DEPARTMENT OF BOTANY

GOVERNMENT DEGREE COLLEGE FOR MEN

SRIKAKULAM

(AFFILIATED TO Dr. BRAU, SRIKAKULAM, AP)

(2022-2023)

Project work

ON

TISSUE CULTURE

Government Degree college for Men
Srikakulam

(Affiliated to Dr BR Ambedkar University, Srikakulam. AP)

DEPARTMENT OF BOTANY

CERTIFICATE

This is to certify that Mr/Kum_____ of III BSc, Group _____has done the project work on "TISSUE CULTURE" with the Registered number _____ of Sem-VI, Paper-VI during the academic year 2022-2023.

Signature of the Lecturer **Signature of the HOD**

Submitted for the exam on - _____

Examiners

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1) HOT AIR OVEN

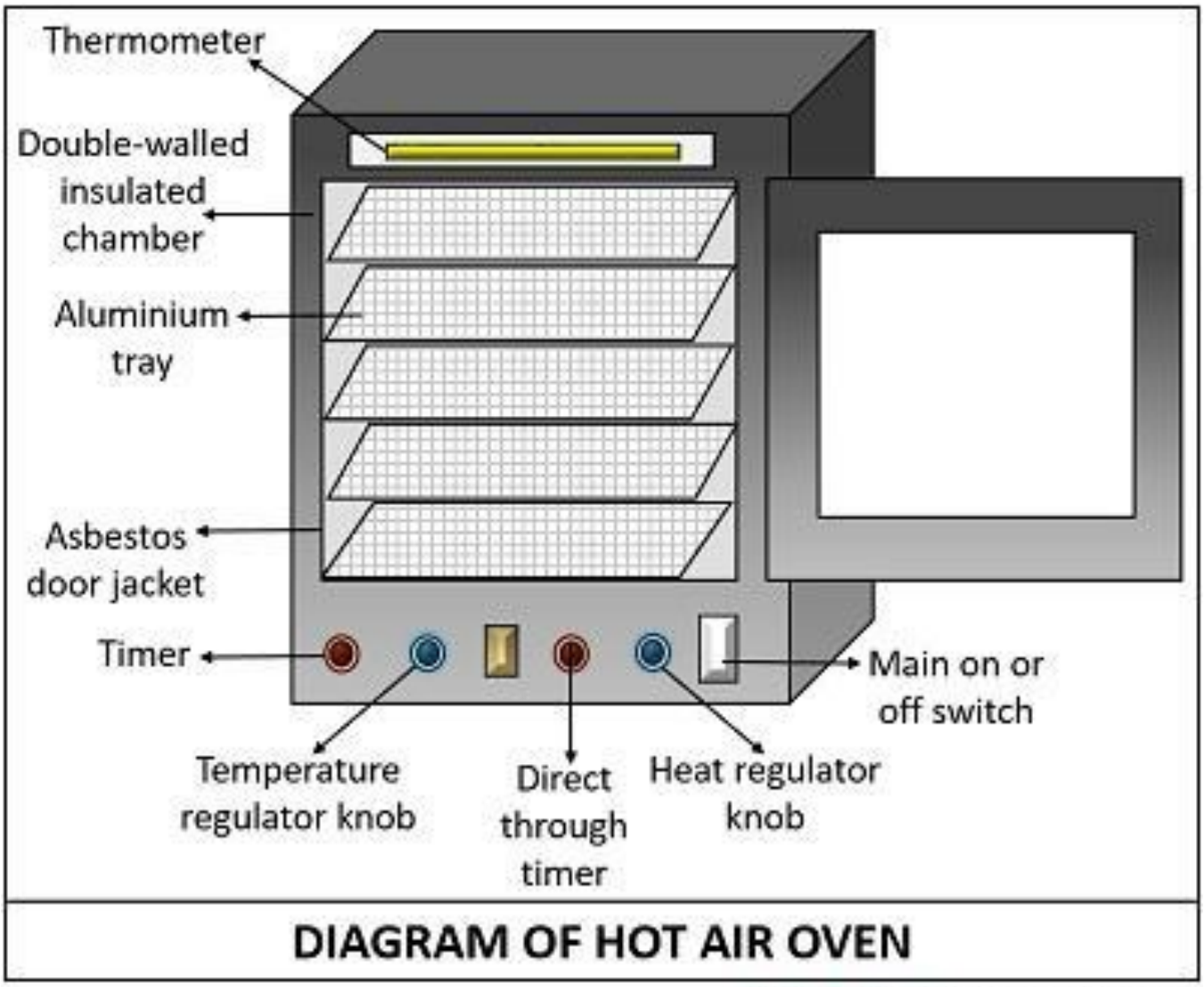


DIAGRAM OF HOT AIR OVEN

1) Hot Air Oven:

This is a dry air type sterilizer with three walls and two air spaces. The outer walls are made up of thick asbestos to reduce the radiation of heat. The hot air steriliser is operated electrically. In this case the heater coil is either be placed at the bottom of the oven or on the side walls. A convection current travel a complete circuit through the wall space and interior of the oven. The temperature inside the oven is controlled by thermostat.

(i) Principle: The hot air steriliser is operated at a temperature of 160 to 180°C for a period of one and a half hour. If the temperature goes above 180°C there is a danger of cotton being charred. The hot air steriliser is used for sterilizing all kinds of laboratory glassware, such as test tubes, Petri dishes, pipettes, flasks, bottles, etc. Other materials which will not be burnt at high temperature may also be sterilized in hot air steriliser. Petri dishes may also be put in metal cans or wrapped with paper and placed inside the steriliser.

(ii) Precaution: It is necessary to check the proper temperature at which the materials are sterilized. Under no circumstances should the hot air oven be used to sterilize culture media, as the liquids will boil to dryness. There should be temperature controlling device for maintaining the temperature required for sterilization.

(iii) Uses: The hot air sterilizer is used for sterilizing laboratory glass ware such as test tubes, Petridishes, pipettes, flasks, bottles and other materials which will not be burned at higher temperature.

2) AUTOCLAVE


Pressure Regulating Device
Pressure Gauge
Safety Valve
Autoclave Lid
Handles
Autoclave Body
Steam Release Valve
Vacuum Release Valve
Outer Stand

2) Autoclave: The autoclave is a cylindrical vessel having double walls around all parts except the upper side. It is built to withstand the steam pressure of at least 30 lb per sq. inch.

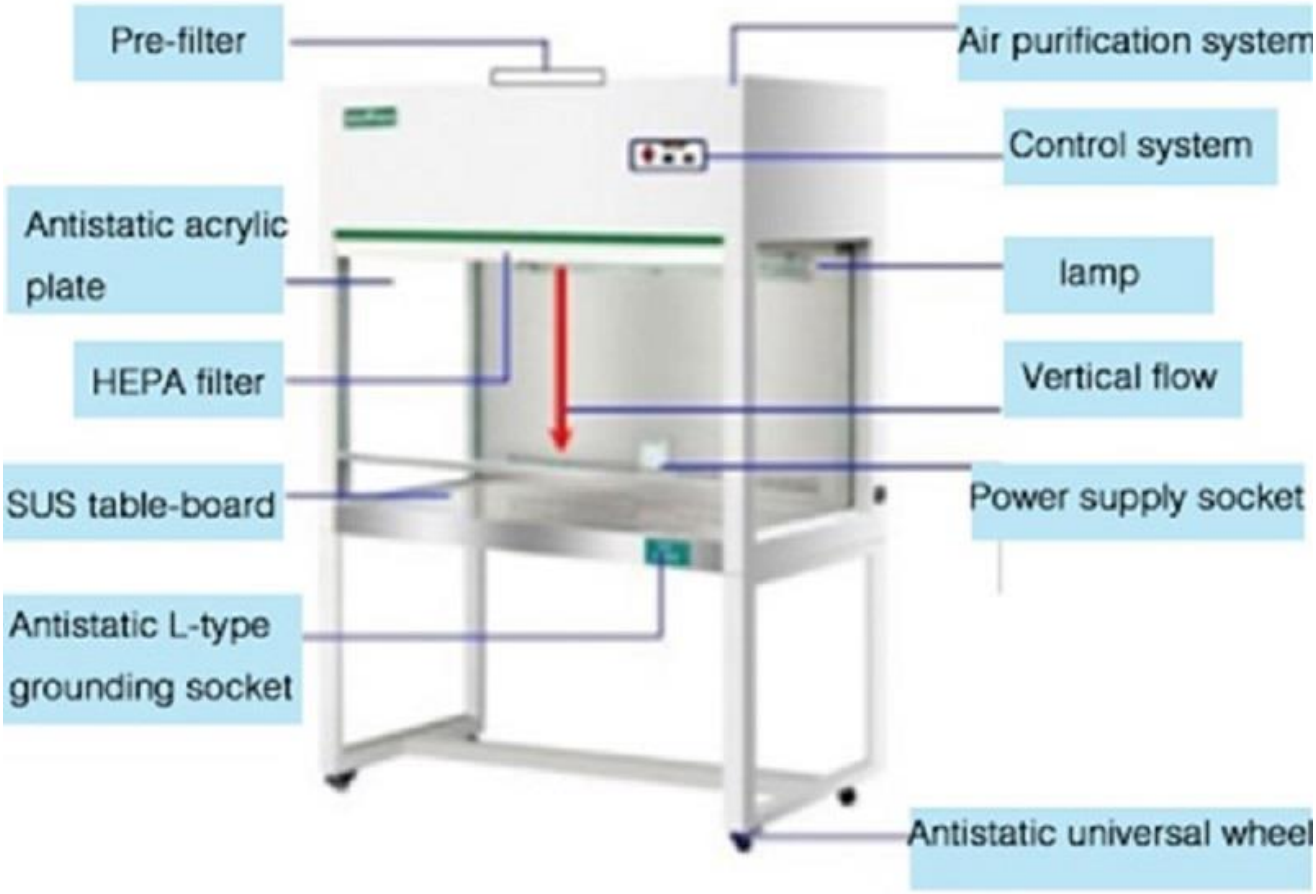
(i) Principle: The principle used here is to increase the temperature of steam (gas) in a closed system that increases its temperature. The water molecules become more aggregated that increases their penetration considerably. The water boils at 100°C depending upon the vapour pressure of the atmosphere. The temperature will be increased if the vapour pressure is increased. The Autoclave is usually operated at 15 Ib/sq. inch steam pressure for 30 min., which as seen from the above table corresponds to 121.5°C. This temperature for a period of 30 min. is sufficient to kill all the spores and vegetative cells of microorganisms.

(ii) Precautions: The following precautions are to be taken: The level of water should be checked before operating. The air should be completely evacuated from the autoclave and the steam must have access to the materials to be sterilized.

iii) Procedure: Sufficient amount of water is placed inside the autoclave. The material is placed inside the autoclave for sterilization. The cotton plug should be covered with a piece of butter paper so that the plug does not wet. The lid of autoclave should be tightened with the help of screws, then switch on the plug. The steam outlet is kept open till we feel that the air from inside the autoclave has been evacuated and then close the steam outlet. The pressure is allowed to remain at 15 Ib/sq inch for 15-30 min., is done by controlling the steam in the valve.After 30 minutes switch the current and let the autoclave cool down and thus the pressure comes down to zero mark.

iv) Uses: The autoclave is used to sterilize usual noncarbohydrate media, bbroths and agar media, contaminated media, aprons, rubber tubings, rubber ggloves etc. This types of sterilization is also used in the commercial canning oof fruits and vegetables and also in order to manufacture sterilized milk.

3) LAMINAR AIR FLOW



3) Laminar Air Flow:

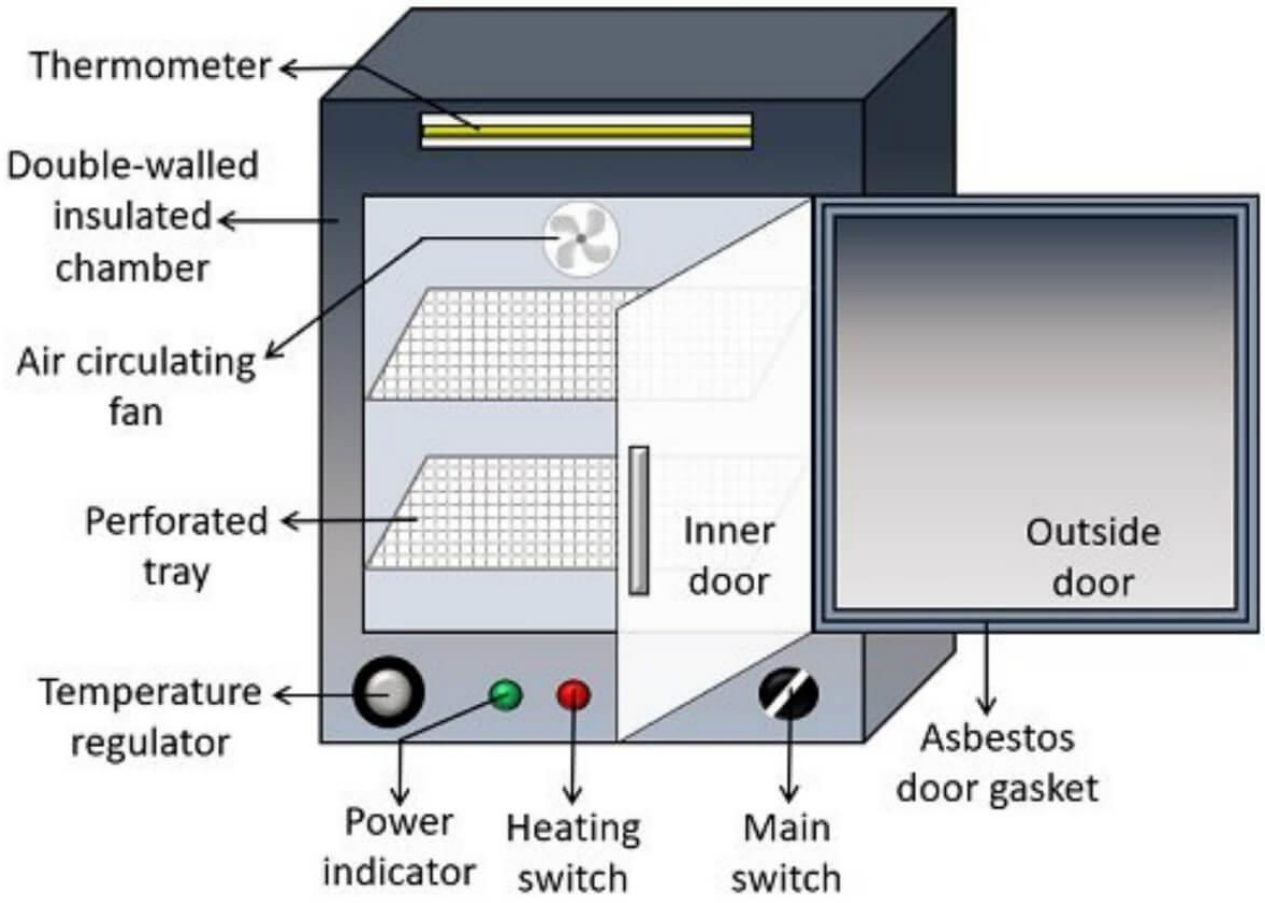
Laminar air flow is an equipment having an air blower in the rear side of the chamber which can produce air flow with uniform velocity alongparallel flow lines. There is a special filter system. high efficiency particulate air filter (HEPA filter) which can remove particles as small as 0.3 mm. In front of the blower,there is also peculiar mechanism from which the air blown from the blower produces uniform air velocity along parallel flow lines. These are horizontal and vertical types.

(i) Principle: Laminar flow can produce dust free air current with uniform velocity along parallel flow lines which help in transferring the culture media bacteria free. Air is passed through these special filters into the enclosure and the filters does not allow any kind of microbes to enter into the system. Due to uniform velocity and parallel flow of air current we can perform pouring, plating slanting, streaking without any kind of contamination.

ii) Precautions: Following precautions should be taken care of before handling the apparatus: We should put off our shoes before entering to operate the apparatus. We should wash our hands with soap and we should not talk inside the chamber while doing experiment, otherwise there will be a chance for contamination with certain bacteria or microorganisms through air of our mouth.

iii) Uses: Within the chamber of laminar flow, we can transfer any media for culturing bacteria or fungi or any microbe without any contamination. The parallel and smooth air flow blown from inside the chamber of the laminar flow is dust free.

4) INCUBATOR



Parts/Components of Incubator

4) Incubator

An incubator is an equipment that consists of copper/steel chamber, naround which warm water or air circulates either by electricity or by ameans of small gas flame. The temperature of the incubator is kept aconstant by thermostat control.

(i) Principle: Incubator is operated to culture or for growing an organism in a suitable medium at proper temperature. In an incubator the variation of temperature should not be more than one degree Celsius. In large incubator the variation of temperature goes up to 2 or 3° C.

(ii) Precautions: The door of the incubator should be opened only when necessary. If the tubes are to be incubated for a long time or at higher temperature, the medium may become too dry due to evaporation. In such case, cotton plug should be pushed inside the neck of the tube and a media rubber cap should be placed 30 to cover the plug. If the Petridishes are to be incubated for a long time these may be placed in moist chamber with a damp sterile cotton wool at the bottom.

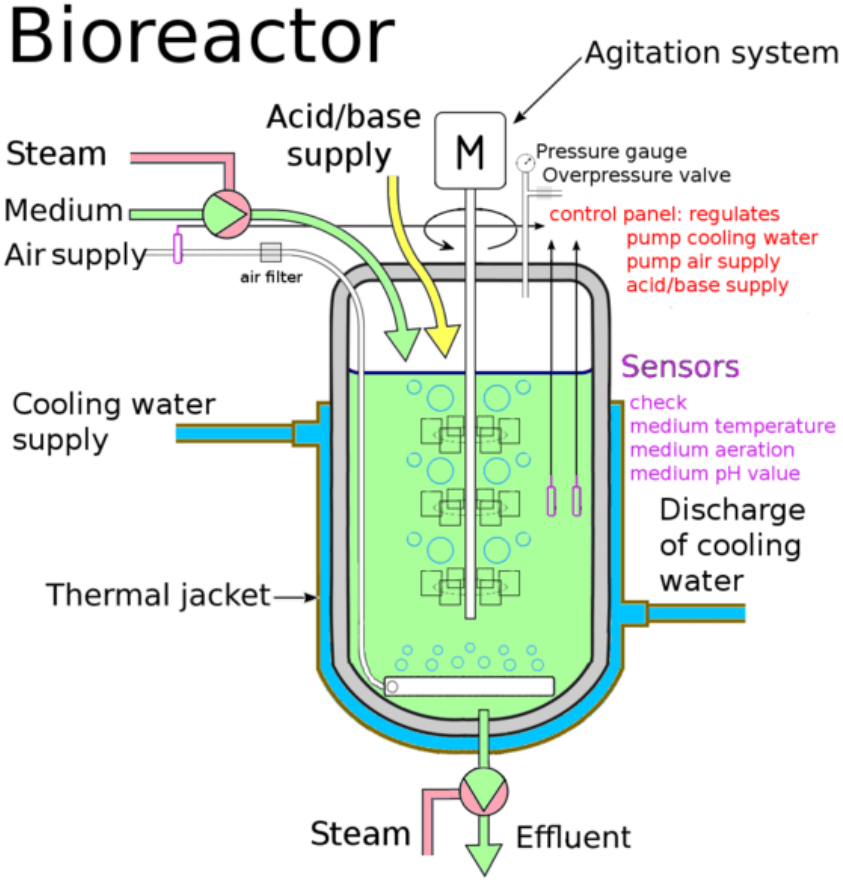
(**iii) Uses:** The method of incubation of culture depends upon the temperature and the oxygen requirements of the organism. For s purpose the incubator is used to maintain different temperature this required for growth of organism in a bacteriological laboratory.

5) GLASS HOUSE

5) Glass house or Green house

Greenhouse, also called **Glasshouse**, building designed for the protection of tender or out-of-season plants against excessive cold or heat. In the 17th century, green houses were ordinary brick or timber shelters with a normal proportion of window space and some means of heating. As glass became cheaper and as more s sophisticated forms of heating became available, the greenhouse evolved into a r roofed and walled structure built of glass with a minimal wooden or metal skeleton. By the middle of the 19th century, the greenhouse had developed from a mere refuge from a hostile climate into a controlled environment, adapted to the needs of particular plants. A huge increase in the availability of exotic plants in the 19th century led to a vast increase in glasshouse culture in England and elsewhere. Large greenhouses are important in agriculture and horticulture and for botanical science, while smaller structures are commonly used by hobbyists, collectors, and home gardeners. Interior of a green house.The modern greenhouse is usually a glass- or plastic-enclosed framed structure that is used for the production of fruits, vegetables, flowers, and any other plants that require special conditions of temperature. The basic structural forms are the span-type greenhouse, which has a double-sloped, or A-shaped, roof, and the lean-to greenhouse, which has only one roof slope and leans against the side of a building. Two or more span-type greenhouses are sometimes joined side by side so that they have fewer external walls, and heating costs are consequently less. A greenhouse has a large expanse of glazing on its sides and roof so that the plants are exposed to natural light for much of the day. Glass has been the traditional glazing material, but plastic films, such as polyethylene or polyvinyl, and fibreglass are also common. The framing of the structure is made of aluminium, galvanized steel, or such woods as redwood, cedar, or cypress. A greenhouse is heated partly by the rays of the Sun and partly by artificial means, such as circulating steam, hot water, or hot air. Because a greenhouse can become too hot as well as too cold, some type of ventilating system is also needed; this usually consists of roof openings, which can be operated mechanically or automatically, and end-wall openings, through which electric fans draw air and circulate it throughout the interior. The plants grown in greenhouses fall into several broad categories based on their temperature requirements during night time hours. In a cool greenhouse, the night time temperature falls to about 7–10 °C (45–50 °F). Among the plants suited to cool greenhouses are azaleas, cinerarias, cyclamens, carnations, fuchsias, geraniums, sweet peas, snapdragons, and a variety of bulbous plants including daffodils, irises, tulips, hyacinths, and narcissi. A warm greenhouse has night time temperatures of 10–13 °C (50–55 °F). Begonias, gloxinias, African violets, chrysanthemums, orchids, roses, coleuses, and many kinds of ferns and cacti and other succulents are suited to such temperatures. In a tropical greenhouse, or hothouse, which has night time temperatures of 16–21 °C (60–70 °F), caladiums, philodendrons, gardenias, poinsettias, Bougainvilleas, passion flowers and many kinds of palms and orchids can be grown. In countries with cool climates, commercial greenhouses are used to grow tomatoes and other warm-weather vegetables.

6) BIOREACTOR



6) Bioreactor

A bioreactor is a type of fermentation vessel that is used for the production of various chemicals and biological reactions. It is a closed container with adequate arrangement for aeration, agitation, temperature and pH control, and drain or overflow vent to remove the waste biomass of cultured microorganisms along with their products.

A bioreactor should provide for the following:

1. **Agitation (for mixing of cells and medium),**
2. **Aeration (aerobic fermenters); for O2 supply,**
3. **Regulation of factors like temperature, pH, pressure, aeration, nutrient feeding, and liquid levelled.**
4. **Sterilization and maintenance of sterility, and**
5. **Withdrawal of cells/medium**

Bioreactors are used for the production of biomass, metabolites, and antibiotics.

Bioreactor Principle

- **The bioreactor is the heart of any biochemical process as it provides an environment for microorganisms to obtain optimal growth and produce metabolites for the biotransformation and bioconversion of substrates into desirable products.**

Applications of Bioreactor

Bioreactors are used in a wide variety of applications, including:

Industrial biotechnology: **Bioreactors are used to produce a variety of bioproducts such as enzymes, antibiotics, and biofuels on a large scale.**

Cell culture: **Bioreactors are used to culture and grow cells, such as stem cells, for research and therapeutic purposes.**

Tissue engineering: **Bioreactors are used to create three-dimensional structures of living tissue for medical applications.**

Environmental biotechnology: **Bioreactors are used to treat waste materials and pollutants, such as sewage and industrial waste, by using microorganisms to break down the contaminants.**

Food and beverage production: **Bioreactors are used to ferment foods such as yogurt, cheese, beer, and bread.**

Pharmaceuticals: **Bioreactors are used to produce vaccines, monoclonal antibodies, and other therapeutics.**

Research and Development: **Bioreactors are used in research to study the behaviour of cells, microorganisms and their metabolic pathways in different conditions.**

7) TISSUE CULTURE-PROCESS

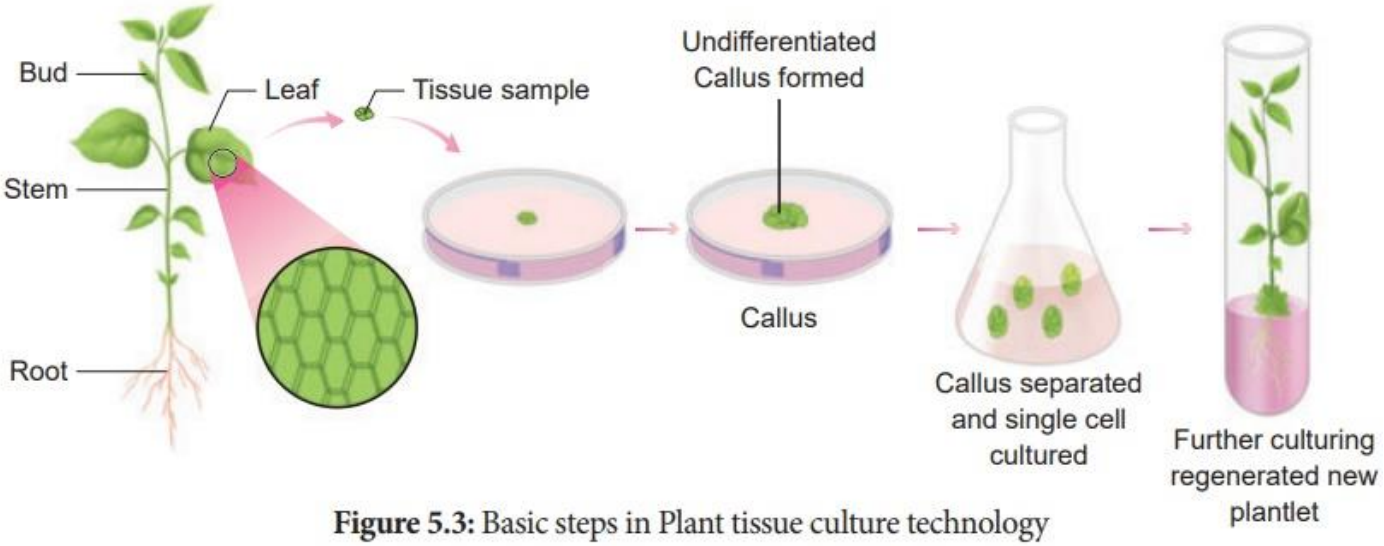
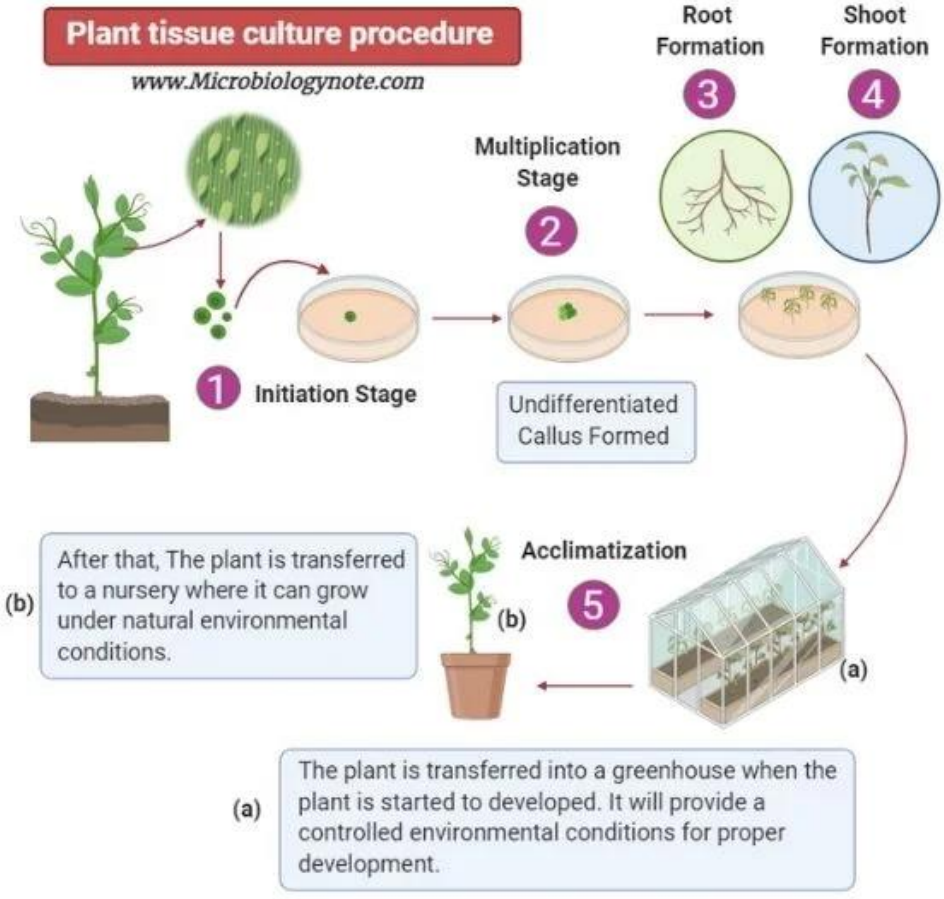
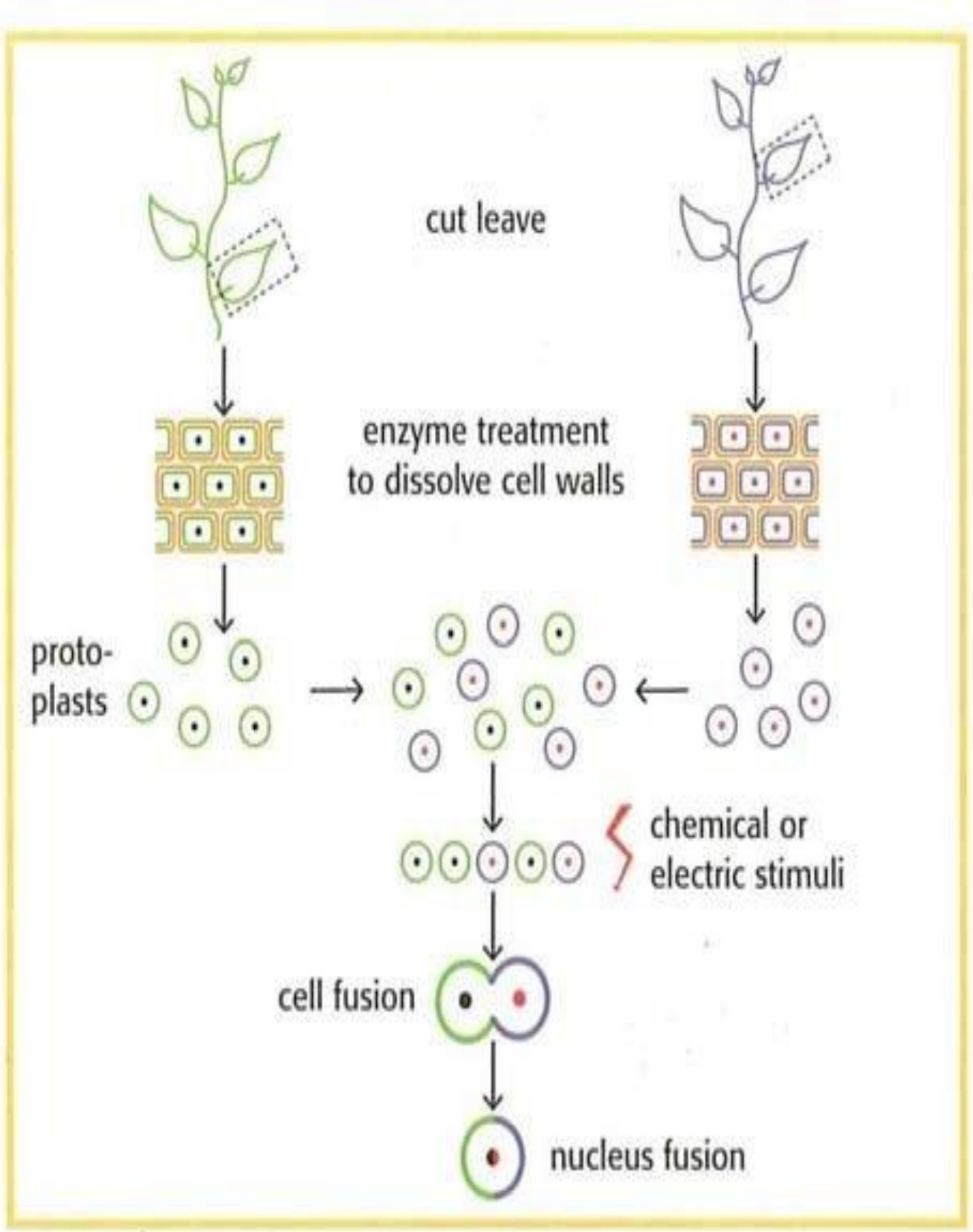


Figure 5.3: Basic steps in Plant tissue culture technology



8) PROTOPLAST FUSION



SOMATIC HYBRIDS

9) POMATO

BROMATO

TRANSGENIC PLANTS

10) Bt-Cotton

Flavr-Savr Tomato

TRANSGENIC PLANTS

11) Golden Rice

Normal rice Golden Rice

Round up ready Soya Bean

THE END