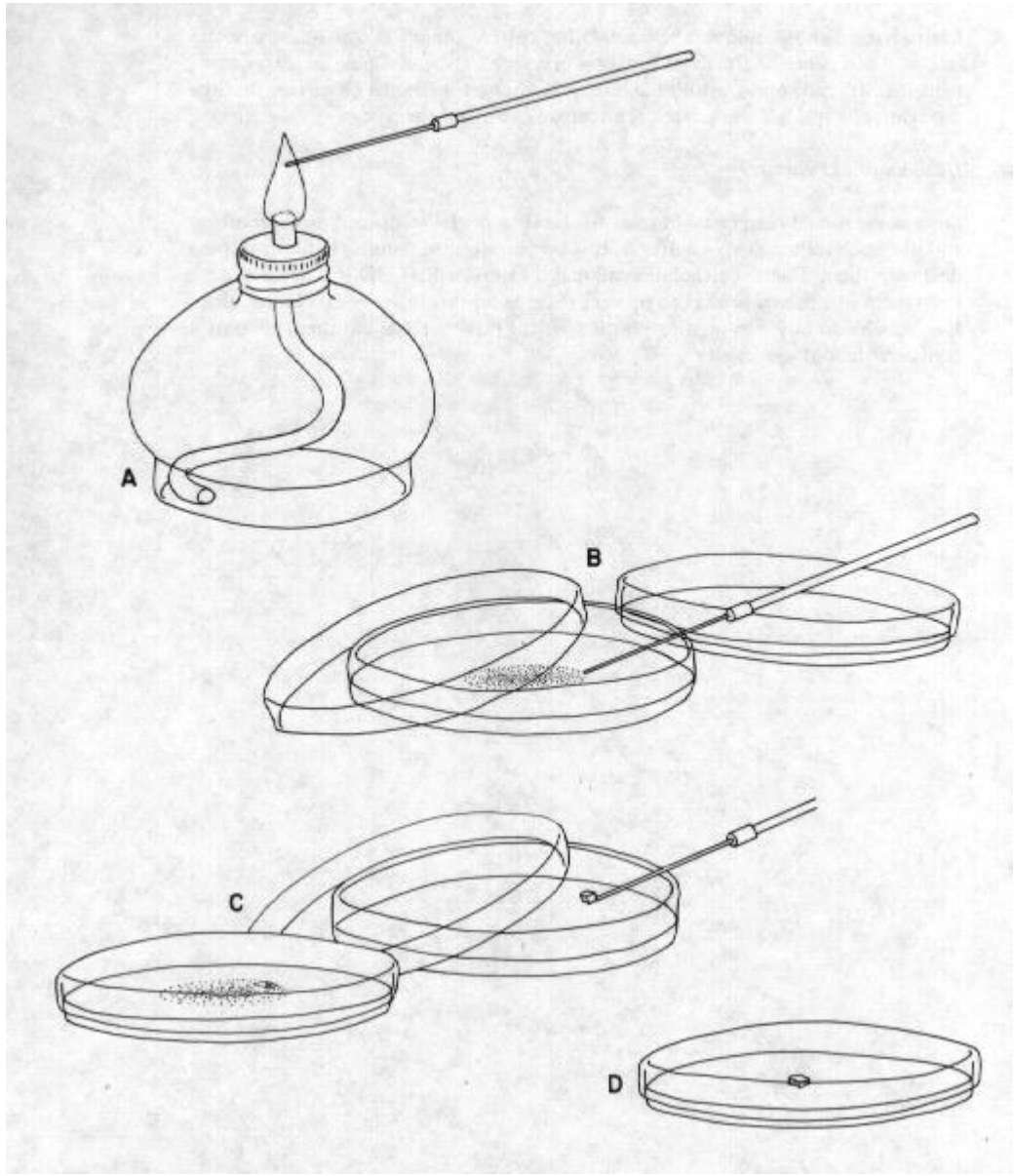
Spread plate technique

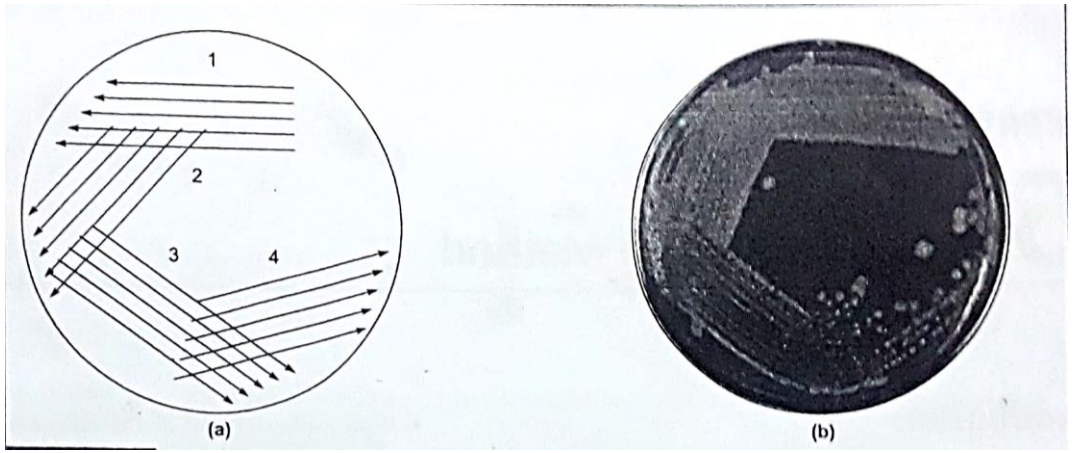


Pour plate technique



Streak plate technique





http://website.nbm-mnb.ca/mycologywebpages/Moulds/Cultivation.html

Discipline Specific Elective
Industrial and Environmental Microbiology
Botany (Honours) Semester VI

List of experiments

1. Principle and functioning of instruments in Microbiology laboratory (Autoclave, Laminar flow, Incubator, types of fermenters)
2. Principle and hands on experimentation for sterilization (wet heat,dry heat, filtration,solvents, radiation) , microbial culturing techniques (Streak plate, spread plate, pour plate, stab culture and glycerol stock) and preparation of different broth/ agar media (Nutrient medium/ Luria Bertani medium/ Potato Dextrose medium/ Czapek Dox medium)
3. Study of VAM from root/ soil samples
4. Study of *Rhizobium* from root of leguminous plants
5. Alcohol production by yeast using sugar/ Gur
6. Serial dilution method for the isolation of microorganism from water and soil and study of aeromicroflora
7. Hydrolysis of casein/ starch by microorganisms
8. Study of Coliform bacteria from water sample using Eosin methylene blue medium
9. Field visit/ Project

IEM practical 2

Glycerol stock

The addition of glycerol stabilizes the frozen bacteria, preventing damage to the cell membranes and keeping the cells alive. A glycerol stock of bacteria can be stored stably at -80°C.

Bacterial samples are critical for research, diagnostic purposes. Although there are many ways to store bacteria, the ideal method is a function of bacterial compatibility, experimental purpose, and cell viability. As a general rule, the viable storage period of bacteria increases as the storage temperature decreases. Once the temperature is below the freezing point, however, cryoprotectants are essential to reduce cell damage caused by the freezing process. The specific length of time that a culture will remain viable in a given storage condition is dependent upon the bacterial strain. Cell death during storage is inevitable but should be minimized as much as possible, which can sacrifice ease of use. Bacterial cultures that are used regularly (i.e., daily/weekly) can be stored on agar plates or in stab cultures in a standard refrigerator at 4°C. If cultures will not be used for more than a few weeks, though, more long-term storage methods should be considered for maximum bacterial viability (Table 1).

Table 1. Approximate time bacterial cultures remain viable in different storage conditions.

Condition	**Temp** (°C)	**Time** (approx.)
Agar plates	4	4 - 6 weeks
Stab cultures	4	3 weeks - 1 year
Standard freezer	-20	1 - 3 years
Super-cooled freezer	-80	1 - 10 years
Freeze dried	≤4	15 years+

Short-term storage

Working bacterial stocks can be streaked onto agar plates and stored at 4°C for daily or weekly use. Culture dishes should be wrapped with laboratory sealing film (plastic or paraffin) and stored upside down (agar side up) to minimize contamination and to keep both the culture and agar properly hydrated. Some bacterial strains can be stored for up to 1 year at 4°C in agar stab cultures, which are especially useful for transporting samples to other research facilities. Stab

cultures are prepared by first sterilizing strain-compatible agar (e.g., luria bertani/ lysogeny broth [LB] agar for E. coli) and then transferring the warm liquid agar to screw-cap vials using the appropriate aseptic technique. After the agar has solidified, a single colony is picked from an actively growing culture using a sterile, straight wire. The wire with the bacteria is then plunged deep into the soft agar several times, and the vial is incubated at 37°C for 8–12 hours with the cap slightly loose. The vial is then sealed tightly and stored in the dark at 4°C.

Long-term storage

As mentioned above, the temperature at which frozen bacteria are stored affects how long they can be stored while remaining viable. Freezing and thawing cells at an appropriate rate and maintaining the frozen stocks at the proper storage temperature help to minimize damage from the freezing process. Also, the greater the cell density, the better the recovery is after thawing the cells. For most bacteria, a density of 10^7 cells/mL will result in adequate recovery if all conditions are properly maintained.

Cryoprotectants: As water in cells is converted to ice, solutes accumulate in the residual free water. This localized increase in salt concentration can denature biomolecules. Furthermore, ice crystal formation can damage cell membranes. Additives that are mixed with the bacterial suspension before freezing lower the freezing point and protect cells during freezing to minimize the detrimental effects of increased solute concentration and ice crystal formation. The most commonly used cryoprotectants are dimethylsulfoxide (DMSO) and glycerol, which are typically used at 5–15% (v/v). Non-permeable additives used as cryopreservants, such as polysaccharides, proteins, and dextrans, adsorb to the surface of microorganisms and form a viscous layer that protects membranes, making these agents particularly useful for cryopreservation. Other commonly used additives include blood serum, ethylene glycol, methanol, skim milk, yeast extracts, and tripticase soy.

Freezing samples: To prepare glycerol stocks, the glycerol is first autoclaved and allowed to cool. The appropriate volume of glycerol is added to a suspension of log-phase bacteria and vortexed to dissociate the cells and ensure even mixing of the bacteria with the glycerol. After aliquoting the suspension into cryogenic screw-cap vials, the cells are snap-frozen by immersing the tubes in either ethanol-dry ice or liquid nitrogen and then stored in freezers (-20 to -80°C) or liquid nitrogen (-150°C). Repeated thawing and refreezing of the bacterial stocks will reduce cell viability and should be avoided. When recovering strains with antibiotic selection markers, culturing them on selective media will ensure that the bacterial stocks were not contaminated.

Freeze-drying: Bacteria can be freeze-dried by suspending log-phase cells in a lyophilization medium and then freeze- drying the suspension. Not all bacteria can be successfully freeze-dried. Certain strains might not survive the process or die rapidly once freeze-dried. The best way to determine if a strain is amenable to freeze-drying is to empirically evaluate its stability post–freeze-drying while maintaining a live culture as a backup. Once freeze-dried, it is best to store the bacteria at or below 4°C.

Stab cultures

Stab cultures are similar to agar plates, but are formed by solid agar in a test tube. Bacteria is introduced via an inoculation needle or a pipette tip being stabbed into the center of the agar. Bacteria grow in the punctured area. Stab cultures are most commonly used for short-term storage or shipment of cultures. A culture into which the organisms are introduced by thrusting a needle deep into the medium. The agar stab is an inoculation technique used when inoculating semi-solid medium for the analysis of motility or oxygen usage and requirement, or when inoculating certain types of solid medium. This particular method of inoculation allows one to culture a microorganism under limited oxygen exposure.

Stab preparation

1. Agar preparation (scale if necessary)- melted LBA medium is poured enough into sterile plastic petri dish or to Eppendorf tubes (for stabs).
2. Stabs: Using a sterile straight, pick a single colony from a freshly streaked plate or from a fresh culture, and stab it deep down into the soft agar several times (Figure 1). Incubate the vial at 37°C for 8–12 h leaving the cap slightly loose. Seal the vial tightly and store in the dark, preferably at 4°C.
3. Flame an inoculaton needle wire inoculating needle to sterilize it. The inoculating needle should be heated to a red glow using a Bunsen burner, ensuring that a large section of the needle has been sterilized. Allow the needle to cool before obtaining a culture. If the needle is too hot, it will cause the cells to burst.
4. With other hand, pick up a culture tube and remove the cap with the fourth and fifth fingers of the hand that is holding the loop. Never lay a cap down on the lab bench, this will result in contamination. Always hold the open tube at an angle so that dust does not fall into the tube and contaminate the culture.
5. Pass the opening of the culture tube through the Bunsen burner flame. This will kill any microorganisms on the lip of the tube.
6. Using this sterilized inoculating needle, pick up a bacterial colony (or piece of a colony) from the surface of the plate culture, and inoculate the media by stabbing the needle into the center of the agar in the tube, and pushing it down to the bottom. Withdraw the needle carefully and try to remove it by following the same stab line that you made pushing the needle down. If there is a broth culture, the inoculating needle can be submerged within the culture.
7. Flame the mouth of the culture tube again, replace the cap, and set it aside.
8. Place the stab subculture in an incubator at the temperature and time specified

Others

For quick sterilization of lab equipment and surfaces, cleaning with a solvent such as ethanol or isopropanol is a convenient option. These solvents work by rupturing the cells and denaturing the cellular proteins. The rupturing is caused by osmosis and requires water, which is why diluted rather than neat solvent is used. Solvents are good for decontaminating microbes, and viruses, but are not effective against spores or tough enzymes like RNases.

Autoclaving is a good option for sterilizing solutions and heat-resistant equipment. Autoclaves kill microbes by hydrolysis and coagulation of cellular proteins, using intense heat from pressurized steam. They do an excellent job of killing microbes, spores and viruses but are only partially effective against RNases and can cause problems with certain solutions containing sugars, phosphates, metals and amino acids.

Effective sterilisation techniques are essential for working with isolated cell lines for obvious reasons you don’t want bugs from the environment growing in your nice culture medium, and equally, cultures must be sterilised before disposal.

WET HEAT (Autoclaving)

The method of choice for sterilisation in most labs is autoclaving; using pressurised steam to heat the material to be sterilised. This is a very effective method that kills all microbes, spores and viruses, although for some specific bugs, especially high temperatures or incubation times are required. Autoclaving kills microbes by hydrolysis and coagulation of cellular proteins, which is efficiently achieved by intense heat in the presence of water. The intense heat comes from the steam. Pressurised steam has a high latent heat; at 100°C it holds 7 times more heat than water at the same temperature. This heat is liberated upon contact with the cooler surface of the material to be sterilised, allowing rapid delivery of heat and good penetration of dense materials. At these temperatures, water does hydrolyzing of proteins.

DRY HEAT (Flaming, baking)

Dry heating has one crucial difference from autoclaving, as there's no water, so protein hydrolysis can't take place. Instead, dry heat tends to kill microbes by oxidation of cellular components. This requires more energy than protein hydrolysis so higher temperatures are required for efficient sterilization by dry heat. For example sterilisation can normally be achieved in 15 minutes by autoclaving at 121°C, whereas dry heating would generally need a temperature of 160°C to sterilize in a similar amount of time.

FILTRATION

Filtration is a great way of quickly sterilizing solutions without heating. Filters, of course, work by passing the solution through a filter with a pore diameter that is too small for microbes to pass through. Filters can be scintered glass funnels made from heat-fused glass particles or, more commonly these days, membrane filters made from cellulose esters. For removal of bacteria, filters with an average pore diameter of 0.2um is normally used. But, viruses and phage can pass through these filters so filtration is not a good option if these are a concern.

SOLVENTS

Ethanol is commonly used as a disinfectant, although since isopropanol is a better solvent for fat it is probably a better option. Both work by denaturing proteins through a process that requires water, so they must be diluted to 60-90% in water to be effective. Although ethanol and IPA are good at killing microbial cells, they have no effect on spores.

RADIATION

UV, x-rays and gamma rays are all types of electromagnetic radiation that have profoundly damaging effects on DNA, so make excellent tools for sterilization. The main difference between them, in terms of their effectiveness, is their penetration. UV has limited penetration in air so sterilisation only occurs in a fairly small area around the lamp. However, it is relatively safe and is quite useful for sterilising small areas, like laminar flow hoods. X-rays and gamma rays are far more penetrating, which makes them more dangerous but very effective for large scale cold sterilization of plastic items (e.g. syringes) during manufacturing.

29 Equipment's Every Microbiology Laboratory Should Have

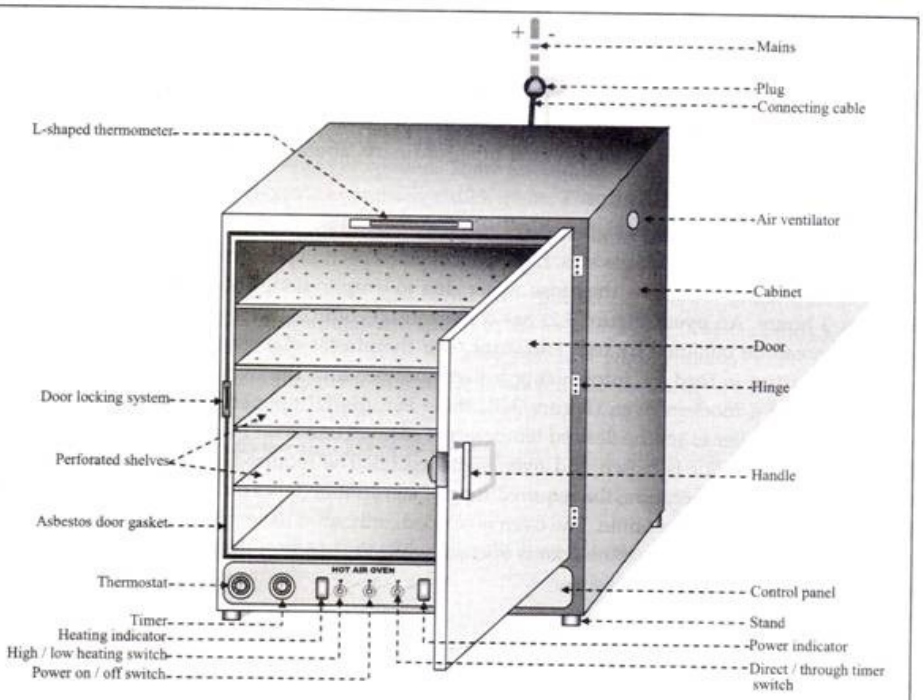
Article shared by : Samiksha S

Aim to study the working Principle and Operation of Equipment's used in Microbiology Laboratory. A modern microbiology laboratory should be furnished with the following equipment.

1. Hot Air Oven for Sterilization:

It is used for sterilization of glassware's, such as test tubes, pipettes and petri dishes. Such dry sterilization is done only for glassware's. Liquid substances, such as prepared media and saline solutions cannot be sterilized in oven, as they lose water due to evaporation.

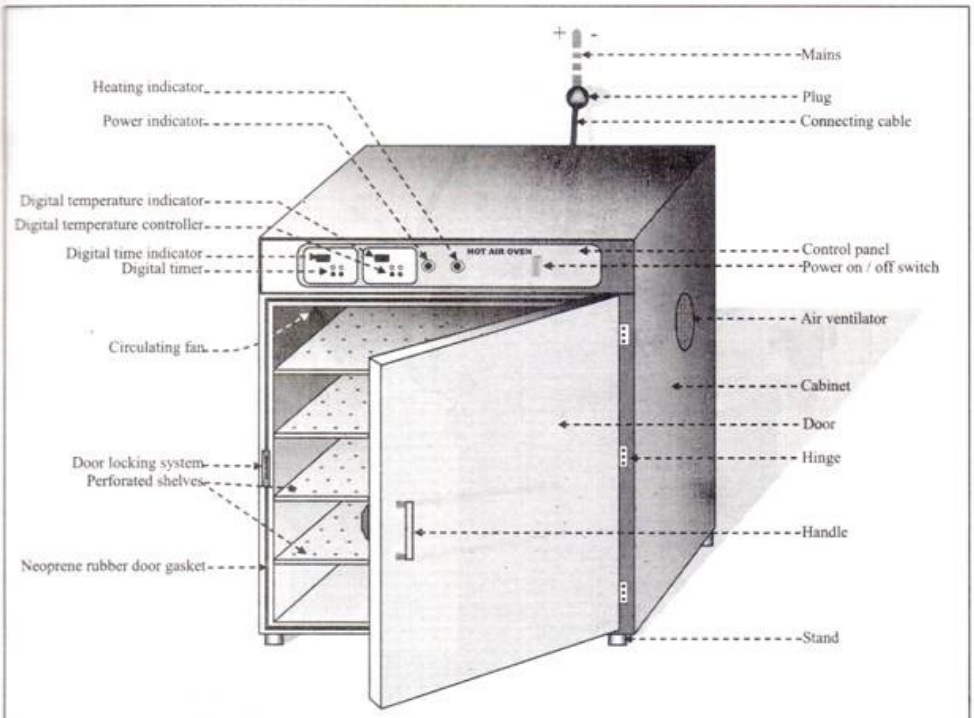
The glassware's are sterilized at 180°C for 3 hours. An oven (Figure 3.2) has a thermostat-control, using which the required constant temperature can be obtained by trial and error. The thermostat dial reading is approximate and the exact temperature is read by introducing a thermometer into the oven or on a built-in L-shaped thermometer.



1. **Mains:** It is the source of electric current for the equipment. The oven runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the oven against electric shock due to any short-circuit in the oven.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the oven and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the oven.
4. **Air ventilator:** Air ventilator is a port (hole), through which the moisture-laden air escapes out from the oven.
5. **Cabinet:** It is the main body of the oven consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets while the inner wall is made of anodised aluminium or stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the oven and reduces consumption of electric current, thereby working the oven efficiently.
6. **Door:** It is the insulated wall, which closes the insulated cabinet of the oven.
7. **Hinge:** It holds the door to the cabinet.
8. **Handle:** It is used to open the door by pulling.
9. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the oven can be controlled.
10. **Stand:** It supports the total weight of the oven along with its contents.
11. **Power indicator:** It indicates supply of electric current to the oven by glowing.
12. **Direct / through timer switch:** This switch gives option to operate the oven directly or through the timer. In case of direct operation, the oven has to be switched off after the required time. Through timer option operates the oven through the timer, which starts functioning and switches off the oven after the set time.
13. **Power on / off switch**: It controls the entry of electric current into the oven.
14. **High/low heating switch**: High heating is done initially to quickly heat the oven near to the desired temperature, after which the low heating is done throughout the operation period to maintain the high temperature.
15. **Heating indicator:** It glows when the heating cycle goes on and the heating elements heat the oven.
16. **Timer:** It is used to set the required time, after which the oven switches off automatically.
17. **Thermostat:** It is used to set the temperature required. After the set temperature is reached, it automatically maintains the oven at that temperature constantly by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the oven. During the no-heating phase, heating by these elements is discontinued and the oven is allowed to cool by radiating heat to the surrounding.
18. **Asbestos door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated hot environment inside the oven, without being influenced by the outer environment.
19. **Perforated shelves:** The materials to be sterilised are kept on the shelves. The perforations ensure proper circulation of the hot air inside the oven. The shelves are removable.
20. **Door locking system:** It locks the door tightly to the cabinet.
21. **L-shaped thermometer:** It is fitted at the top panel of the oven. One of its arms having graduations for temperature reading, remains along the top panel, so that the temperature can be easily read and the other arm, which has the mercury bulb at its end, remains protruded slightly into the oven.

Figure 3.2: A conventional hot air oven

In a modern oven (Figure 3.3), there is a digital temperature display and automatic temperature controller to set the desired temperature easily. Time is set by a digital timer. After loading the glassware's, the door is closed and oven switched on.



1. **Mains:** It is the source of electric current for the equipment. The oven runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the oven against electric shock due to any short-circuit in the oven.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the oven and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the oven.
4. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the oven can be controlled.
5. **Power on / off switch**: It controls the entry of electric current into the oven.
6. **Air ventilator :** Air ventilator is a port (hole), through which the moisture-laden air escapes out from the oven.
7. **Cabinet:** It is the main body of the oven consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets while the inner wall is made of stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the oven and reduces consumption of electric current, thereby working the oven efficiently.
8. **Door:** It is the insulated wall, which closes the insulated cabinet of the oven.
9. **Hinge:** It holds the door to the cabinet.
10. **Handle:** It is used to open the door by pulling.
11. **Stand:** It supports the total weight of the oven along with its contents.
12. **Neoprene rubber door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated hot environment inside the oven, without being influenced by the outer environment.
13. **Perforated shelves:** The materials to be sterilised are kept on the shelves. The perforations ensure proper circulation of the hot air inside the oven. The shelves are removable.
14. **Door locking system:** It locks the door tightly to the cabinet.
15. **Circulating fan:** It is a small fan fitted inside the cabinet, which circulates the hot air inside the oven, thereby helping in uniform distribution of temperature throughout the oven.
16. **Digital timer:** It is used to set the required time, after which the oven switches off automatically.
17. **Digital time indicator:** It digitally displays the time left to reach the set (required) time.
18. **Digital temperature controller:** It is a microprocessor based PID digital temperature controller used to set the temperature required. After the set temperature is reached, it automatically maintains the oven at that temperature constantly. The oven maintains the temperature by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the oven. During the no-heating phase, heating by these elements is discontinued and the oven is allowed to cool by radiating heat to the surrounding.
19. **Digital temperature indicator:** It digitally displays the actual temperature inside the oven throughout its period of operation.
20. **Power indicator:** It indicates supply of electric current to the oven by glowing.
21. **Heating indicator:** The heating indicator glows when the heating cycle goes on and the heating elements heat the oven.

Figure 3.3: A modern hot air oven

The required temperature is set. After the oven attains the set temperature, the required time of sterilization is set on the timer. The oven switches off automatically after the set time. The oven is opened, only after its temperature comes down near to room temperature. Otherwise, if the door is opened, while the inside of the oven is still very hot, cold air may rush in and crack the glassware's.

2. Drying Oven:

For preparation of certain reagents, the glassware's, after proper cleaning and rinsing with distilled water, are required to be dried. They are dried inside the drying oven at 100°C till the glassware's dry up completely.

3. Autoclave:

Autoclave is the nucleus of a microbiology laboratory. It is used not only to sterilize liquid substances such as prepared media and saline (diluents) solutions, but also to sterilize glassware's, when required.

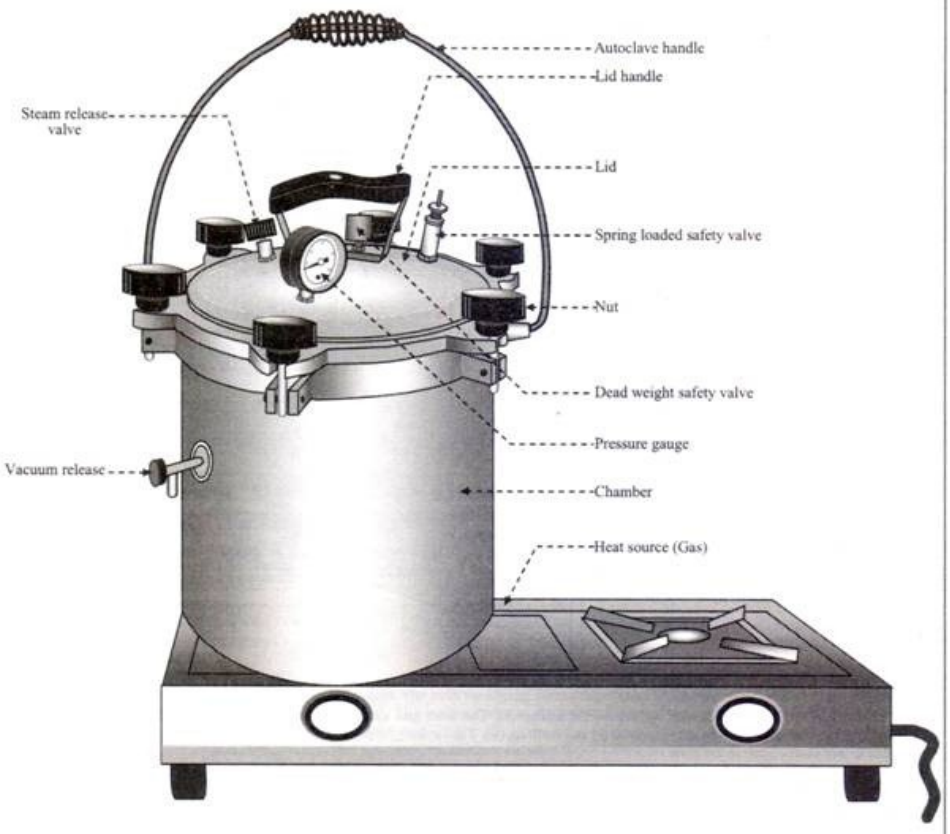
It has the same working principle as a domestic pressure cooker. The maximum temperature that can be obtained by boiling water in an open container is 100°C (boiling point of water).

This temperature is sufficient to kill only the non-spore formers, but it is difficult to kill the spore-forming bacteria at this temperature, as they escape by forming heat resistant spores. It takes very long time to kill the spores at this temperature.

On the other hand, when water is boiled in a closed container, due to increased pressure inside it, the boiling point elevates and steam temperature much beyond 100°C can be obtained. This high temperature is required to kill all the bacteria including the heat resistant spore-formers. Steam temperature increases with increase in steam pressure (Table 3.1).

Table 3.1: Temperatures attainable at different steam pressures:

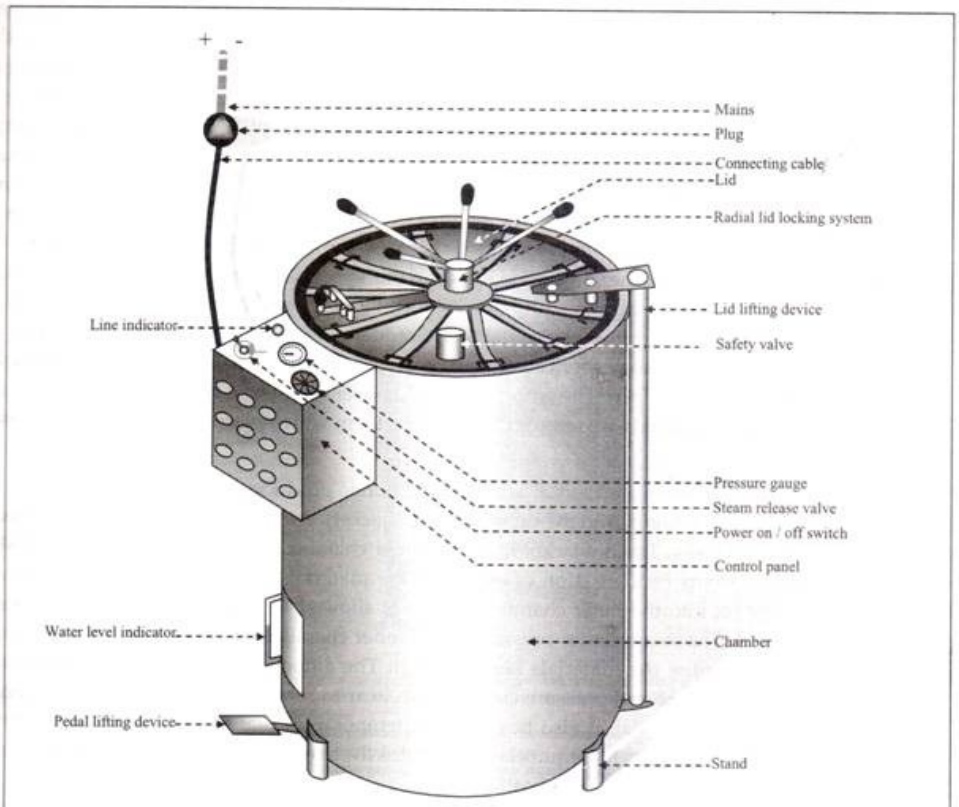
In operating a standard vertical autoclave, (Figure 3.4) sufficient water is poured into it. If water is too less, the bottom of the autoclave gets dried during heating and further heating damages it.



1. **Autoclave handle:** It is a metal rod bent to a half circle and hinged to the top of the chamber. It is used to move the chamber whenever required.
2. **Lid handle:** It is an insulated handle used to lift the lid and separate it from the chamber.
3. **Lid:** It is the cover, which closes the chamber of the autoclave. It is made of heavy gauge aluminium. A neoprene rubber gasket provides airtight seal between the chamber and the lid, thereby preventing leakage of steam to outside from the autoclave.
4. **Spring loaded safety valve:** It allows steam to escape, in case the pressure inside the autoclave becomes very high beyond the limit. Thus, it prevents bursting of the autoclave and thereby, helps in its safety.
5. **Nut:** These help in holding the lid tightly on the chamber so as to make it airtight and prevent leakage of steam from the autoclave to outside. These nuts are tightened manually after keeping the lid in position. Opposite nuts must be tightened simultaneously so as to make the autoclave perfectly airtight.
6. **Dead weight safety valve:** It blows out in case the pressure inside the autoclave becomes very high beyond the limit. Thus, it prevents bursting of the autoclave and thereby helps in the safety of the autoclave. It occurs when the pressure inside the autoclave goes beyond the capacity of the spring loaded safety valve.
7. **Pressure gauge**: It indicates the steam pressure inside the chamber.
8. **Chamber:** It is the main body of the autoclave, in which water is heated by an external heating source (LPG gas or electric heater) to produce steam. In the chamber, the materials to be sterilised are kept and sterilised at a steam pressure of 15 psi (121°C) for 15 minutes. It is made of heavy gauge aluminium. A stand is put at the bottom of the chamber on which the materials are to be kept. Pressure is adjustable from 10-20 psi. For safety reasons, all pressure vessels are tested hydraulically upto 40 psi.
9. **Vacuum release valve (Vacuum breaker):** It breaks the vacuum, if formed, inside the chamber due to condensation of steam when the hot autoclave is allowed to cool to room temperature in closed airtight condition.
10. **Steam release valve:** It is the knob, which is rotated to release steam from the chamber to outside after the sterilisation process. Using this knob, air is also allowed to escape from the chamber before sterilisation so that steam completely fills the chamber.
11. **Heat source:** Usually the external heat source is either gas or electric heater. In some portable autoclaves, in addition, there is an in-built electric heating system. In these autoclaves, a heating element is fixed on the wall of the chamber towards the bottom. It always remains submerged under water and heats it to generate steam.

Figure 3.4: A portable externally-heated autoclave

If it has in-built water heating element, (Figure 3.5) water level should be maintained above the element. On the other hand, if there is too much water, it takes long time to reach the required temperature.



1. **Mains:** It is the source of electric current for the equipment. The autoclave runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the autoclave against electric shock due to any short-circuit in the autoclave.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the autoclave and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the autoclave.
4. **Lid:** It is the heavy gauge stainless steel cover, which closes the chamber of the autoclave. A neoprene rubber gasket provides airtight seal between the chamber and the lid, thereby preventing leakage of steam from the autoclave to outside.
5. **Radial lid locking system:** It locks the lid tightly to the chamber.
6. **Lid lifting device:** It helps in lifting the heavy lid by pressing the paddle on the opposite side.
7. **Safety valve:** It blows out, in case the pressure inside the autoclave becomes very high beyond the limit. Thus, it prevents bursting of the autoclave and thereby, helps in its safety.
8. **Pressure gauge :** It indicates the steam pressure inside the chamber.
9. **Steam release valve :** It is the knob, which is rotated to release steam from the chamber to outside after the sterilisation process. Using this knob air is also allowed to escape from the chamber before sterilisation so that steam completely fills the chamber.
10. **Power on / off switch**: It controls the entry of electric current into the autoclave.
11. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the autoclave can be controlled.
12. **Chamber:** It is the main body of the autoclave consisting of a two-layered cylindrical enclosure. The space between the two layers is filled with glass wool to provide thermal insulation. Water is heated in this chamber by electric heating elements to produce steam. The insulation minimises heat loss from the autoclave and reduces consumption of electric current, thereby working the autoclave efficiently. In the chamber, the materials to be sterilised are kept and sterilised at a steam pressure of 15 psi (121°C) for 15 minutes. It is made of heavy gauge stainless steel. It is made leak proof by argon arc wielding, so as make the autoclave airtight during sterilisation. Pressure is adjustable from 10-20 psi. For safety reasons, all pressure vessels are tested hydraulically upto 40 psi.
13. **Stand:** It supports the total weight of the autoclave along with its contents.
14. **Pedal lifting device:** It is a pedal, which is pressed by leg to lift the heavy lid by the lid lifting device located on the opposite side.
15. **Water level indicator:** The level of water must remain sufficiently above the heating element. If water level goes down too much exposing the heating elements, dry heating damages them. The water level indicator indicates the level of water inside the autoclave, so that water can be poured into the autoclave before the heating elements get exposed
16. **Line indicator:** It indicates supply of electric current to the autoclave by glowing.

Figure 3.5: A conventional vertical autoclave

The materials to be sterilized are covered with craft paper and arranged on an aluminium or wooden frame kept on the bottom of the autoclave, otherwise if the materials remain half-submerged or floating, they tumble during boiling and water may enter. The autoclave is closed perfectly airtight only keeping the steam release valve open.

Then, it is heated over flame or by the in-built heating element. Air inside the autoclave should be allowed to escape completely through

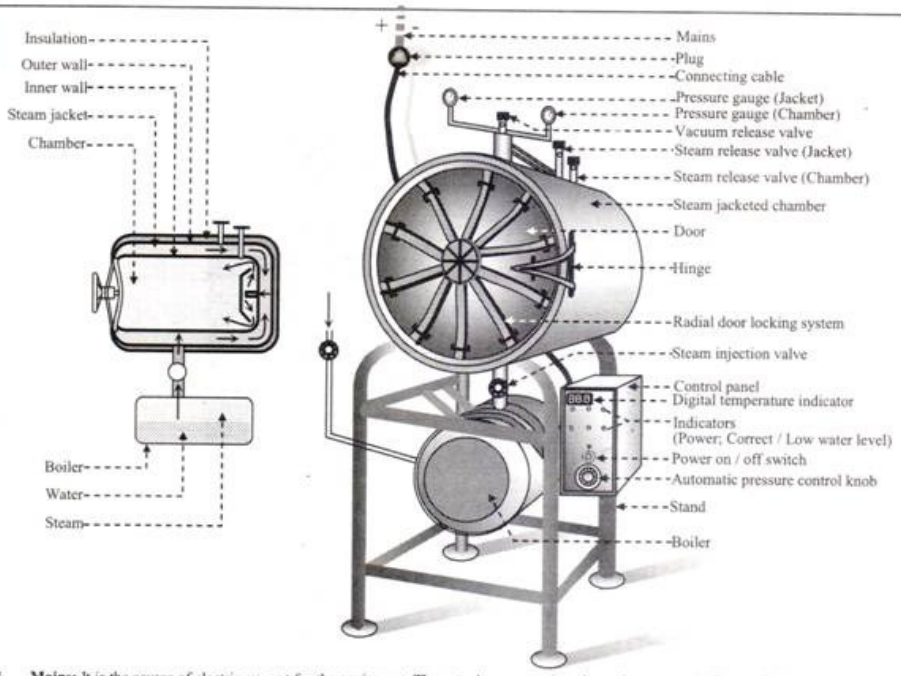
this valve. When water vapour is seen to escape through the valve, it is closed.

Temperature and pressure inside goes on increasing. The pressure increase is observed on the pressure dial. Usually sterilization is done at 121 °C (a pressure of 15 pounds per square inch i.e. 15 psi) for 15 minutes. The required time is considered from the point, when the required temperature-pressure is attained.

Once required temperature-pressure is attained, it is maintained by controlling the heating source. After the specified time (15 minutes), heating is discontinued and steam release valve slightly opened. If fully opened immediately, due to sudden fall in pressure, liquids may spill out from the containers.

Gradually, the steam release is opened more and more, so as to allow all steam to escape. The autoclave is opened only after the pressure drops back to normal atmospheric pressure (0 psi). The autoclave should never be opened, when there is still pressure inside. The hot sterilized materials are removed by holding them with a piece of clean cloth or asbestos- coated hand gloves.

In case of a steam-jacketed horizontal autoclave, a boiler produces the steam (Figure 3.6). It is released at a designated pressure, into the outer chamber (jacket). Air is allowed to escape and then its steam release valve is closed.



1. **Mains:** It is the source of electric current for the equipment. The autoclave runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the autoclave against electric shock due to any short-circuit in the autoclave.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the autoclave and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the autoclave.
4. **Pressure gauge (Jacket):** It indicates the steam pressure inside the outer chamber (jacket).
5. **Pressure gauge (Chamber):** It indicates the steam pressure inside the chamber.
6. **Vacuum release valve (Vacuum breaker):** It breaks the vacuum inside the chamber, in case vacuum is formed due to condensation of steam.
7. **Steam release valve (Jacket):** Steam that enters from the boiler into the jacket, removes the air present inside it through this valve. After the sterilisation process, steam present in the jacket is also allowed to escape to outside through this valve.
8. **Steam release valve (Chamber):** Steam that enters from the jacket into the chamber, removes the air present inside it through this valve. After the sterilisation process, steam present in the chamber is also allowed to escape to outside through this valve.
9. **Steam-jacketed chamber:** It is the main body of the autoclave, consisting of a double-walled cylindrical enclosure. The outer wall encloses the steam jacket. It is again two-layered and is made of heavy gauge stainless steel. The space between the two layers is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the autoclave and reduces consumption of electric current, thereby working the autoclave efficiently. The inner wall encloses the chamber, in which the materials to be sterilised are kept and sterilised at a steam pressure of 15 psi (121°C) for 15 minutes. It is made of heavy gauge stainless steel. The chamber and jacket are made leak proof by argon arc wielding, so as make the autoclave airtight during sterilisation. Pressure is adjustable from 10-20 psi. For safety reasons, all pressure vessels are tested hydraulically upto 40 psi.
10. **Door:** It is the heavy gauge stainless steel lid, which closes the chamber of the autoclave. A neoprene rubber door gasket provides airtight seal between the chamber and the door, thereby preventing leakage of steam to outside from the autoclave.
11. **Hinge:** It holds the door to the chamber.
12. **Radial door locking system:** It locks the door tightly to the chamber. It also has an automatic shutting system, which does not allow the door to be opened, unless the temperature and pressure comes down near to room conditions.
13. **Steam injection valve:** Steam enters from the boiler into the steam jacket through this valve.
14. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the autoclave can be controlled.
15. **Digital temperature indicator:** It digitally displays the actual temperature inside the chamber throughout its period of operation.
16. **Indicators:** The power indicator indicates supply of electric current to the autoclave by glowing. The correct / low water level indicators glow according to the level of water in the boiler.
17. **Power on / off switch**: It controls the entry of electric current into the autoclave.
18. **Automatic pressure control knob:** It is used to set the pressure required. After the set pressure is reached, it automatically maintains the autoclave at that steam pressure constantly by alternate closing and opening of the valve, injecting steam into the chambers from the jacket.
19. **Stand:** It supports the total weight of the autoclave along with its contents.
20. **Boiler:** It is an airtight horizontal cylindrical enclosure, in which water is allowed to boil to produce steam. It is located below the steam jacketed chamber. It receives water form the water supply system and heats it by electrical heating elements. The steam produced goes to the steam jacket through the steam injection valve. It is fitted with water level indicator, pressure gauge, low water level cut-off device and automatic pressure control device.

Figure 3.6: A modern steam-jacketed horizontal autoclave

The hot jacket heats the inner chamber, thereby heating the materials to be sterilised. This prevents condensation of steam on the materials. Now, steam under pressure is released from the jacket into the inner chamber and air is allowed to escape from it.

Then, its steam release valve is closed. The steam under pressure in the inner chamber reaches temperatures in excess of 100°C, which can sterilise the materials kept inside it. The autoclave also has

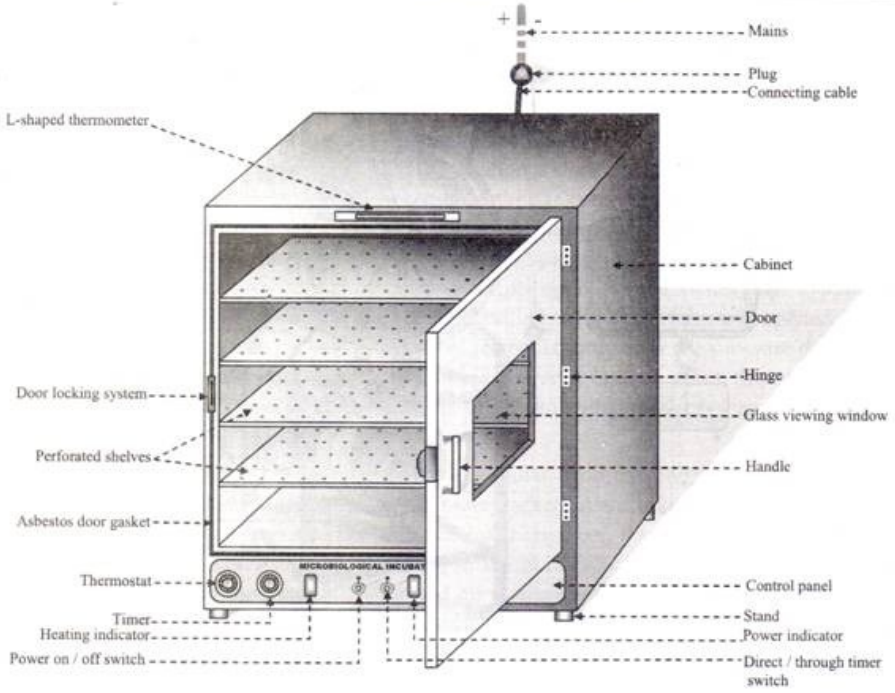
automatic shutting system i.e. unless temperature and pressure comes down near to room conditions, the door cannot be opened.

Besides the pressure dial, it also has separate temperature dial to indicate the temperature inside the inner chamber. Moreover, the autoclave maintains the temperature and pressure automatically and switches off after the set time of sterilization.

4. Microbiological Incubator:

Profuse growth of microbes is obtained in the laboratory by growing them at suitable temperatures. This is done by inoculating the desired microbe into a suitable culture medium and then incubating it at the temperature optimum for its growth.

Incubation is done in an incubator (Figure 3.7), which maintains a constant temperature specifically suitable for the growth of a specific microbe. As most of the microbes pathogenic to man grow profusely at body temperature of normal human being (i.e. 37°C), the usual temperature of incubation is 37°C.



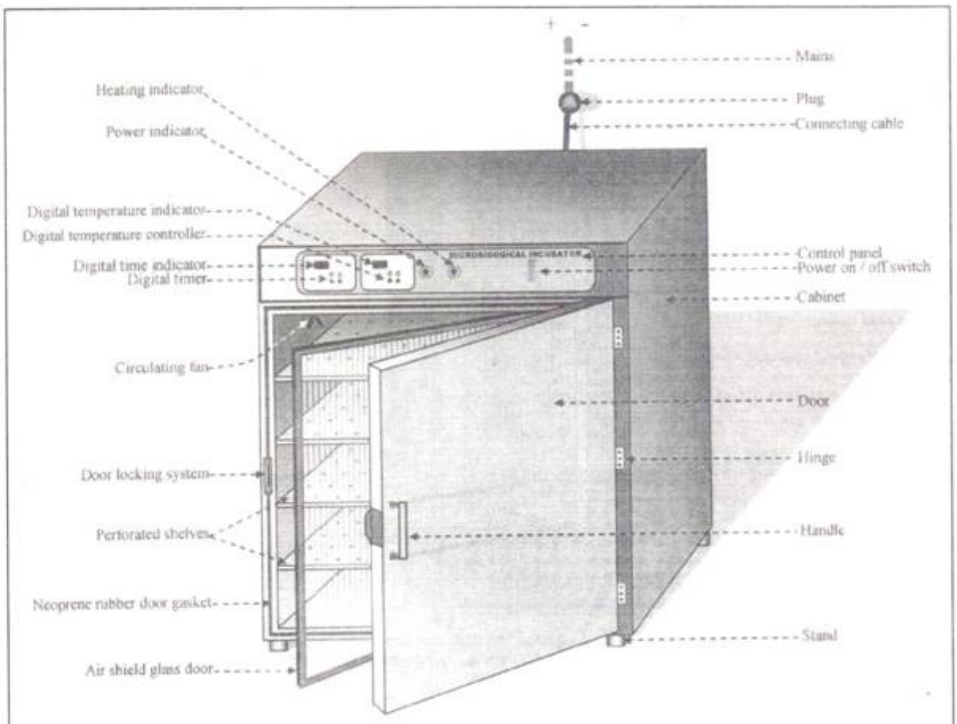
1. **Mains:** It is the source of electric current for the equipment. The incubator runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the incubator against electric shock due to any short-circuit in the incubator.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the incubator and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the incubator.
4. **Cabinet:** It is the main body of the incubator consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets while the inner wall is made of anodised aluminium or stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the incubator and reduces consumption of electric current, thereby working the incubator efficiently.
5. **Door:** It is the insulated wall, which closes the insulated cabinet of the incubator.
6. **Hinge:** It holds the door to the cabinet.
7. **Glass viewing door:** It enables visualisation of the interior of the incubator during incubation without disturbing the interior environment.
8. **Handle:** It is used to open the door by pulling.
9. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the incubator can be controlled.
10. **Stand:** It supports the total weight of the incubator along with its contents.
11. **Power indicator:** It indicates supply of electric current to the incubator by glowing.
12. **Direct / through timer switch:** This switch gives option to operate the oven directly or through the timer. In case of direct operation, the oven has to be switched off after the required time. Through timer option operates the oven through the timer, which starts functioning and switches off the oven after the set time.
13. **Power on / off switch**: It controls the entry of electric current into the incubator.
14. **Heating indicator:** The heating indicator glows when the heating cycle goes on and the heating elements heat the incubator.
15. **Timer:** It is used to set the required time, after which the incubator switches off automatically.
16. **Thermostat:** It is used to set the temperature required. After the set temperature is reached, it automatically maintains the incubator at that temperature constantly by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the incubator. During the no-heating phase, heating by these elements is discontinued and the incubator is allowed to cool by radiating heat to the surrounding.
17. **Asbestos door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated hot environment inside the incubator without being influenced by the outer environment.
18. **Perforated shelves:** The microbes to be cultured, after inoculation to proper growth media, are kept on the shelves. The perforations ensure proper circulation of the hot air inside the incubator. The shelves are removable.
19. **Door locking system:** It locks the door tightly to the cabinet.
20. **L-shaped thermometer:** It is fitted at the top panel of the oven. One of its two arms, having graduations for temperature reading, remains along the top panel, so that the temperature can be easily read and the other arm, which has the mercury bulb at its end, remains protruded slightly into the chamber of the incubator.

Figure 3.7: A conventional microbiological incubator

The incubator has a thermostat, which maintains a constant temperature, set according to requirement. The temperature reading on the thermostat is approximate. Accurate temperature can be seen on the thermometer fixed on the incubator. Exact temperature, as per requirement, is set by rotating the thermostat knob by trial and error and noting the temperature on the thermometer.

Most of the modern incubators (Figure 3.8) are programmable, which do not need trial and error temperature setting. Here, the

operator sets the desired temperature and the required period of time.



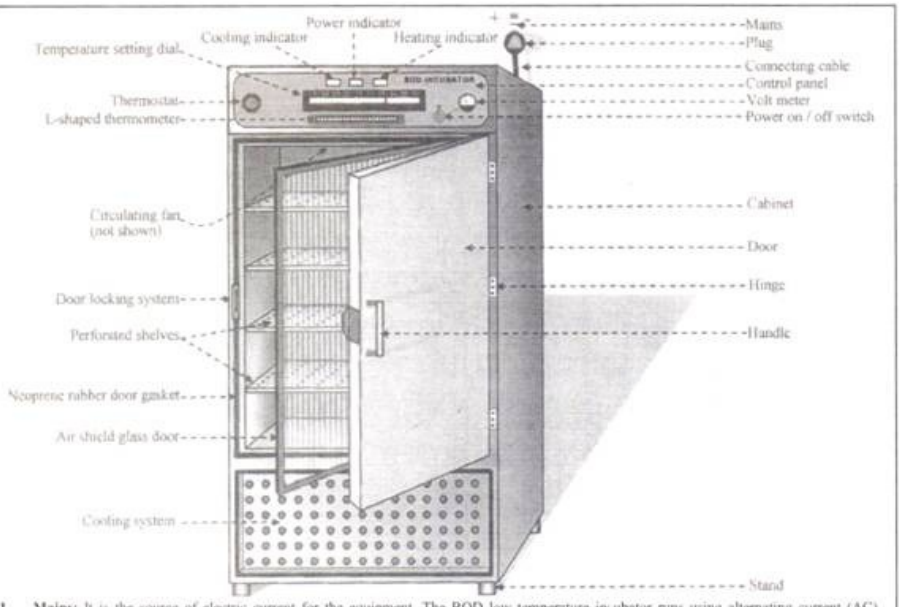
1. **Mains:** It is the source of electric current for the equipment. The incubator runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the incubator against electric shock due to any short-circuit in the incubator.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the incubator and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the incubator.
4. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the incubator can be controlled.
5. **Power on / off switch**: It controls the entry of electric current into the incubator.
6. **Cabinet:** It is the main body of the incubator consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets, while the inner wall is made of stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the incubator and reduces consumption of electric current, thereby working the incubator efficiently.
7. **Door:** It is the insulated wall, which closes the insulated cabinet of the incubator.
8. **Hinge:** It holds the door to the cabinet.
9. **Handle:** It is used to open the door by pulling.
10. **Stand:** It supports the total weight of the incubator along with its contents.
11. **Air shield glass door:** This glass door enables clear visualisation of the interior of the incubator during incubation and at the same time prevents draft of cool outside air from entering in and reducing its temperature below the set temperature.
12. **Neoprene rubber door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated hot environment inside the incubator without being influenced by the outer environment.
13. **Perforated shelves:** The microbes to be cultured, after inoculation to proper growth media, are kept on the shelves. The perforations ensure proper circulation of the hot air inside the incubator. The shelves are removable.
14. **Door locking system:** It locks the door tightly to the cabinet.
15. **Circulating fan:** It is a small fan fitted inside the cabinet, which circulates the hot air inside the incubator, thereby helping in uniform distribution of temperature throughout the incubator.
16. **Digital timer:** It is used to set the required time, after which the incubator switches off automatically.
17. **Digital time indicator:** It digitally displays the time left to reach the set (required) time.
18. **Digital temperature controller:** It is a microprocessor based PID digital temperature controller used to set the temperature required. After the set temperature is reached, it automatically maintains the incubator at that temperature constantly. The incubator maintains the temperature by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the incubator. During the no-heating phase, heating by these elements is discontinued and the incubator is allowed to cool by radiating heat to the surrounding.
19. **Digital temperature indicator:** It digitally displays the actual temperature inside the incubator throughout its period of operation.
20. **Power indicator:** It indicates supply of electric current to the incubator by glowing.
21. **Heating indicator:** The heating indicator glows when the heating cycle goes on and the heating elements heat the incubator.

Figure 3.8: A modern microbiological incubator

The incubator automatically maintains it accordingly. Moisture is supplied by placing a beaker of water in the incubator during the growth period. A moist environment retards the dehydration of the media and thereby, avoids spurious experimental results.

5. BOD Incubator (Low Temperature Incubator):

Some microbes are to be grown at lower temperatures for specific purposes. The BOD low temperature incubator (Figure 3.9), which can maintain temperatures from 50°C to as low as 2-3°C is used for incubation in such cases.

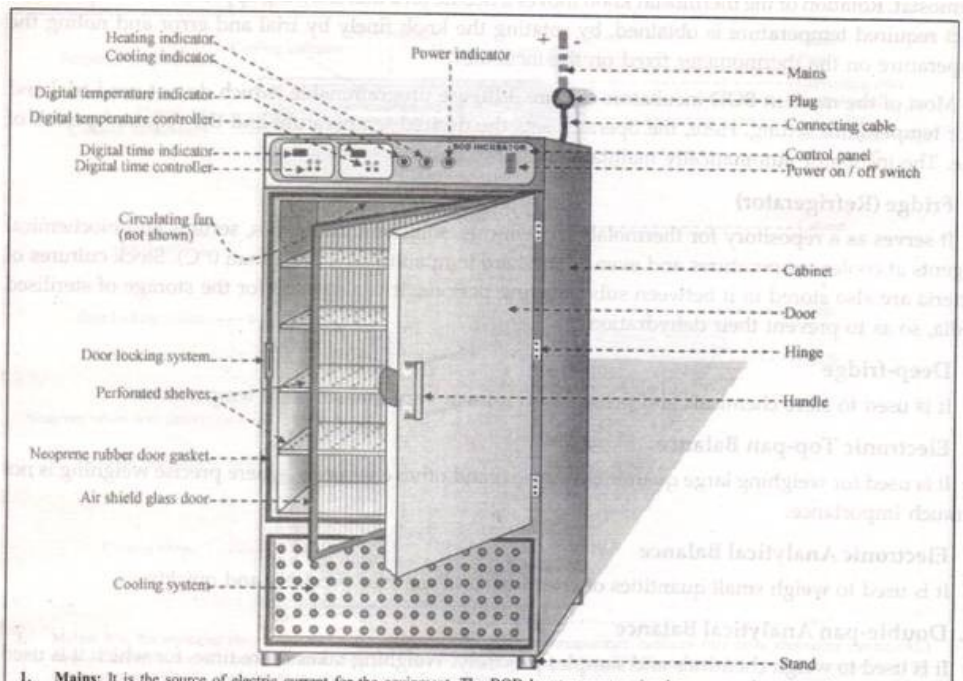


1. **Mains:** It is the source of electric current for the equipment. The BOD low temperature incubator runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the incubator against electric shock due to any short-circuit in the incubator.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the low temperature incubator and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the incubator.
4. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the incubator can be controlled.
5. **Volt meter:** It indicates the voltage of the AC current going into the incubator.
6. **Power on / off switch**: It controls the entry of electric current into the incubator.
7. **Cabinet:** It is the main body of the incubator consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets, while the inner wall is made of stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the incubator and reduces consumption of electric current, thereby working the incubator efficiently.
8. **Door:** It is the insulated wall, which closes the insulated cabinet of the incubator.
9. **Hinge:** It holds the door to the cabinet.
10. **Handle:** It is used to open the door by pulling.
11. **Stand:** It supports the total weight of the incubator along with its contents.
12. **Cooling system:** It reduces the temperature of the incubator, when it has to be maintained below the ambient temperature.
13. **Air shield glass door:** This glass door enables clear visualisation of the interior of the incubator during incubation and at the same time prevents draft of outside air from entering in and changing its temperature away from the set temperature.
14. **Neoprene rubber door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated environment inside the incubator without being influenced by the outer environment.
15. **Perforated shelves:** The microbes to be cultured, after inoculation to proper growth medium, are kept on the shelves. The perforations ensure proper circulation of the air inside the incubator. The shelves are removable.
16. **Door locking system:** It locks the door tightly to the cabinet.
17. **Circulating fan:** It is a small fan fitted inside the cabinet, which circulates the air inside the incubator, thereby helping in uniform distribution of temperature throughout the incubator.
18. **L-shaped thermometer:** It is fitted at the top panel of the incubator. One of its two arms, having graduations for temperature reading, remains along the top panel, so that the temperature can be easily read and the other arm, which has the mercury bulb at its end, remains protruded slightly into the chamber of the incubator.
19. **Thermostat:** It is used to set the temperature required. After the set temperature is reached, it automatically maintains the incubator at that temperature constantly. If the desired temperature is above the ambient temperature, then the incubator maintains it by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the incubator. During the no-heating phase, heating by these elements is discontinued and the incubator is allowed to cool by radiating heat to the surrounding. On the other hand, if the desired temperature is below the ambient temperature, then the incubator maintains it by alternate refrigeration and no-refrigeration cycles. During the refrigeration phase, the cooling coils cool the incubator. During the no-refrigeration phase, cooling by these cooling coils is discontinued and the incubator is allowed to warm up by absorbing heat from the surrounding.
20. **Temperatare setting dial:** When the thermostat is rotated to set the temperature, a needle moves on the temperature setting dial and indicates the set temperature.
21. **Cooling indicator:** The cooling indicator glows, when the refrigeration cycle goes on and the cooling coils cool the incubator.
22. **Power indicator:** It indicates supply of electric current to the incubator by glowing.
23. **Heating indicator:** The heating indicator glows, when the heating cycle goes on and the heating elements heat the incubator.

Figure 3.9: A conventional BOD low temperature incubator

The constant desired temperature is set by rotating the knob of the thermostat. Rotation of the thermostat knob moves a needle on a dial showing approximate temperature. Exact required temperature is obtained, by rotating the knob finely by trial and error and noting the temperature on the thermometer fixed on the incubator.

Most of the modern BOD incubators (Figure 3.10) are programmable, which do not need trial and error temperature setting. Here, the operator sets the desired temperature and the required period of time. The incubator automatically maintains it accordingly.



1. **Mains:** It is the source of electric current for the equipment. The BOD low temperature incubator runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the incubator against electric shock due to any short-circuit in the incubator.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the low temperature incubator and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the incubator.
4. **Control panel:** It is the panel or switchboard containing all the switches and indicators, with which the functions of the incubator can be controlled.
5. **Power on / off switch:** It controls the entry of electric current into the incubator.
6. **Cabinet:** It is the main body of the incubator consisting of a double-walled cuboidal enclosure. The outer wall is made of mild steel sheets, while the inner wall is made of stainless steel. From the inner wall, there are inward projections (battens) to support the sliding shelves. The space between the two walls is filled with glass wool to provide thermal insulation. The insulation minimises heat loss from the incubator and reduces consumption of electric current, thereby working the incubator efficiently.
7. **Door:** It is the insulated wall, which closes the insulated cabinet of the incubator.
8. **Hinge:** It holds the door to the cabinet.
9. **Handle:** It is used to open the door by pulling.
10. **Stand:** It supports the total weight of the incubator along with its contents.
11. **Cooling system:** It reduces the temperature of the incubator, when it has to be maintained below the ambient temperature.
12. **Air shield glass door:** This glass door enables clear visualisation of the interior of the incubator during incubation and at the same time prevents draft of outside air from entering in and changing its temperature away from the set temperature.
13. **Neoprene rubber door gasket:** It provides an almost airtight seal between the cabinet and the door, thereby preventing the outside air from entering into the cabinet. This ensures an isolated environment inside the incubator without being influenced by the outer environment.
14. **Perforated shelves:** The microbes to be cultured, after inoculation to proper growth medium, are kept on the shelves. The perforations ensure proper circulation of the air inside the incubator. The shelves are removable.
15. **Door locking system:** It locks the door tightly to the cabinet.
16. **Circulating fan:** It is a small fan fitted inside the cabinet, which circulates the hot air inside the incubator, thereby helping in uniform distribution of temperature throughout the incubator.
17. **Digital timer:** It is used to set the required time after which the incubator switches off automatically.
18. **Digital time indicator:** It digitally displays the time left to reach the set (required) time.
19. **Digital temperature controller:** It is used to set the temperature required. After the set temperature is reached, it automatically maintains the incubator at that temperature constantly. If the desired temperature is above the ambient temperature, then the incubator maintains it by alternate heating and no-heating cycles. During the heating phase, the heating elements heat the incubator. During the no-heating phase, heating by these elements is discontinued and the incubator is allowed to cool by radiating heat to the surrounding. On the other hand, if the desired temperature is below the ambient temperature, then the incubator maintains it by alternate refrigeration and no-refrigeration cycles. During the refrigeration phase, the cooling coils cool the incubator. During the no-refrigeration phase, cooling by these cooling coils is discontinued and the incubator is allowed to warm up by absorbing heat from the surrounding.
20. **Digital temperature indicator:** It digitally displays the actual temperature inside the incubator throughout its period of operation.
21. **Cooling indicator:** The cooling indicator glows, when the refrigeration cycle goes on and the cooling coils cool the incubator.
22. **Heating indicator:** The heating indicator glows, when the heating cycle goes on and the heating elements heat the incubator.
23. **Power indicator:** It indicates supply of electric current to the incubator by glowing.

Figure 3.10: A modern BOD low temperature incubator

6. Fridge (Refrigerator):

It serves as a repository for thermo labile chemicals, solutions, antibiotics, serums and biochemical reagents at cooler temperatures

and even at sub-zero temperatures (at less than 0°C). Stock cultures of bacteria are also stored in it between sub-culturing periods. It is also used for the storage of sterilized media, so as to prevent their dehydration.

7. Deep-fridge:

It is used to store chemicals and preserve samples at very low sub-zero temperatures.

8. Electronic Top-pan Balance:

It is used for weighing large quantities of media and other chemicals, where precise weighing is not of much importance.

9. Electronic Analytical Balance:

It is used to weigh small quantities of chemicals and samples precisely and quickly.

10. Double-pan Analytical Balance:

It is used to weigh chemicals and samples precisely. Weighing takes more time, for which it is used in emergency only.

11. Distilled Water Plant:

Water is used in the preparation of media and reagents. If the media are prepared using tap water, the chemical impurities present in it may interfere with the growth of the microorganisms in the media. Moreover, the higher is the bacteria content of the media, the longer is the time required for their sterilization and greater is the chance of survival of some bacteria.

Distilled water, though not bacteria- free, contains less number of bacteria. That is why; it is preferred in the preparation of microbiological media. It is also used in the preparation of reagents, because the chemical impurities present in tap water may interfere with the proper functioning of the reagent chemicals.

As manufacture of distilled water by Liebig condenser is a time-taking process, in most laboratories, it is prepared by 'distilled water

plants'. Usually a distilled water plant is made of steel or brass. It is also called distilled water still.

It has one inlet to be connected to the water tap and two outlets, one for distilled water to drop into a container and the other for the flow out of hot cooling water into the sink. The still is installed on the wall. It is heated by in-built electric heating elements (immersion heater).

The still works efficiently, when the water in-flow is so adjusted that, the temperature of the cooling water flowing out from the still into the sink is neither too high nor too low i.e., warm water should flow out. The distilled water may contain traces of metals corroded from the steel or brass container.

To get metal-free distilled water, glass distillation apparatus is used and still better is quartz distillation apparatus. However, for a microbiology laboratory, a steel or glass distillation apparatus is sufficient. For precision analyses, double- or triple- distilled water is used.

12. Ultrapure Water Purification System:

For precision analytical works, now-a-days, instead of using double- or triple-distilled water, micro- filtered water is used. In case of distilled water, there is chance that, few volatile substances present in the water get volatilized during heating of the water and subsequently get condensed into the distilled water collected.

Thus, there may be traces of such substances in the distilled water. To overcome this, ultrapure water is used. Here, water is allowed to pass through very fine microscopic pores, which retain the microscopic suspended particle including the microbes.

Then, the water passes through two columns of ion exchange resins. The anion exchange resin adsorbs the captions present in the water, while the caption exchange resin adsorbs the anions. The water that comes out is extremely pure.

13. Homogeniser:

For microbiological analysis, liquid samples are directly used, whereas solid samples have to be mixed thoroughly with a diluents (usually physiological saline), so as to get a homogenous suspension of bacteria. This suspension is assumed to contain bacteria homogenously.

The mixing of solid samples and diluents is done by a homogenizer, in which a motor rotates an impeller with sharp blades at high speed inside the closed homogenizer cup containing the sample and the diluents. It has a speed regulator for controlling the speed of rotation of the impeller.

In some laboratories mixing is done manually by sterilized pestle and mortar. In modern laboratories, a disposable bag is used, inside which the solid sample and liquid diluents are put aseptically and mixed mechanically by peristaltic action of a machine on the bag. This machine is called stomacher.

14. pH Meter:

A pH meter is an instrument for determining the pH of liquid media, liquid samples and buffers. It has a glass pH electrode. When not in use, it should be kept half immersed in water contained in a small beaker and preferably be covered by a bell jar to avoid dust accumulation in the water and loss of water through evaporation.

Before use, the meter is calibrated using two standard buffers of known pH. Usually buffers of pH 4.0, 7.0 and 9.2 are available commercially. The instrument is switched on and left for 30 minutes to warm up. The temperature calibration knob is rotated to the temperature of the solutions whose pH is to the measured.

Then, the electrode is dipped into the buffer (pH 7.0). If the reading is not 7.00, the pH calibration knob is rotated till the reading is 7.00. Then, the electrode is dipped in another buffer (pH 4.0 or 9.2).

If the reading is same as the pH of the buffer used, the instrument is working properly. Otherwise, the electrode is activated by dipping in 0.1 N HC1 for 24 hours. After calibration, the pH of samples is

determined by dipping the electrode into them and noting the reading.

Every time, before dipping into any solution, the electrode should be rinsed with distilled water. The samples should not contain any suspended sticky materials, which may form a coating on the tip of the electrode and reduce its sensitivity.

The old model pH meters have double electrodes (one of them acting as reference electrode), while new models have single combined electrode. Moreover, to overcome the problem of temperature correction, now pH meters with automatic temperature correction are available.

Here, another 'temperature electrode' is also put into the solution along with the pH electrode, which measures the temperature of the solution and automatically corrects the influence of temperature variations.

Sophisticated pH meters have single gel electrode. Such electrodes have very little chance of breakage, as they are almost completely enclosed in a hard plastic casing except at the tip. The tip has both pH and temperature sensors.

Moreover, they are easy to maintain, as they do not require constant dipping in distilled water, because the electrode tip is closed with a plastic cap containing saturated solution of potassium chloride, when not in use. However, in preparation of microbiological media, pH is determined by narrow-range pH papers and is adjusted to the required pH by adding acids or alkalis as required.

15. Hot Plate:

Hot plate is used to heat chemicals and reagents. The hot plate is made of an iron plate, which gets heated by an electric heating element from below. The required degree of heating is obtained by a regulator.

16. Shaking Water Bath:

Sometimes, heating at very precise temperatures is required. Such precise temperatures cannot be obtained in an incubator or oven, in which temperature fluctuates, though slightly. However, precise temperatures can be maintained in a water bath, which provides a stable temperature.

A water bath consists of a container containing water, which is heated by electric heating elements. The required water temperature is obtained by increasing or decreasing the rate of heating by rotating the thermostat by trial and error.

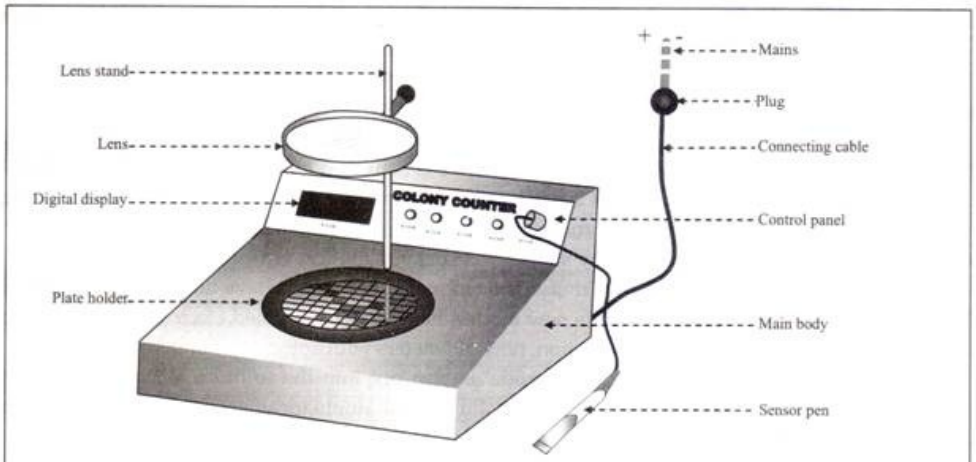
In a shaking water bath, the substance is heated at the required temperature and at the same time, it is shaken constantly. Shaking is done by a motor, which rotates and moves the containers to and fro in each rotation. The rate of shaking is again controlled by a regulator. Shaking agitates the substance and enhances the rate of the process.

Most modern water baths are programmable and do not need trial and error temperature setting. A desired water temperature can be maintained over a desired period of time by programming accordingly. It is used for cultivation of bacteria in broth medium at a specific temperature.

17. Quebec Colony Counter:

In enumeration of bacteria in samples, it is assumed that a single bacterium gives rise to a single visible colony, when grown on a plate of solidified nutrient medium. Thus, by counting the number of colonies, the number of bacteria in a sample can be estimated.

Sometimes, colonies are very small and too much crowded making it difficult to count. Counting becomes easy, when a mechanical hand counter, called Quebec colony counter (Figure 3.11), is used. It divides the plate into several square divisions and the colonies are magnified 1.5 times by a magnifying glass, which makes counting easy.



1. **Mains:** It is the source of electric current for the colony counter. The equipment runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the equipment against electric shock due to any short-circuit.
2. **Plug:** A three-pin plug (5 ampere capacity) provides non-sparking interface between the equipment and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the equipment.
4. **Control panel:** It is the panel or switchboard containing all the switches and indicators with which the functions of the colony counter can be controlled.
5. **Main body:** It encloses the parts of the equipment. It is made of metal sheets.
6. **Sensor pen:** It is an electronic sensor, which passes an electric impulse to the equipment, each time its tip touches the surface of the petri dish containing colonies of bacteria. Its tip is touched once on each colony. The inking tip marks the counted colonies, thereby helping in proper counting.
7. **Plate holder:** It is a circular area, which tightly holds the plate containing colonies of bacteria. The plate is kept here upside down so that the colonies can be seen and marked on the glass surface by the sensor pen. The area can be illuminated from below for clear visibility of the colonies. The area is also divided into several small squares to help in the counting process.
8. **Digital display:** It displays the number of colonies. Each time the tip of the sensor pen touches the surface of the plate, it increases by one.
9. **Lens:** A convex lens fitted above the plate holder magnifies the area 1.5 times thereby helping in counting very small colonies.
10. **Lens stand:** It holds the lens in position. The height of the lens above the plate can be adjusted.

Figure 3.11: A Quebec colony counter

18. Electronic Colony Counter:

Electronic colony counter is of two types:

(1) Hand-held electronic colony counter and

(2) Table-top electronic colony counter.

The hand-held electronic colony counter is a pen-style colony counter with an inking felt-tip marker. For counting of colonies of bacteria grown in a petri dish, it is kept in an inverted position, so that the colonies are visible through the bottom surface of the petri dish.

The colonies are marked by touching the glass surface of the petri dish with the felt-tip of the colony counter. Thus, each colony is marked by a dot made by the ink of the felt-tip on the bottom surface of the petri dish. In a single motion, the electronic colony counter marks, counts and confirms with a beep sound.

The cumulative count of colonies is displayed on a four-digit LED display. In case of table-top electronic colony counter, the petri dish

containing the colonies of bacteria is placed on an illuminated stage and the count bar is depressed. The precise number of colonies is instantly displayed on a digital read out.

19. Magnetic Stirrer:

In the preparation of solutions, certain chemicals require stirring for long time, to be dissolved in certain solvents. Magnetic stirrer is used to dissolve such substances easily and quickly. A small teflon- coated magnet, called 'stirring bar', is put into a container containing the solvent and the solute.

Then, the container is placed on the platform of the magnetic stirrer, below which a magnet rotates at high speed by a motor. Attracted by the rotating magnet, the teflon-coated magnet rotates inside the container and stirs the contents. Now, the solute dissolves quickly.

The teflon coating prevents the magnet from reacting with the solution, which comes in contact with it. After complete dissolution, the teflon-coated magnet is removed from the solution by mean of a long retriever, called 'stirring bar retriever'.

20. Sonicator:

It is used to rupture cells using high frequency waves.

21. Vortex Mixer:

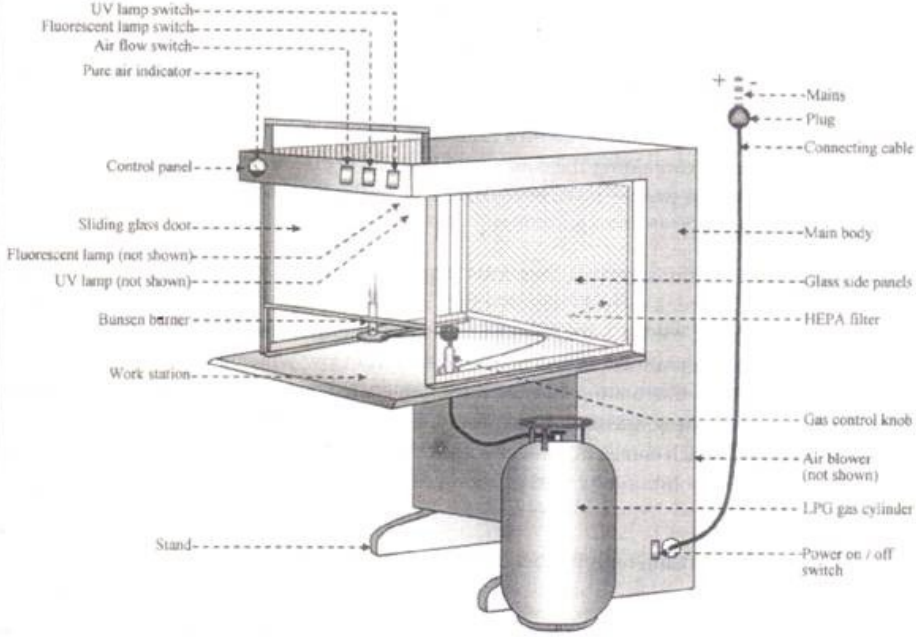
It is an instrument used for thorough mixing of liquids in test tubes. It has a rotor, whose speed can be controlled. On the tip of the rotor is a foam-rubber top. When the bottom of a test tube is pressed upon this foam-rubber top, the rotor starts rotating, thereby rotating the bottom of the test tube at high speed.

Due to centripetal force, the solution gets mixed thoroughly. It is particularly helpful during serial dilution in enumeration of bacteria, which needs homogenous suspension of bacteria cells.

21. Laminar Flow Chamber:

It is a chamber (Figure 3.12) used for aseptic transfer of sterilized materials, as well as for inoculation of microbes. Dust particles

floating in the air harbour microbes. These microbe-laden dust particles may enter into the sterilized media and contaminate them, when they are opened for short periods of time during inoculation of microbe or transfer from one container to another.



1. **Mains:** It is the source of electric current for the laminar flow chamber. The equipment runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the equipment against electric shock due to any short-circuit.
2. **Plug:** A three-pin plug (15 ampere capacity) provides non-sparking interface between the equipment and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the equipment.
4. **Main body:** It encloses the parts of the equipment. It is made of metal sheets or laminated wood.
5. **Glass side panels:** The two glass side panels help in viewing the interior of the chamber.
6. **HEPA filter:** The high efficiency particulate air filter (HEPA filter), fixed at the backside of the chamber, filters out the dust particles, thereby allowing dust-free air to enter into the chamber in a laminar manner from the rear side towards the front door.
7. **Gas control knob:** It is fixed to the workstation and controls the flow of gas from the cylinder to the burner.
8. **Air blower:** It blows the air at high speed, which passes through the HEPA filter to produce a laminar flow.
9. **LPG gas cylinder:** It provides gas to the burner.
10. **Power on / off switch:** : It controls the entry of electric current into the equipment.
11. **Stand:** It supports the total weight of the autoclave along with its contents.
12. **Work station:** It is the rectangular bottom of the chamber on which the things required during aseptic transfer are kept.
13. **Bunsen burner:** It provides the flame for the flame-sterilisation of needles and loops as well as of mouths of containers.
14. **UV lamp:** It is fitted to the top panel. It is switched on for 10 minutes after closing the front sliding door to sterilise the interior of the chamber.
15. **Fluorescent lamp:** It is fitted to the top panel. It lightens the interior of the chamber.
16. **Sliding glass door:** It closes the chamber when not in operation.
17. **Control panel**: It is the panel or switchboard containing all the switches and indicators, with which the functions of the laminar flow chamber can be controlled.
18. **Pure air indicator:** It indicates the purity of the air entering into the chamber through the HEPA filter, based on which the filter is removed and cleaned periodically.
19. **Air flow switch:** It controls the entry of electric current into the air blower.
20. **Fluorescent lamp switch:** It controls the entry of electric current into the fluorescent lamp.
21. **UV lamp switch:** It controls the entry of electric current into the UV lamp.

Figure 3.12: A laminar flow chamber

To overcome this, when inoculation is done in open air, the air of the small inoculating area is sterilized by the flame of a bunsen burner. The heated air becomes light and moves upwards, thereby

preventing the dust particles from falling on the media during the short opening process.

To further reduce the chance of contamination by the microbe-laden air, a laminar flow chamber is used. It is a glass-fitted cuboidal chamber. An air blower blows air from the surrounding and passes it through a HEPA filter (High Efficiency Particulate Air filter), so as to make it dust free (microbe-free).

This microbe-free air passes through the chamber in a laminar manner and comes out from the chamber through the open front door. This laminar flow of microbe-free air from the chamber to outside through the open door prevents the outside air from entering into the chamber.

Thus, the chamber does not get contaminated with the microbes present in the outside air, though the door is kept opened during inoculation or transfer of media. An UV lamp fitted inside the chamber sterilizes the chamber before operation.

It has a stainless steel platform with provision for gas pipe connection for a bunsen burner. Before use, the platform is cleaned and disinfected with lysol, the bunsen burner is connected and then the glass door is closed.

The UV light is switched on for 10 minutes to sterilise the environment inside the chamber and then switched off. The glass door should never be opened when the UV light is on, because UV light has detrimental effect on skin and vision. The blower is switched on and then the glass door is opened.

Now, the bunsen burner is lighted and media transfer or inoculation is carried out in the chamber aseptically. If extremely hazardous microbes are to be handled, a laminar flow chamber with gloves projecting into the chamber from the front glass door is used, as inoculation has to be done keeping the front door closed.

22. Electronic Cell Counter:

It is used to directly count the number of bacteria in a given liquid sample. An example of electronic cell counter is the 'Coulter counter'. In this equipment, a suspension of bacteria cells is allowed to pass through a minute orifice, across which an electric current flows.

The resistance at the orifice is electronically recorded. When a cell passes through the orifice, being non-conductor, it increases resistance momentarily. The number of times resistance increases momentarily is recorded electronically, which indicates the number of bacteria present in the liquid sample.

23. Membrane Filtration Apparatus:

Certain substances like urea disintegrate and lose their original properties, if sterilized by heat. Such substances are sterilized by membrane filtration apparatus. In this apparatus, the solution of the substance to be sterilized is filtered through a membrane filter, which does not allow bacteria cells to pass down. Filtration is done under suction pressure to increase the rate of filtration (Figure 2.19, page 30).

24. Microscopes:

Different types of microscopes are used for visual observation of morphology, motility, staining and fluorescent reactions of bacteria.

25. Computers:

Computers are generally used for analysis of results. They are also used for identification of bacteria easily within few hours. Otherwise, identification of bacteria is a tedious process and takes days together to identify one bacteria species.

The computers used for identification of bacteria are Apple II, IBM PC and TRS-80 and their modern variants. Each research personnel of the laboratory should be provided with a computer, along with internet facility.

26. Spectrophotometer:

It is an instrument for measuring the differences in color intensities of solutions. A beam of light of a particular wavelength is passed

through the test solution and the amount of light absorbed (or transmitted) is measured electronically.

A simple visible spectrophotometer can pass light with wavelengths within visible range, whereas a UV-cum-visible spectrophotometer can pass light with wavelengths in ultraviolet as well as in visible range. In microbiology lab, it is used for direct counting of bacteria in suspension as well as for other purposes.

27. Electrical Devices:

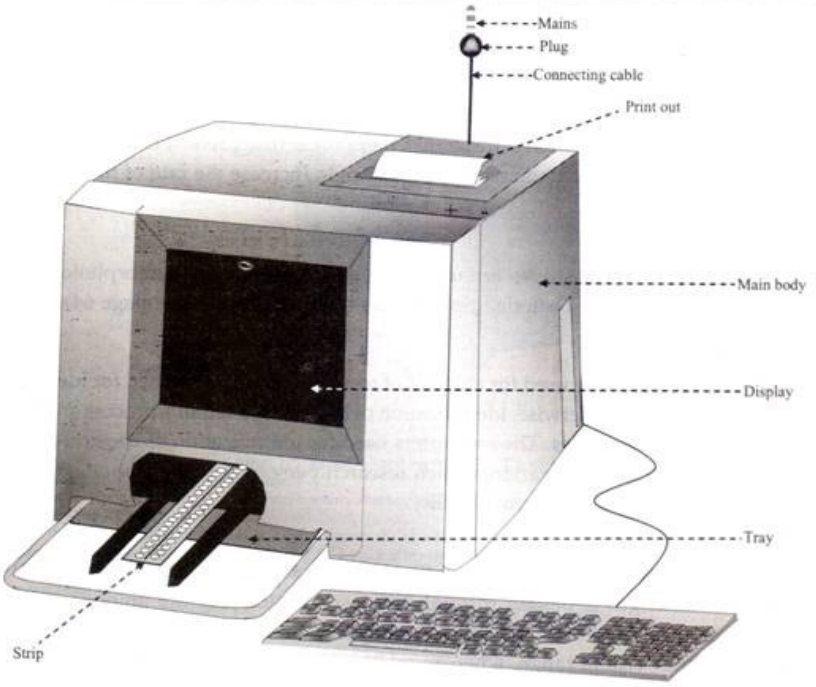
A fluctuation of electric voltage in the laboratory is one of the most important reasons, which reduces the longevity of the equipments and sometimes damage them. Therefore, all the voltage-sensitive equipments should be provided with voltage protection devices like stabilizers, servo stabilizers or constant voltage transformers (CVT) as per the recommendations of the manufacturers of the equipments.

Computers, balances and some sophisticated equipments should be connected through uninterrupted power supply (UPS), as any breakdown in the electric power supply during their operation may severely damage some of their sensitive components.

The laboratory should have a high capacity generator to supply electric current to the whole laboratory in case of power failure. This is because, power failure not only brings the activities of the laboratory to a standstill, it also brings about undesirable irreversible changes in the samples stored in the deep-fridges and refrigerators.

28. Automatic Bacteria Identification System:

It is an instrument used for automatic computer-assisted identification of bacteria (Figures 3.13 and 3.14). The conventional method of identification of bacteria is very lengthy and cumbersome.

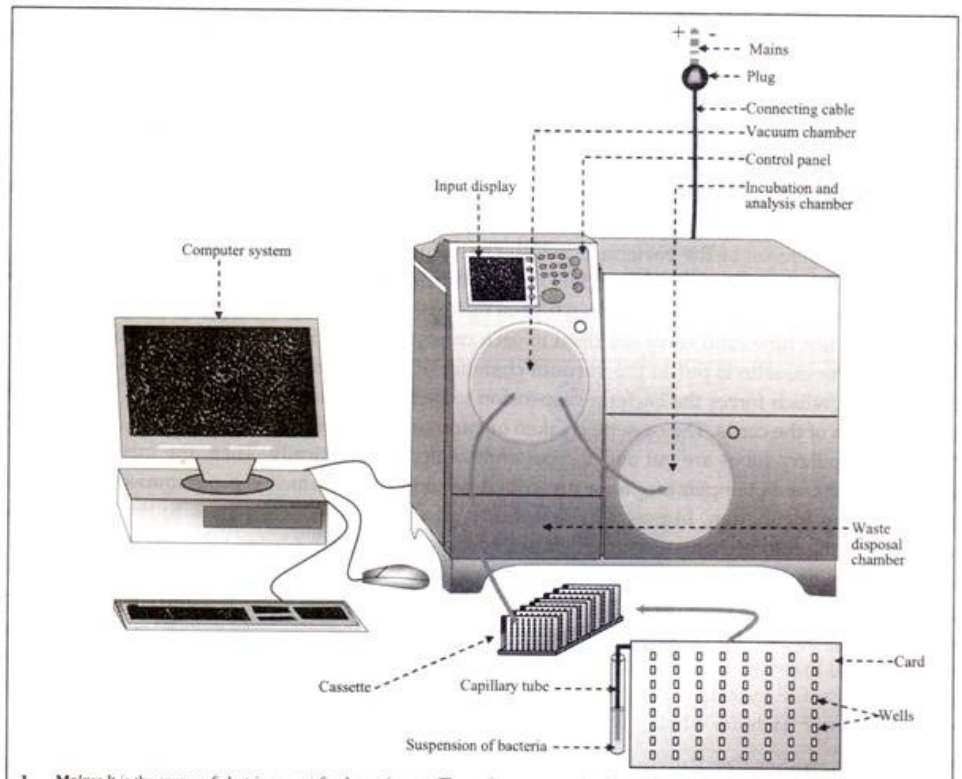


1. **Mains:** It is the source of electric current for the equipment. The equipment runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the equipment against electric shock due to any short-circuit.
2. **Plug:** A three-pin plug provides non-sparking interface between the equipment and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the equipment.
4. **Print out:** It gives the print out of the results and other relevant information regarding the genus and species of the unknown bacteria.
5. **Main body:** It encloses the parts of the equipment. It is made of metal sheets. It houses the colour reader, which reads the colour changes in the test strips. The results of colour changes are compared automatically in the system, with those available in its library. Finally, the name of the unknown bacteria upto genus and species level is displayed.
6. **Display:** It displays the input data and the output results of identification, as well as other relevant informations.
7. **Tray:** It carries the inoculated and incubated strips into the system for colour reading and further comparison, leading to identification.
8. **Strip:** Identification of each bacteria needs a disposable strip. Each strip has a series of cupules (usually 32 numbers), each containing a definite medium required for a biochemical test. The unknown bacteria culture to be identified is first made into a suspension of a particular density as read by a densitometer. This suspension is then inoculated manually into all the cupules. The inoculated test strip is incubated at a prescribed temperature for a prescribed period of time in an incubator. The bacteria grows in the media in the cupules and produces colour changes, which are characteristic of that bacteria. Then the strip is put on the tray, which carries it into the system for colour reading and further comparison leading to the identification of the bacteria.

Figure 3.13: Automatic bacteria identification system (MINI API)

It mainly involves staining, motility test, cultural characteristics, a series of biochemical tests and finally searching the name of the bacteria in ‘Bergey’s Manual of Determinative Bacteriology’ by matching the results with those available in the manual. The automatic bacteria identification system automatically identifies the bacteria in very short time.

The system, like VITEK 2 (Figure 3.14) uses disposable cards. One card is required for the identification of one bacteria. The system can accommodate a series of cards, which can be arranged on a cassette, thus enabling the identification of several bacteria at a time.



1. **Mains:** It is the source of electric current for the equipment. The equipment runs using alternating current (AC) supplied through a three-ply cable. The three wires are phase (+), neutral (-) and earthing (E). The earthing helps in the safe operation of the equipment against electric shock due to any short-circuit.
2. **Plug:** A three-pin plug provides non-sparking interface between the equipment and the mains.
3. **Connecting cable:** A three-ply cable connects the mains with the equipment.
4. **Control panel:** It is the panel or switchboard containing all the switches, with which the functions of the system can be controlled. The different parameters like temperature and time of incubation required for the operation of the system can be programmed on the panel.
5. **Vacuum chamber:** It is the chamber, into which the cassette containing the cards and the bacteria suspensions to be identified is put. A high vacuum is created inside the chamber, which forces the bacteria suspensions to be sucked into the capillary tubes and dispensed into the wells of the cards.
6. **Incubation and analysis chamber:** In this chamber, incubation and colour reading takes place simultaneously. The cassette containing the cards dispensed with the bacteria suspensions is put into it. After scanning the bar codes on the cards, the capillary tubes are cut and the cut ends sealed automatically. Then, the cards are positioned onto a carosol (a wheel-like structure), which moves in such a way that, in every 15 minutes each card comes in front of the colour reader. The colour reader reads the changes in colour at three different wavelengths. The changes in colour of the media in the wells of each card are recorded and sent to the computer for comparison of the results with the database and display of the names of the bacteria in terms of genus and species.
7. **Waste disposal chamber:** After analysis, the used cards automatically get collected in the waste disposal chamber for further disposal to outside after sterilisation.
8. **Card:** The identification of each unknown bacteria requires one disposable card. There are few types of cards each specific for a particular group of bacteria. The type of card to be used for a particular unknown bacteria is determined by a preliminary test like gram staining.
9. **Wells:** Each card has several rows of well. Usually there are 8 rows of 8 well each (8 X 8 =64 wells). The wells contain different dehydrated media for different biochemical tests required for the identification of bacteria. When bacteria suspension is dispensed into all the wells, the dehydrated media become hydrated by the suspension liquid. The hydrated media allow the bacteria to grow. The growth of the bacteria in the wells results in the change in the colour of the media in a particular way, which is characteristic for each bacteria species.
10. **Suspension of bacteria:** The unknown bacteria, grown as isolated colony on a plate or as pure culture grown on a slant is taken. A loopful of the bacteria is transferred aseptically into sterile saline solution in a test tube and a suspension of the bacteria is made. The suspension should contain a prescribed density of bacteria as determined by a densitometer.
11. **Capillary tube:** A capillary tube is fixed to each card, which sucks the suspension of bacteria and dispenses into all the wells.
12. **Cassette:** It can hold a series of cards along with their respective test tubes containing bacteria suspensions. The test tubes and cards are fixed in such a way that the tips of the suction capillary tubes of the cards remain deeply submerged in the suspensions.
13. **Computer system:** The results of colour changes recorded by the analysis chamber go to the computer, which automatically compares them with those available in its library (database) for different bacteria. Finally, it gives the names of the bacteria with definite probabilities.
14. **Input display:** It displays the input programme required for operation of the system.

Figure 3.14: Automatic bacteria identification system (VITEK 2)

Each card has several rows of wells. Usually there are 8 rows of 8 wells each (8X8 =64 wells). The wells contain different dehydrated media required for different biochemical tests. A capillary tube is fixed to each card, which sucks the suspension of bacteria to be identified and dispenses into all the wells.

The dehydrated media in the wells become hydrated by the suspension liquid, thereby allowing growth of the bacteria. After a

prescribed period of incubation, the colour changes in all the wells are recorded automatically in the system.

The results of the color changes go to a computer attached to the system. The computer automatically compares the results with those available in its library for different bacteria and finally gives the name of the bacteria with a definite probability.

For identification, the given bacteria, grown as isolated colony on a plate or as pure culture grown on a slant are taken. A lapful of the bacteria is transferred aseptically into sterile saline solution in a test tube and a suspension of the bacteria is made.

The suspension should contain a prescribed density of bacteria, as determined by a densitometer. The test tube is fixed to the cassette and a card is fixed near it, such that the tip of the suction capillary tube of the card remains deeply submerged in the suspension.

Several such test tubes and cards are fixed to each cassette, depending on the number of bacteria to be identified. The cassette is put in the vacuum chamber of the system. A high vacuum is created inside the chamber, which forces the bacteria suspension to be sucked into the capillary tubes and dispensed into the wells of the cards.

The cassette is taken out and put inside the incubation and analysis chamber. Here, the capillary tubes are cut and the cut ends sealed automatically. Then, the incubation process starts at a prescribed temperature for a prescribed period of time, which is programmed by the control panel. During incubation, in every 15 minutes, each card automatically goes to the color reader, which reads the color changes in the wells and records them.

The recorded results go to the computer, which automatically compares them with those, available in its library for different bacteria. Finally, it gives the names of the bacteria with definite probabilities. The used cards fall into the waste disposal chamber of the system for removal and final disposal after sterilization.

The renowned automatic bacteria identification systems are VITEK 2 and API. While VITEK 2 works on the above principle, the API (Analytical Profile Indexing) system (Figure 3.13) uses a slightly different method for the automatic identification of bacteria, which involves manual inoculation and external incubation.

29. PCR Thermocycler, Refrigerated Centrifuge, Ultra-centrifuge, Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography (TLC), Paper Chromatography, Column Chromatography and Electrophoresis Unit:

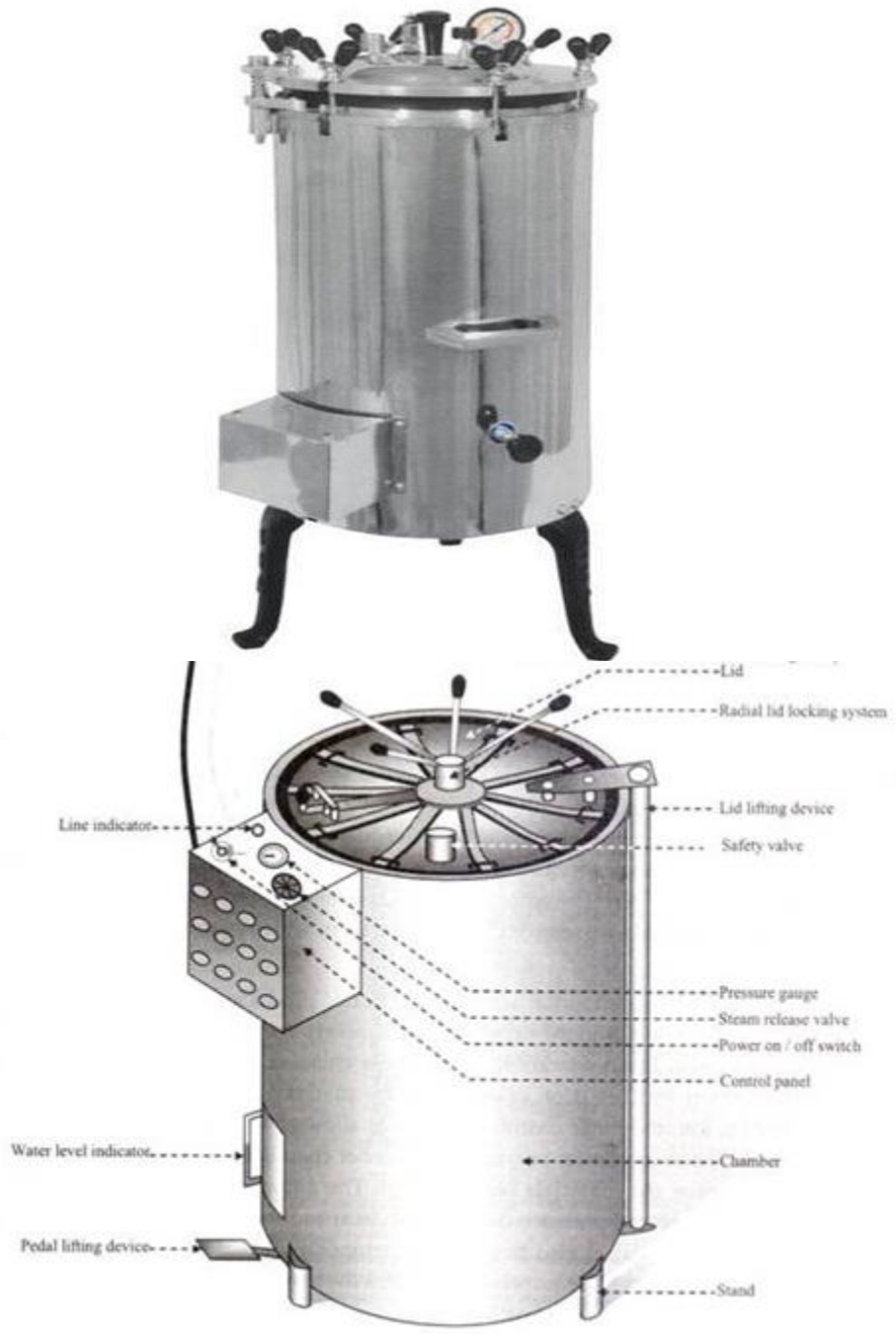
These are instruments used for isolation, purification and identification of biochemical substances, such as bacterial DNA, plasmids, microbial toxins etc. Polymerase chain reaction (PCR) is an important tool in nucleic acid based methods. It is a workhorse in modern microbiology and biotechnology laboratories.

10 Reasons Why Paris Should be on Your Travel Bucket List
SOTC

Home ›› Microbiology

Autoclave

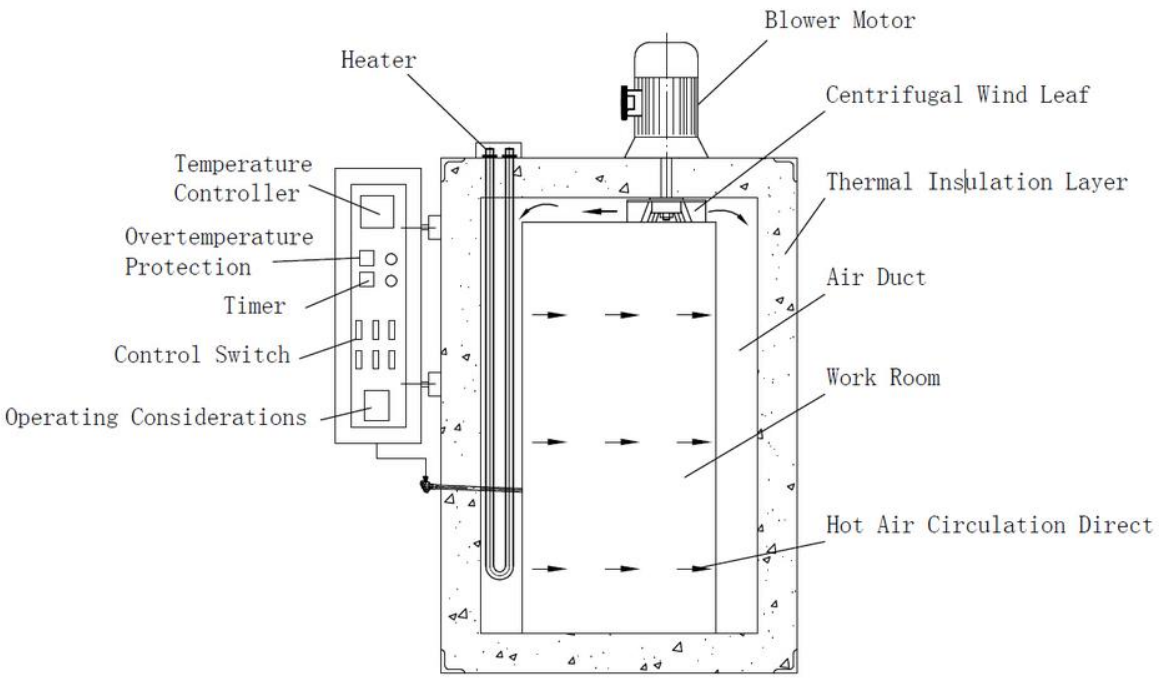
- An autoclave is a device somewhat like a fancy pressure cooker for moist heat or steam sterilization of equipment and growth media. This type of sterilization is a kind of **moist heat sterilization.**
- The development of the autoclave was firstly carried out by **Chamberland in 1884**.
- In this instrument, water is boiled to produce steam, which is released through the jacket and into the autoclave's chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed. Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually **121°C and 15 psi or lb (1 atm or 100 kPa)** of pressure.
- At this temperature, saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minutes. Treatment is continued for about **15 minutes** to provide a margin of safety. However, larger containers of liquid such as flasks and carboys will require much longer treatment times.
- Autoclaves heat their contents to 121°C, which is 21°C higher the boiling point of water. To prevent the solutions from boiling over/vaporizing, the elevated pressure in the autoclave increases the boiling temperature of water during this process.
- Moist heat sterilization is carried out at very high temperatures in order to destroy living cells and various heat labile compounds.
- Moist heat kills microorganisms by coagulating proteins, denaturing enzymes and other essential proteins and degrading nucleic acids. It may also disrupt cell membranes.
- Autoclaves are operated under high pressures, therefore a safety devices i.e., safety valve is present to avoid any danger.
- As precaution, insulated thermal gloves should always be put on when removing glassware from autoclaves.
- Flammable, reactive, corrosive, toxic or radioactive materials, liquids in sealed containers, Paraffin-embedded tissue sections should never be autoclaved.


Lid
Radial lid locking system
Lid lifting device
Line indicator
Safety valve
Pressure gauge
Steam release valve
Power on / off switch
Control panel
Water level indicator
Chamber
Pedal lifting device
Stand

Autoclave

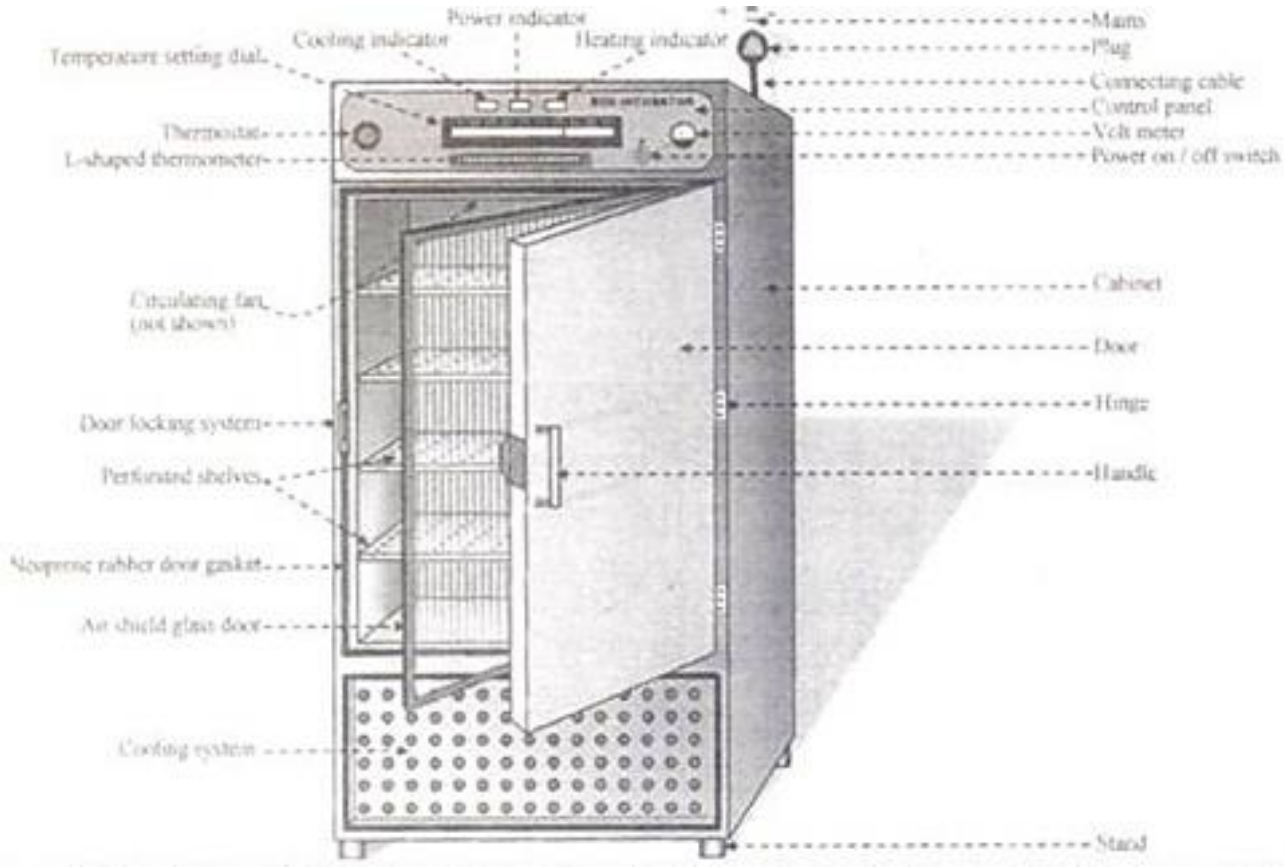
Dry Oven

- Dry oven involves the dry air of higher temperature and for the longer time to sterilize the items and this process is a kind of Dry Heat Sterilization.
- It is used to sterilize items that might be damaged by moist heat or that are impenetrable to moist heat (e.g., powders, petroleum products, sharp instruments).
- This process is maintained by conduction and thermostat, and was first developed by **Pasteur**.
- This electric device is a double walled jackets the outer layer is high quality steel shell and the inner layer is a poor conductor, where insulation keeps the heat in and conserves energy
- There is also an air filled space in between to aid insulation. An air circulating fan helps in uniform distribution of the heat.
- The standard settings for a hot air oven are 1.5 to 2 hours at 160 °C (320 °F) and 6 to 12 minutes at 190 °C.
- It is time consuming method because of slow rate of heat penetration and microbial killing.
- Dry heat does most of the damage by oxidizing molecules. The essential cell constituents are destroyed and the organism dies. The temperature is maintained for almost an hour to kill the most difficult of the resistant spores.
- However, high temperatures are not suitable for most materials.
- As precaution, insulated thermal gloves should always be put on when removing glassware from oven.



BOD Incubator

- The full from of BOD incubator is Biological Oxygen Demand incubator. It is a device for incubation process at predetermined temperature to provide and maintain all artificial optimal conditions for growth of microbial and tissue cultures.
- It is widely used in microbiology laboratories for the applications that include cell culture and fungal growth, BOD test, fermentation, crop and physiology and various pharmaceutical tests etc.
- **Chamber**: BOD incubators are made up of durable and double walled chamber. The exterior wall is usually made of thick mild steel and powder coated; while the interior wall is usually made of stainless steel. The gap between these two walls is filled with insulation. Outer door is solid and there is also an inner door made of glass to view samples. Outer door has mechanical door lock and key.
- **Temperature Control** (Thermostat): Standard temperature range of our BOD Incubators is 5°C to 60°C. In incubation process, temperature accuracy and uniformity both are important; therefore, our BOD incubators are equipped with digital PID controller supported by PT100 sensor for temperature settings that displays both SV (set value) and PV (process value). Temperature accuracy is ± 0.5°C and uniformity remains ± 1°C. . The incubator has a thermostat which maintains a constant temperature by creating a thermal gradient.
- **Safety Measures**: There are some safety features in our BOD Incubators such as over temperature and over current protection and temperature low and high limit buzzer alert etc.
- **Compressor:** compressor is used to lower down the temperature of BOD Incubator. For this purpose, CFC free refrigeration system has been used in the instrument.
- **Heaters**: Heating is done by ISI mark U shaped tubular air heaters.
- **Fan**: Air circulation is mandatory to spread uniform temperature inside the chamber; for this axial fans or motorized blower system is being used.
- **Trays**: Each BOD Incubator is provided with 2 to 5 removable trays. These trays are removable and height adjustable and made of steel wire mesh cable.
- Principle and working of incubator: Incubator depends on the principle of thermo-electricity. The predetermined temperature is maintained by the compatibility operation of the temperature sensor, temperature controller and temperature contactor. When the switch on, current flows into the system thereby energizing the contactor that powers the bulbs which serves as heating elements to the system, the fan ensures distribution of hot air in the entire system. When the temperature in the system gets to desirable (37℃), the digital temperature controller sends an electrical signal to contactor, which de-energized the contactor by switching off the heaters temporarily. Also when temperature reduces beyond desired temperature, the contactor will be energized again by switching on the system**.**



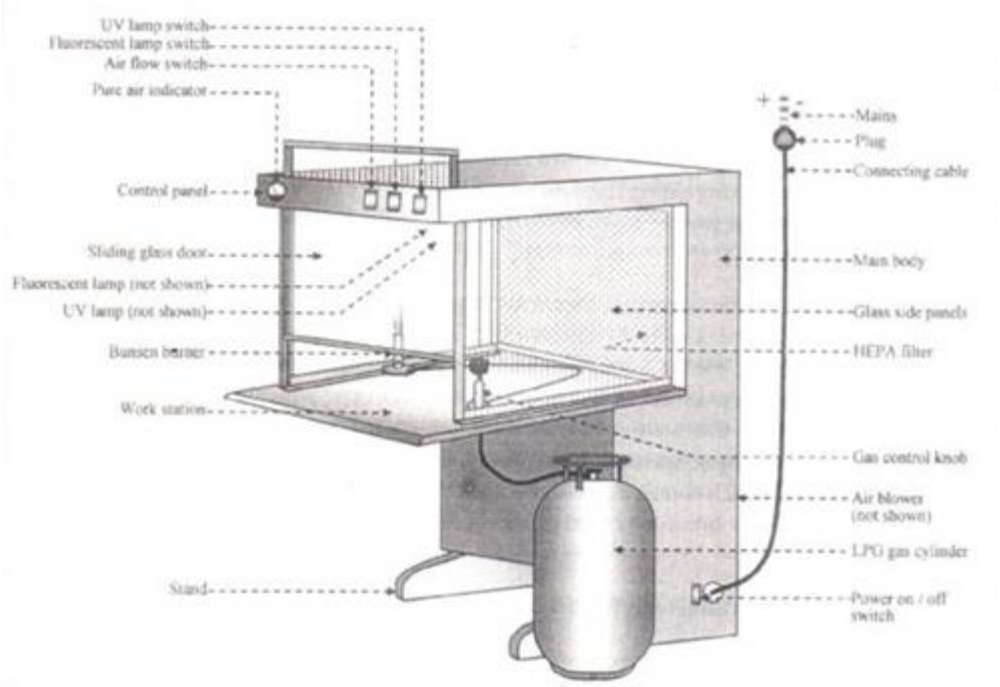
BOD incubator

BOD incubator shaker

- Incubator shaker is often used to mix, blend, or agitate substances in tube or flask by shaking for cell culturing, cell aeration, and solubility studies.
- It involves temperature controlled conditions containing an oscillating board.
- Shaking is necessary to provide agitation or incorporate oxygen and for evenly distribution of nutrients throughout the culture media in order to provide optimal conditions for cell growth.
- The circular shaking motion works at usually range of 25-500 rpm (rotation per minute).
- Other than these facilities, this instrument functions on the same principle of BOD.

Laminar Flow hood or cabinet

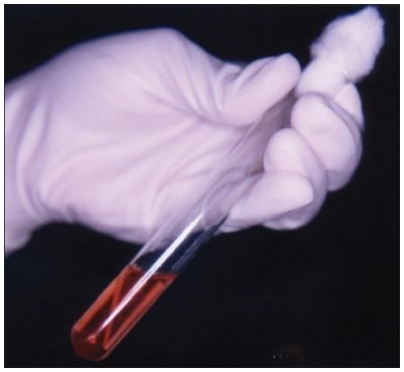
- Laminar flow hood is a biological safety cabinet and one of the most important Air filtration systems, which provides an aseptic work area.
- This instrument employs **high-efficiency particulate air** (**HEPA**) filters.
- These filters trap air contaminants in a complex web of fibers and removes 99.97% of 0.3 µm particles.
- An air pump is required to develop an air flow towards the HEPA filters. It forces air through HEPA filters, then project sterile air across the cabinet opening, which later on create positive pressure inside the Laminar chamber.
- Laminar Flow Cabinets can be produced as both horizontal and vertical cabinets.
- In the **Vertical Laminar Air Flow**, the air blows down from the top of the cabinet. The air can leave the working area via holes in the base and provide greater operator protection. Therefore, Vertical Laminar Air Flow is best for working with hazardous specimens.
- While in **Horizontal Laminar Flow**, the direction of air flow comes from above but then changes direction and is processed across the work in a horizontal direction and hence are not useful for working with hazardous specimens.
- Laminar flow hood is also incorporated with a UV-C germicidal lamp (emits radiation concentrated around 254 nm wave length (i.e. short wavelength)) to sterilize the interior and contents.
- Germicidal lamps are usually turned on for 15 minutes to sterilize the interior and contents before use.
- The UV light should never be on while the cabinet is in use as its exposure damages eyes and skin.
- Work surfaces should be wiped down with 70% ethanol, before and after each use and between handling different cell lines.
- Avoid unnecessary talking while working in the hood may generate microbe-laden aerosols that can enter into the hood.

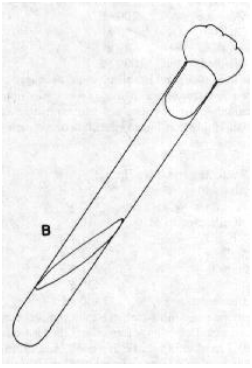


Laminar flow chamber/ cabinet

Cotton plugs

- Cotton plugs are fitting caps on culture vessels that allow the free passage of air but keep microorganisms out.
- H.G.F. Schroder bush and T. von Dusch in 1853 were the first scientists, who made use of cotton-wool as the method of air sterilization for food preservation.
- The meshwork of the cotton fibers acts as an excellent filter preventing entry of fungal spores or bacteria.
- They are cheap and can be recycled for use in a new batch and re-autoclaved.
- Cotton plugs should be loose-fitting on culture vessels for proper aeration.



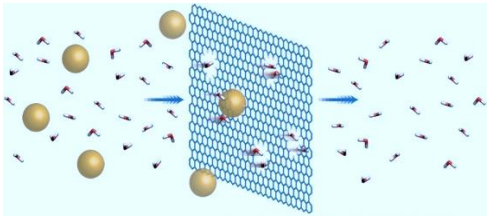


Inoculating needle and inoculating loop

- They are simple tools of plastic handle with **nichrome wire needle or loop** (with a diameter of about 5 mm) to transfer bacteria from a culture to a petri dish.
- Nowadays, they are often made up of plastic resin and can be disposable.
- Generally, inoculating needle is used for the inoculation and isolation of very defined regions of the cultures and at the least disturbance sites between two closely crowded microbial colonies.
- During inoculation, the re-usable needle or loop is sterilized between transfers using a flame and heating until it glows and treating with 70% ethanol in amidst.

Membrane Filters

- Certain thermolabile substances like urea and other carbonic molecules disintegrate and lose their original properties, if sterilized by heat. Such substances are sterilized by membrane filtration. In this apparatus, the solution of the substance to be sterilized is filtered through a membrane filter, which does not allow microbia cells to pass down larger than the filter size.
- Ideally, the pore size of bacterial filteration is 0.22 µm.
- It is used associated with sterilized syringe.
- Initially, the barrel of the syringe is filling with unsterilized solution by sucking with plunger. Further, needle of the syringe is replaced by sterilized membrane filters and the solution passed through the syringe is sterilized.
- However, filtration of large volumes is time-consuming and expensive.



Aim: To study of vesicular arbuscular mycorrhizae (VAM) from rhizosphere soil

Requirements:
(see Sabarna’s file)

Principle:

Mycorrhizae (sing., mycorrhiza) are the symbiotic (mainly mutualistic) associations between root of vascular plants and fungi. It can be of two different types: ectomycorrhizae and endomycorrhizae, where colonization of fungi in root tissues can be extracellular and intracellular, respectively.

Endomycorrhizae are variable and one of its type vesicular-arbuscular mycorrhizae (VAM) (or arbuscular mycorrhizas (AM)) are the ancestral and predominant forms. They are present in 85% of plant families (80% of plant species). In this association, hyphae enter into the plant cells, producing structures that are either balloon-like (vesicles) or dichotomously branching invaginations (arbuscules). The fungal components are obligate symbionts.

The mycorrhizal mutualistic association provides plants with increased assess of water and mineral nutrients, and in return, fungal partner receives carbohydrates.

Procedures:

- VAM spores were extracted by wet sieving and decanting method developed by Gerdemann and Nicolson (1963).
- In this method, 5 g of rhizosphere soil sample was mixed with 200 ml of water to make a suspension.
- The solution was kept for 15 min to settle down the heavy soil particles.
- Further, the supernatant was decanted through a series of sieves of different sizes (100, 200 and 300 mesh).
- The remaining filtrate (heavy soil particles) was re-suspended into water and the same procedure was followed two more times.
- The material remained on the sieves were treated with a slow jet of water from both sides and their shallow suspension was collected in a beaker.
- The suspension was passed through the filter paper (Whatman no. 1) using funnel by a ring formation.
- The filter paper was viewed in a dissecting microscope for spores.
- Few spores were picked up using brush on a glass slide and mounted using glycerin to observe in light microscope for higher magnification.

Results and Discussion:

Spores (Glomerospores) are large, brownish yellow and spherical structures (globose-subglobose) with diameters of 50-750 μm. These structures contain lipids, cytoplasm and many nuclei. Spores usually develop thick walls with more than one layer and can function as propagules. Spores are germinated by emergence of the germ tube. They are formed as swellings on one or more subtending hypha in the soil. They belong to only division Glomeromycota (primitive fungi) and few common genera include *Glomus*, *Acaulospora*, *Gigaspora* and *Entrophospora*, former being the most common one. The associations of VAM in plants provide following benefits:

(i). Increase nutrient uptake (nitrogen, sulfur, phosphorus, potassium, etc.)

(ii). Relieve plants of drought and salinity stress

(iii). Increase root and shoot growth

(iv). Increase water uptake

(v). Heavy metal stress alleviation (rhizoremediation)

(vi). Disease resistance

(vii). Restoration of land plants

Moreover, paleological and molecular evidences suggest that development of the AM played a key role for the origin of land plants in Devonian period (400-460 mya).

Precautions: (from Sabarna's file)

Precautions:

(i) Mixing of the suspension should be proper

(ii) The soil suspension should settle down, before using it for isolation of spores

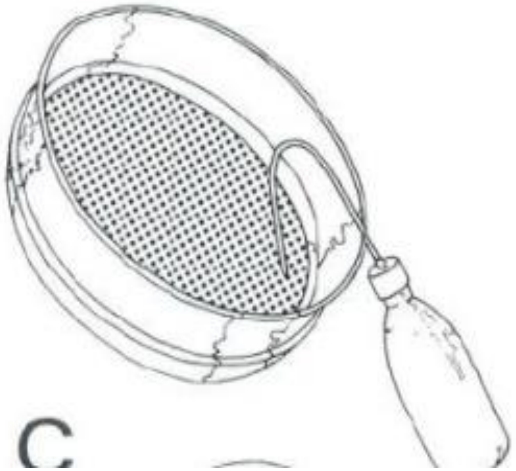
(iii) The flow of water should be adjusted in such a manner, such that it doesn't wash out the spores instead.

(iv) The sieves must be placed in ascending order of mesh sizes, 100, 200, and 300

(v) While transferring the spores from petriplate to slide, care should be taken in order not to crush the spore.

A

B



C

And draw a photograph of ring formation as well showing funnel, filter paper, stand and beaker

- Draw few spores as you have seen in microscope and also paste a photo if you have clicked it (but that is not mandatory).

Aim: To study of rhizobia from root nodule of a leguminous plant

Requirements:

Principle

Nitrogen is a macronutrient and essential for all living as inorganic nitrogen compounds are required for the biosynthesis of nucleotides for DNA and RNA, the coenzyme nicotinamide adenine dinucleotide and amino acids for proteins. Nitrogen fixation is an important process to convert inert nitrogen gas (N_2) into biologically available form, which can be occurred by two different ways:

1. Biological nitrogen fixation
2. Lightening and industrial processes

All biological nitrogen fixation is done by bacteria and archaea, usually called as diazotrophes. Metalloenzymes called nitrogenases containing Fe-Mo are responsible for the catalytic activity of nitrogen fixation. Both autotrophic and heterotrophic bacteria are reported for nitrogen fixation. Some nitrogen fixing archaea are *Methanococcus* and *Desulfovibrio.* Biological nitrogen fixation is possible in free living bacteria and symbiotic association of bacteria with plants. Examples of free living bacteria for nitrogen fixation are *Azotobacter*, *Clostridium*, *Azospirillum*, *Klebsiella*, etc. Some cynobacteria like *Nostoc* and *Anabaena* are also reported for the capability of nitrogen fixation in both free state and symbiotic association. The symbiotic association results into nodule formation in plants and can be categorized in two different types: leguminous and actinorhizal plants. In leguminous plants (Family Fabaceae), the main infecting microorganisms are rhizobia and in actinorhizal plants (e.g. *Betula*, *Casurina*, *Alnus*, etc.), it involves a bacterium, *Frankia.*

$$N_2 + 8\,H^+ + 8\,e^- + 16ATP \xrightarrow{\text{nitrogenases}} 2\,NH_3 + H_2 + ADP + Pi$$

Procedure

1. Take a clean root nodule on the slide.
2. Gently press the nodule and made a smear on the slide with the help of other slide.
3. Then gently heat the slide for few minutes so that the bacteria get sticked to the slide.
4. Then dip the slide in the crystal violet solution for one min and then wash gently with water to remove the extra stain.
5. Then dry out the slide with the help of the dryer.
6. Then dip the slide in iodine which can act as an mordant (increase affinity b/w bacteria and stain).
7. Then dip the slide in alcohol for 30 sec as it can act as decolorizing agent. Wash the slide and dried up.
8. Then dip the slide in safranin for one min, which act as counterstain. Wash the slide and dried up.
9. Clear the other end of the slide, put the cover slip and observe under light microscope.

Results and Discussion

Rhizobia were observed red in colour after Gram staining. They were of rod shape and Gram negative type of bacteria. These bacteria are responsible for nodule formation in leguminous plants and *nod* genes in bacteria are responsible for nodulation. In this symbiotic association, bacteria benefits plants by nitrogen fixation and in return, plants provide rhizobia with sugar (i.e., carbon). Rhizobia may belong to two different phyla: α-Proteobacteria and β-Proteobacteria. Examples of α-Proteobacteria are different species of *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, etc., and for β-Proteobacteria, *Burkholderia*. The microbial genes, *nif* genes are required for nitrogen fixation and are diverse and widely distributed in the environment. Nitrogen fixation is strictly anaerobic process and involves leghaemoglubin protein.

Precautions

1. Slide should be ultra clean
2. Always made a thin and uniform smear
3. Heating should be done carefully as extensive heating may cause distortion of bacteria
4. Washing should be done gently at each step
5. Slide should be dried up properly before proceeding to next step
6. Base of the slide should be cleaned up properly before observing it under microscope

Aim: To study of alcohol fermentation of glucose by yeast

Requirements:

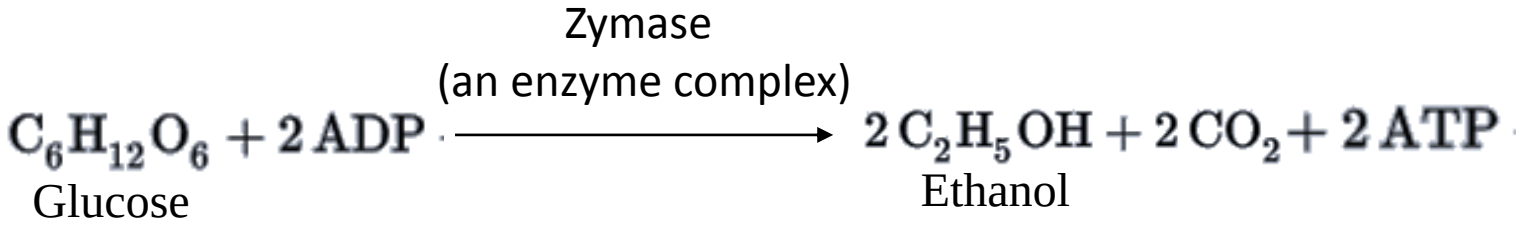
Principle:

Alcohol fermentation is anaerobic biochemical process using different carbohydrates like glucose, fructose, sucrose, etc. as the substrates to breakdown into ethanol (alcohol) and carbon dioxide. This process also generate small amount of cellular energy in form of ATP. An enzyme complex, zymase present in the brewer's/baker's yeast cell (*Saccharomyces cerevisiae*) can efficiently catalyze this reaction for the production of alcohol using glucose.

In this process, glucose is first converted into pyruvate passing through glycolysis. Further, pyruvate is transformed into acetaldehyde, and then alcohol using enzymes pyruvate decarboxylase and alcohol dehydrogenase, respectively.

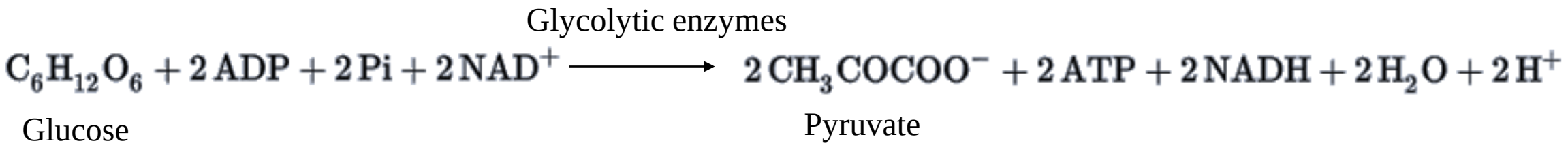
Reaction:

Overall reaction

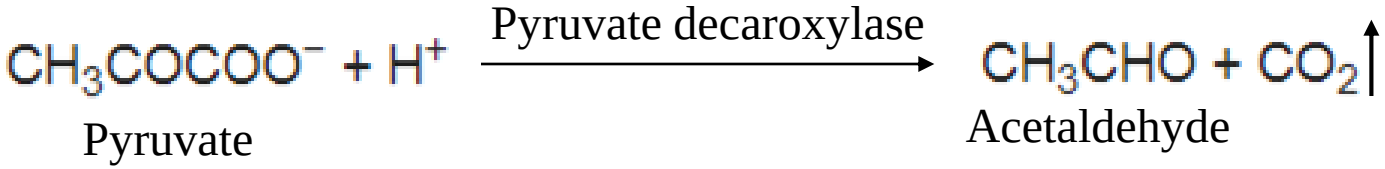


Different steps in the reaction

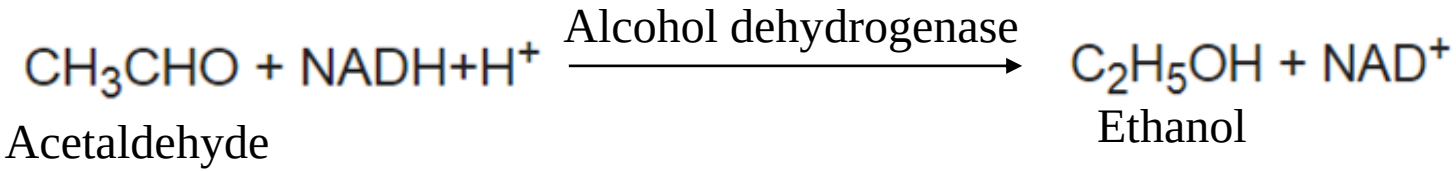
Step 1



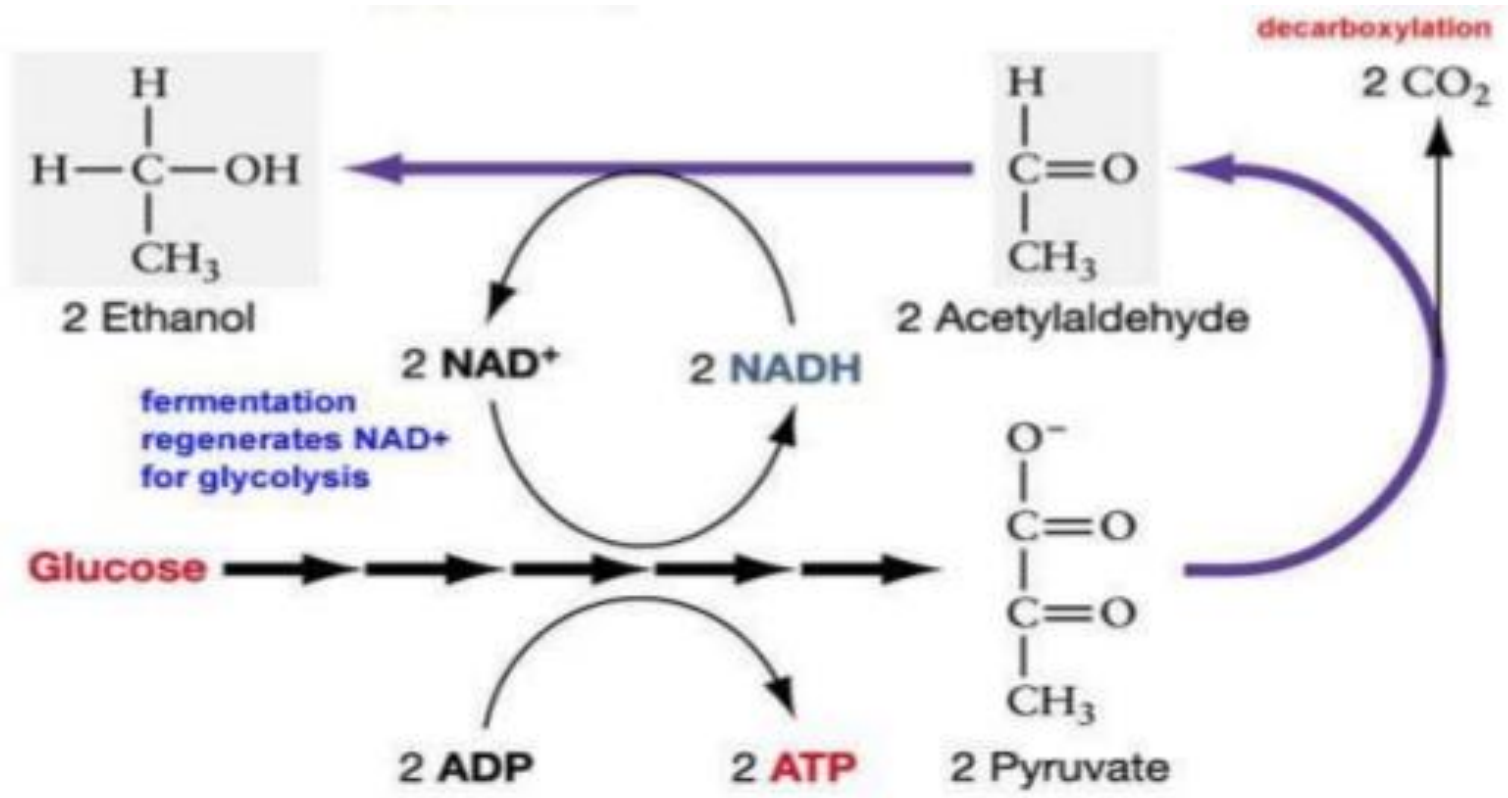
Step 2



Step 3



In other way, the complete process



Procedures:

- In this experiment, 2.5 g of glucose were added in 50 ml water in 100 ml flask.
- The solution was warmed to remove the dissolved oxygen and plugged with aluminium foil to develop anaerobic condition.
- Further, 2.5 g of yeast was added in the solution and kept it for 2 h at 35-37 °C for the fermentation process.
- Similarly, the control and blank were also set in absence of glucose and yeast, respectively.
- After incubation, the solutions were filtered using Whatman paper 41 (or 1). The solution of blank was not filtered, as it did not contain any solid residue.
- After this, two different tests were performed to detect alcohol in the solution.
- The first test was ceric ammonium nitrate test. Few drops of the reagent was added to the test solutions and change in colour and duration of colour stability was observed.
- Anther test was iodoform test. In this process, approximately equal volume of iodine solution was added into test solution and then NaOH (10% w/v) was added drop by drop by shaking the solution until the colour of iodine disappears.
- If nothing happens in the cold, warm the mixture very gently (at 50-60 °C for 2-3 min) and observe the pale yellow precipitate.

Results and Discussion:

Both alcohol and glucose give intense red colour for ceric ammonium nitrate test. However, this colour is discharged quickly for glucose, whereas the colour production in the primary, secondary and tertiary aliphatic alcohols remain stable for a long duration. This has been observed in this result in the treatment solution as compared to blank and control solution.

In another test of iodoform too, the formation of a pale yellow precipitate of iodoform is a positive result for alcohol production.

Alcohol is a colorless, volatile liquid with slight odor. It has the property to solubilize in both water and many organic solvents. Alcohol fermentation by yeast is an important method for its production because it is used in various application as follow:

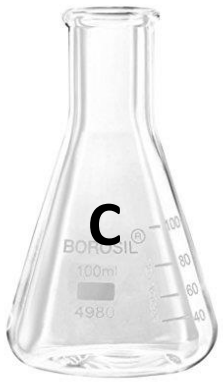
1. As an ultimate solvent for the synthesis of organic compounds
2. Employed in paints, lacquers and varnish and household cleaning products.
3. In preparation of pharmaceutical drug and organic synthesis.
4. In preparation of alcoholic beverages like beer, wine, rum, whisky, vodka, etc.
5. Used as antiseptic, hand sanitizer and antidone
6. Used as moter fuel and rocket fuel
7. Used to produce methylated spirit in spirit lamp and house hold fire
8. Preparation of bread

Precautions:

- All the glassware should be properly cleaned.
- All the reagents should be made in proper solvent.
- The mouth of the flasks should be properly covered to develop anaerobic condition.
- Yeast should not added in the solution, when it is very hot.
- Temperature of incubation should not be too high or low.
- Production of high concentration of alcohol requires long incubation period.

Protocol: Alcohol fermentation of glucose by yeast

100 ml flask



2.5 g glucose
+ 50 ml water
(Treatment)

50 ml water
(Control)

2.5 g glucose
+ 50 ml water
(Blank)

Warm it at 60-80 °C for 3 min and cover with foil
(to remove all the dissolved oxygen)

Cool down the solution up to 40 °C
(by keeping the solution for 5-7 min)

Add yeast in the solution with minimum exposure of air and cover tightly with foil to develop anaerobic condition)

Aluminum foil

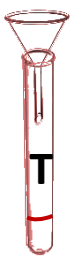
2.5 g yeast
(Treatment)

2.5 g yeast
(Control)

No yeast
(Blank)

Keep them for 2 h at 35-37 °C in water bath

Filter the suspension and collect 5-10 ml clean solution
(using what man paper 41 (better) or 1 (if 41 is not available))



No need to filter
(already a clear solution)

Alcohol test

Protocol: ceric ammonium nitrate test

(for the detection of alcohol in the solution) (Detection of R-OH)

Mix 5-10 drops of ceric ammonium nitrate solution* (reagent) in approximately 5 ml solution.

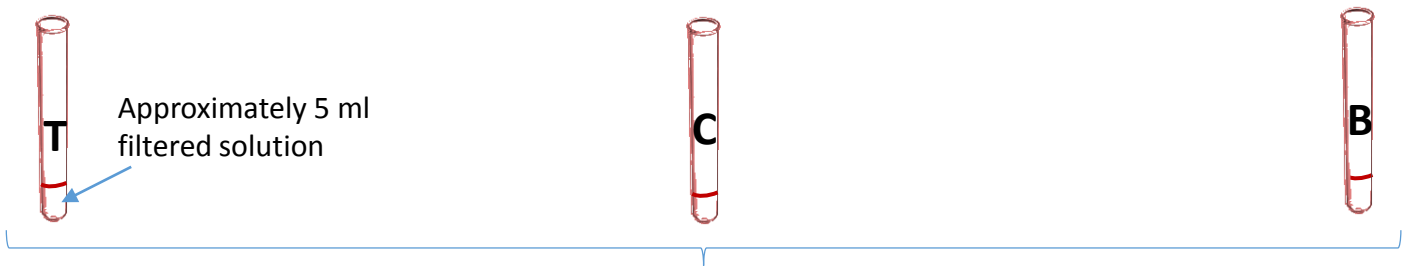
↓

For carbohydrates, it give an intense red colour that is discharged quickly. For Primary, secondary and tertiary aliphatic alcohols, it give red colour, which is stable for a long time.

***Preparation of ceric ammonium nitrate solution:** Dissolve 20 g ceric ammonium nitrate in 500 ml of 2N nitric acid

Protocol: Iodoform test (for the detection of alcohol in the solution)

(Detection of ethanol and secondary alcohol)



↓

Add approximately equal volume of **iodine solution*** and add NaOH (10% w/v) drop by drop by shaking the solution until the colour of iodine disappears.

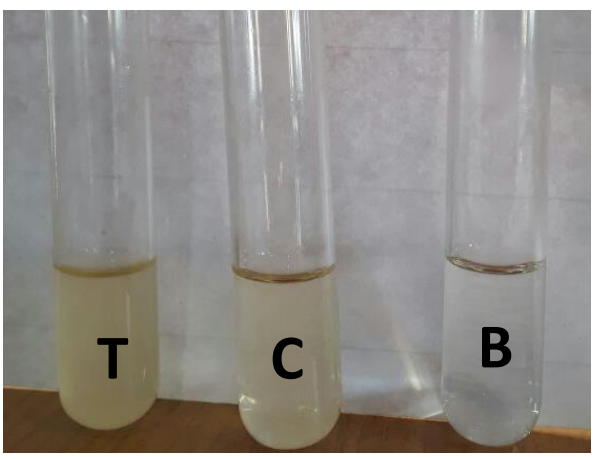
↓

The formation of a pale yellow precipitate of iodoform is a positive result.

↓

(If nothing happens in the cold, warm the mixture very gently (at 50-60 °C for 2-3 min) and observe the pale yellow precipitate (**positive result**).

↓



* **Preparation of iodine solution:** Dissolve 10 g iodine and 25 g potassium iodide in 100 ml distilled water

Reaction for alcohol fermentation

Overall Process

Zymase (enzyme complex, which involve many enzymes)

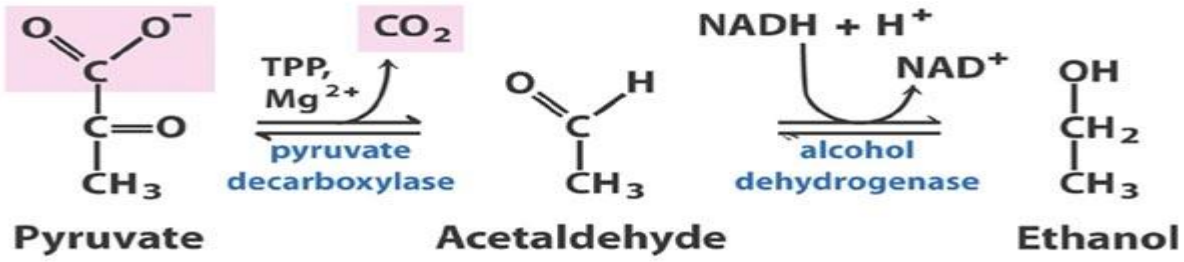
$$C_6H_{12}O_{6\,(aq)} \longrightarrow 2CH_3CH_2OH_{\,(aq)} + 2CO_{2\,(g)}$$

Glucose — Ethanol — Carbon dioxide

Glycolysis

$$C_6H_{12}O_6 + 2\,ADP + 2\,Pi + 2\,NAD^+ \longrightarrow 2\,CH_3COCOO^- + 2\,ATP + 2\,NADH + 2\,H_2O + 2\,H^+$$

Glucose — Pyruvate



Reaction for ceric ammonium nitrate test:

$$(NH_4)_2Ce(NO_3)_6 + RCH_2OH \longrightarrow [\text{red complex}] \qquad (1)$$

yellow

$$[\text{red complex}] \longrightarrow RCH_2O\cdot + (NH_4)_2Ce(NO_3)_5 + HNO_3$$

colourless (2)

This red colour disappears after a reason-able time due to completing the oxidation of this intermediate.

Reaction for Iodoform test:

$$CH_3CH_2OH + 4I_2 + 6\,NaOH \xrightarrow{\Delta} CHI_3 + HCOONa + 5NaI + 5\,H_2O$$

Triiodomethane
(Pale yellow precipitate)