MICROBIOLOGY 18KP2Z07

UNIT - I

SCOPE OF MICROBIOLOGY:

Microbiology is a branch of science that deals with microbes, the microscopic forms of life. Microbes are invisible creature. They are the micro organisms They include viruses, bacteria, algae, fungi and protozoa. Microorganisms have gained tremendous significance. Today, microorganisms are the basic tools of genetic engineering and Biotechnology.

Biogeochemical cycles:

The flow of chemicals between living and non-living things is called biogeochemical cycles. Microbes decompose dead plants and animals into simple chemical nutrients that can be used by plants and photosynthetic organisms.

The plants use these simple chemical nutrients and incorporate them into complex organic compounds which are the ultimate source of food for all animals.

cellulose Digestion:

Ruminants cannot digest cellulose present in plants because they do not contain the enzyme cellulase.

Food Production:

Some of our foods are actually the by-products of microbial growth.

cheese - Leuconostoc citrovorum, Streptococcus Lactis. Penicillium roqueforti.

yoghurt - Lactobacillus Bulgericus and Streptococcus thermophilus.

Energy production:

Microbial methane generators are used to convert manure to combustible fuel for powering vehicles and heaters.

Industrial products:

Microorganisms are extensively used in industries to produce products useful to mankind. These products include fermented foods, alcohol, alcoholic beverages, such antibiotics, pharmaceuticals, steroids, vaccines, vitamins, enzymes, proteins etc.

Microbes in Medicine:

Some important drugs synthesized by microorganisms are antibiotics such as penicillin, streptomycin, chloramphenicol, tetracyclins, neomycin, actinomycin etc.. ergotin, ephedrine, vitamins, glycerin, steroids etc.

Microbes in pesticides:

Certain microbes like bacteria, fungi, viruses, protozoa and even nematodes infect insects and kill them. They may be called microbial pesticides and are used as biopesticides.

Microbes in Improvement of soil:

Most of the bacteria and fungi live saprophytically on dead organic matter of soil. They decompose complex organic matter into simpler one. In fact they bring about decay by their various digestive and respiratory processes. Nostoc, Anabaena, Nitrosomonas, Nitrobacter, Rhizobium.

Microbes to Better Sanitation:

Microbes like bacteria, algae, fungi and protozoa are used in improvement of sanitory methods.

Microbes in Retting of Fibres.

Retting is the process in which plant fibres such as coconut husk, jute, hemp, flax, etc., are separated by the activity of microorganisms. Clostridium butyricum which hydrolyses the pectic substances. Consequently the fibres get separated and are then used in preparing ropes, sacks etc.

Microbes and Genetic Engineering:

* Genetic engineering technique involves the use of microorganisms – recombinant DNA is produced.
* Applying this techniques nitrogen fixing genes are transferred from nitrogen fixing bacteria to cultivated plants.
* Insulin synthesizing genes are transferred from vertebrates to microbes and insulin is synthesized on a large scale.

Biomining:

Microbes are now used in extracting valuable metals like uranium from rocks.

Pseudomonas and Baker's yeast – Heavy metals.

Thiobacillus ferrooxidans – iron sulphide

Use of microbes in mining reduces the cost of 75%.

HISTORY OF MICROBIOLOGY :-

Antoni van Leeuwenhock is the father of Microbiology. He assembled simple microscopes and in 1674 through his own microscopes, he brought to light for the first time.

Linnaeus - 1767 - Chaos infusoria

Muller - 1773 & 1788 - Vibrio and monas.

Edward Jenner - 1798 - Small pox vacin

Ehrenberg - 1829 - Spirillum Spirochete.

Agostino Bassi - 1835 - Silworm disease - fungus Botrytis

Theodore Schwann - 1836 - yeast causes fermentation

Louis Pasteur - 1822 - 1895. He is the father of Immunology.

Anthrax (disease of cattle) - Pasteurella aviseptica.

Chicken Cholera (disease of the fowls) - pasteurella aviseptica.

rabies - rabies vaccine.

Robert Koch - 1843 - 1910 - discovered Bacilli

- 1882 - Tuberculosis - Mycobacterium tuberculosis

- 1883 - cholera - Cholera vibrio

Louis Pasteur - 1866 - pasteurization.

Weigert - 1875 - Methyl violet dye.

Albert Neisser - 1879 - Gonorrhea - Gonococcus

Gram - 1884 - Gram Staining techniques for bacteria.

Karl Joseph Eberth - 1880 - Typhoid bacillus.

Charles Louis Alphonse laveran - 1880 - parasite of malaria.

Carl Friedlander - 1882 - bacillus Klebsiella Pneumoniae

Biejernick - 1888 - root nodule bacteria.

Winogradsky - 1893, 1994 - Clostridium - Atmospheric N_2 in the soil

Ivanowski - 1892 - Tobacco mosaic virus

Alexanderyersin - 1894 - plague bacillus.

Kiyoshi Shiga - 1898 - Dysentery bacillus.

Ronald Ross - 1898 - Malarial parasite - Anopheles Mosquito

Sir Alexander Fleming - 1929 - Penicillin.
He was father of antibiotics.

Enders, Robbins and weller - 1949 - Poliomyelitis

Salk - 1950 - polio vaccine.

Waksman - 1973 - Streptomycin.

PROKARYOTES AND EUKARYOTES :

Based upon the presence or absence of nuclear membrane, microbes are classified into two categories, namely prokaryotes and Eukaryotes.

The prokaryotes do not contain a nuclear membrane. The nuclear material are not separated from the cytoplasm and the nuclear material is suspended in the cytoplasm. This type of nuclear material without a nuclear membrane is called a nucleoid or incipient nucleus. (eg) Bacteria.

Eukaryotes contain a nucleus. The nuclear materials are surrounded by a nuclear membrance and the nuclear materials are well separated from the cytoplasm. (eg) protozoa, algae and fungi

Viruses Lack a cellular structure and hence they are non-cellular Particles.

CHARACTERISTICS AND ULTRA STRUCTURE OF BACTERIUM:

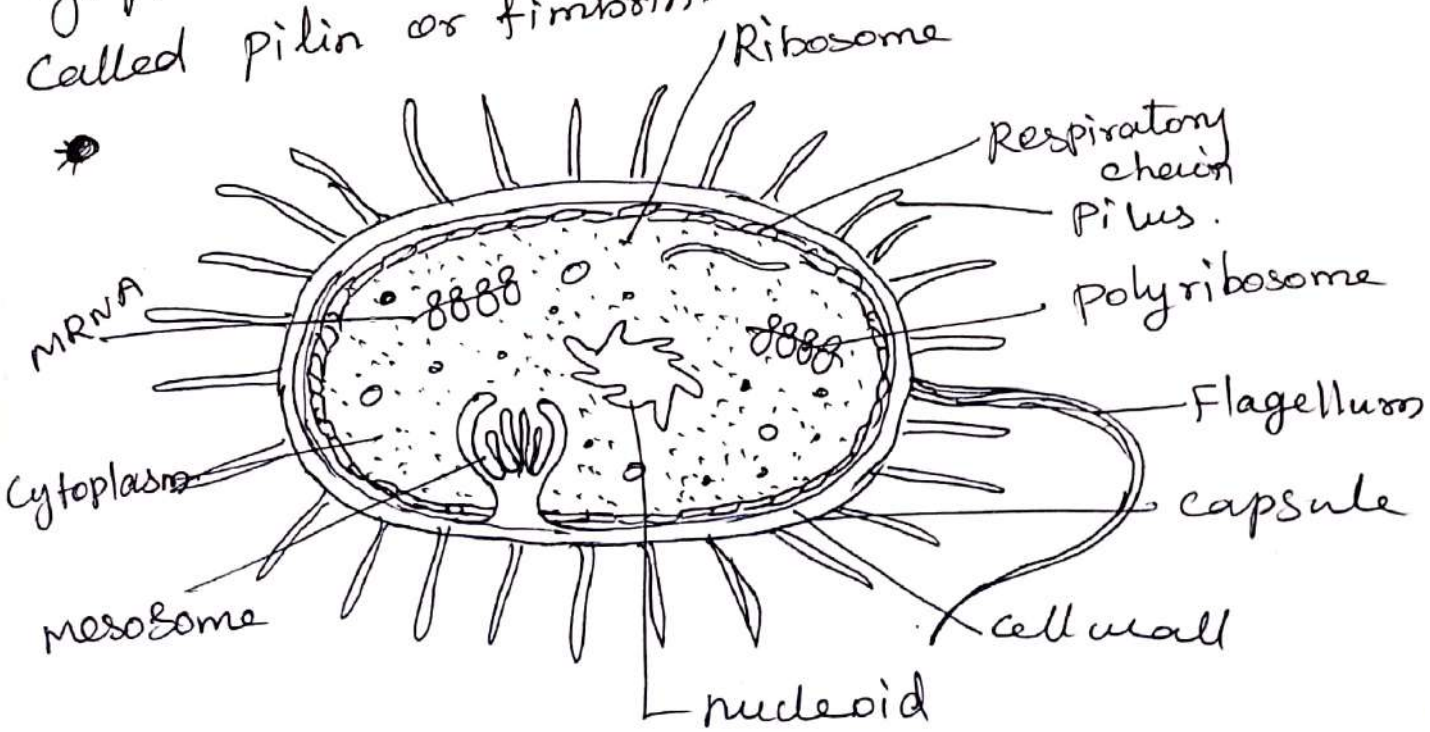
Bacteria are microscopic, unicellular, Prokaryotic Organisms. The study of bacteria is called Bacteriology. Ehrenberg (1829) established the genus Bacterium. Bacteria are present everywhere, in the water, in the soil, in the air, on our body and in our body. Eg - E. coli, Lactobacillus, streptococcus etc.

STRUCTURE OF E. coli :-

* E. coli is Escherichia coli. It is a colon bacterium. It lives in the colon of man. It is the indigenous flora of intestine.
* It is rod-shaped. Hence it is a Bacillus.
* It is 1.5µm broad and 6.5 µm long
* It lives individually or in pairs
* It is Gram negative. It appears red in Gram staining.
* It is a faculative anaerobe.
* It is Petritrichous having flagella all over the bod. It is motile.
* It is chemoorganotrophic in nutrition.
* 37°C temperature is the optimum for its growth
* It can ferment glucose and produce acid and gas
* It is harmless. But occasionally it attains virulence and produce enterotoxin.
* It produces diseases like diarrhoea, septicemia, meningitis and urinary tract infections.

* It is a prokaryote.
* It is covered by a capsule. Hence it is a capsulated bacterium. It is tightly attached to the underlying cell wall. It is made up of disaccharides and polypeptides.
* Below the capsule there is cell wall. It gives shape and rigidity to the cell. The cell wall is composed of peptidoglycan. The cell wall contai bacterial antigens.
* Below the cell wall, there is a plasma membra It is an unit membrane. It is composed of phospholipids, proteins and polysaccharides.
* Plasma membrane serves as a barrier to lysoso -me, that could damage the cell.
* Unit membrance acts as an endotoxin.
* plasma membrane is selectively permeable.
* The cell membrane encloses the cytoplasm. It is colloidal in nature. It contain ribosomes, mesosom etc.
* The cytoplasm Contains a circular double standed DNA. It is the bacterial chromosome. It does not contain a nuclear membrane.
* cytoplasm contains 70S ribosomes.
* Ribosomes are the centres of protein Synthesis.

* Mesosomes are intracytoplasmic membranous structures. It is involved in septum formation during binary fission. It is involved in DNA replication.
* Small circular double stranded DNA-s are also found in the cytoplasm. They are called plasmids.
* plasmids produce toxins, They resist various antibiotics, They resist environmental factors and They use unusual chemical compounds as nutrients
* The surface of bacteria contains numerous flagella used for locomotion. The flagellum is made up of protein subunits called flagellin.
* In between the flagella, there are short appendages called pilli. pili arise from the cytoplasm. They are made up of protein subunits called pilin or fimbrin.



* Bacteria need enough suitable nutrients for its growth and reproduction.
* Bacteria respire by Aerobic, Anaerobic and Faculative respiration.
* Bacteria reproduce by binary fission, budding, fragmentation, endospore and conidiospores.

CHARACTERISTICS AND ULTRA STRUCTURE OF VIRUSES:

* Viruses are defined as sub-microscopic, self reproducing particles capable of being introduced into living cells and reproducing inside such cells only.
* The existence of virus was first proved by Ivanowski in 1892. The first virus was discovered by Ivanowski in 1899.

Viruses are omnipresent. The viruses living on animals are called animal viruses. The viruses that live on plants are called plant viruses. The viruses that live on bacteria are called bacterial viruses or bacteriophages.

The shape of viruses varies. An individual viral particle capable of infecting a specific host is called a virion. The protein free pathogenic RNA (nucleic acid) of virus is called viroid.

Animal virus :- Adenovirus

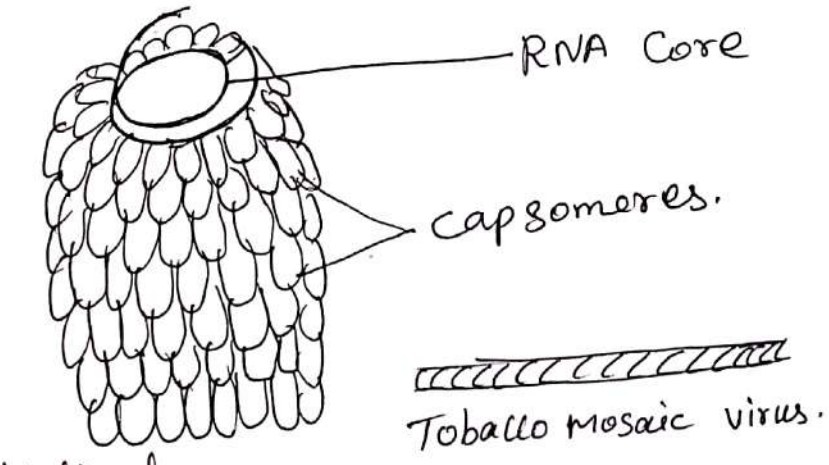
Plant virus :- Tobacco Mosaic virus

Bacterial virus :- Bacteriophage.

Tobacco Mosaic Virus :-

It is Plant virus. It causes a disease called tobacco mosaic in tobacco plants. It is an RNA virus. It is a helical virus. It is rod shaped. The TMV is made up of two components, namely an outer capsid and an inner RNA molecule.

The capsid is the outer coat. It is formed of proteins. The capsid is made up of small subunits called capsomeres. There are about 2,130 capsomeres in a TMV. The capsomeres are helically arranged around the RNA. There are about 129 complete spirals.

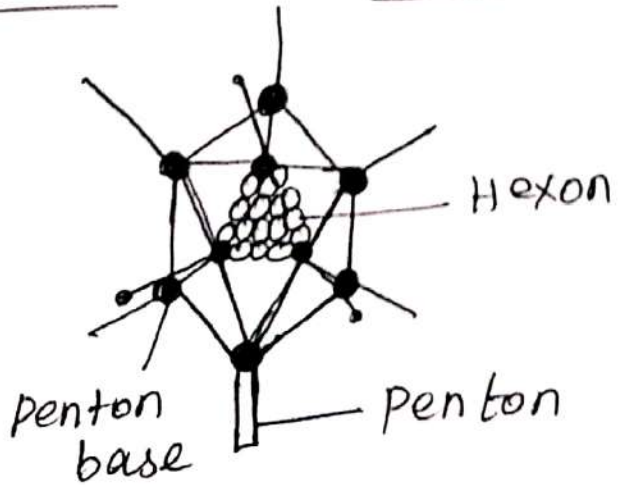


Adenovirus :

Adenovirus is a typical animal virus. It infects eye and alimentary canal. It is a non-envoloped virus. It is a polyhedral virus. It is a DNA virus. It is made up of two components. namely an outer coat the capsid and an inner core the DNA.

There are about 252 capsomeres. The capso-meres are arranged in the form of an icosahedron having 20 triangular facets and 12 vertices. The 12 capsomeres at the vertices have 5 neighbours and are called pentons. The remaining 240 capsomeres have six neighbours and are called hexons. Each penton contains a fibre ending in a knob.

The capsomeres present in the facets are called hexons because they are surrounded by 6 capsomeres. In Adenovirus, there are 240 hexons and 12 pentons.



BACTERIOPHAGE:

It is a bacterial virus. It is a virus living inside bacterial cells. Bacteriophage means bacteria eating agent. It was first described by Twort in 1915. T_4 bacteriophage is an parasitic on human colon bacteria, Escherichia coli. It is also known as coliphage.

The T_4 phage is tadpole-shaped. It consists of 3 parts, namely, a head, a neck and a tail. The head is polyhedral. It is covered by a Protein Coat called capsid. The capsid is made up of about 2,000 protein sub units called capsomeres.

A double stranded DNA is present inside the head. The DNA is highly coiled and tightly packed. It contains more than 75 genes. The neck is very short and it contain collar. It connects the head with the tail.

The tail consists of a central hollow core tube. Through this core tube the DNA of the phage can pass into the bacterial cell. The core tube is covered by a sheath or tube made up of about 144 protein contractile subunits. The free end of the core tube has a hexagonal end plate. The end plate has 6 spikes and 6 fibres.

The spikes are used for penetration and the fibres are used for attachment on the host. The bacteriophage has a complex symmetry. The bacteriophage has 2144 capsomeres. Of these 2000 capsomeres are present in the head and 144 Capsomeres are in the tail.

Multiplication of Bacteriophages:-

T4 bacteriophage infects and multiplies only inside the bacterium, E. coli.

(i) Attachment of phage on bacterium.
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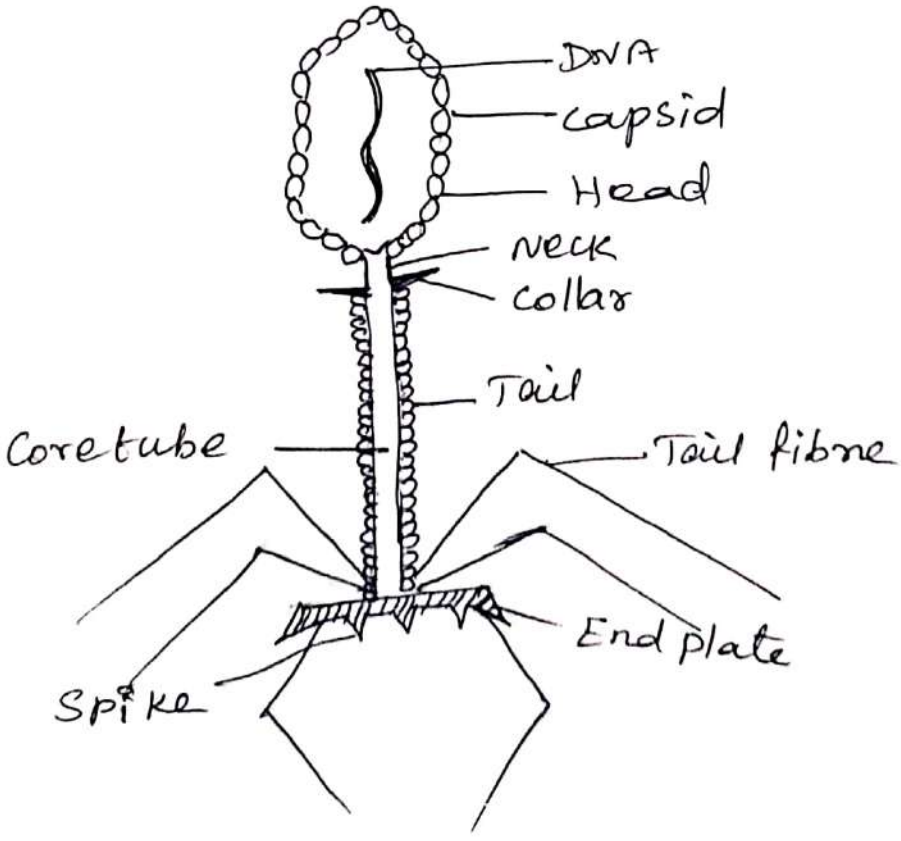
(iv) Assembly of phage particles.
(v) Lysis of Bacterium.



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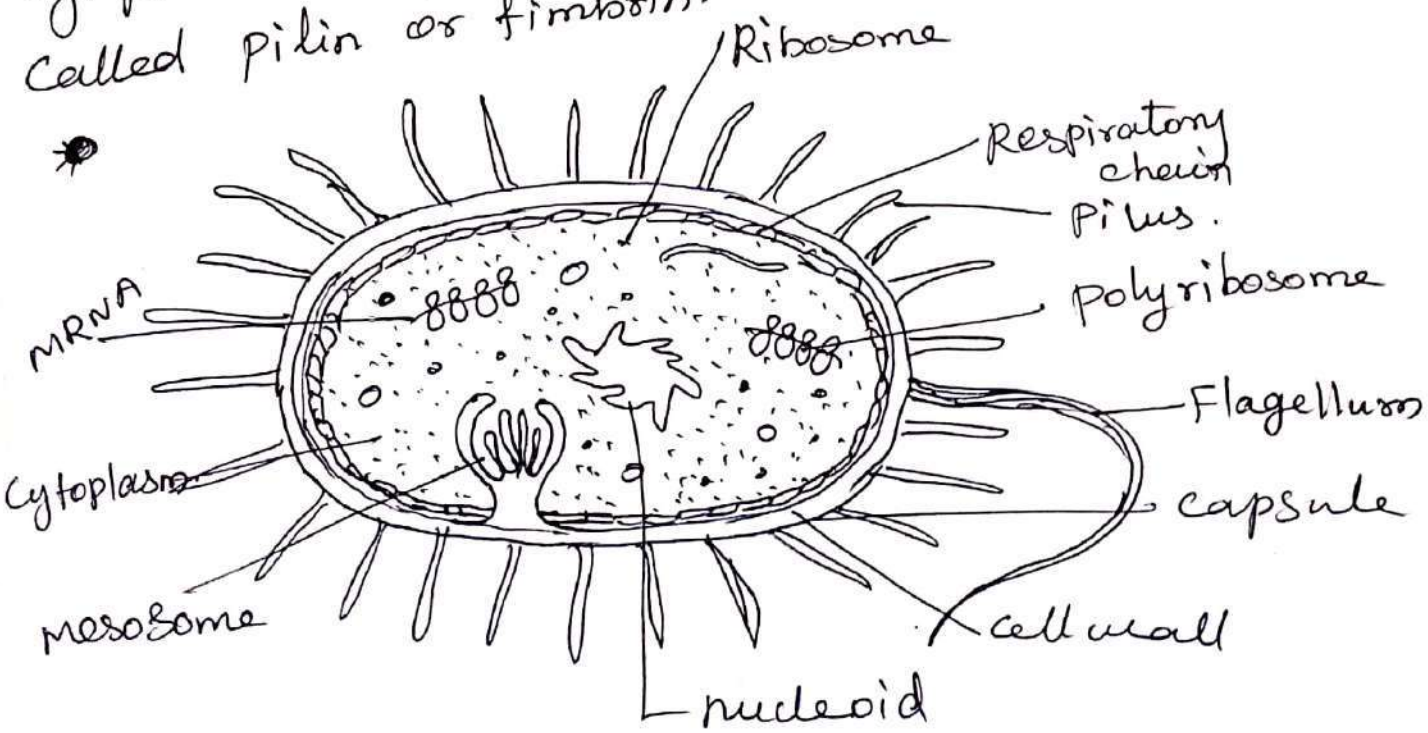

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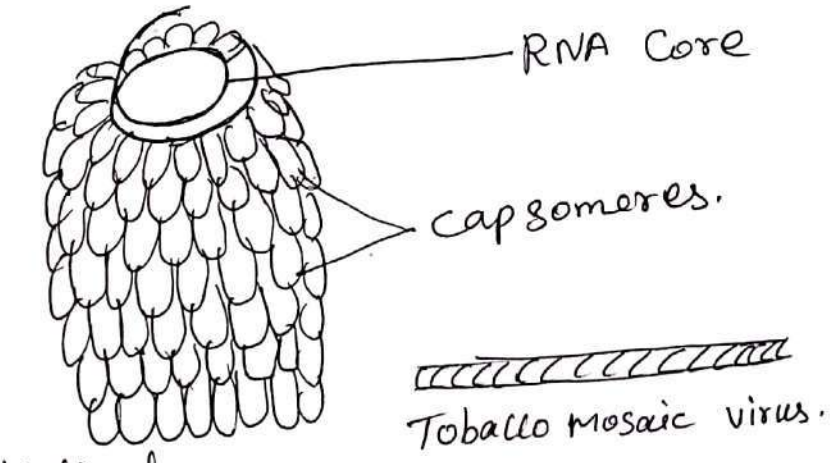

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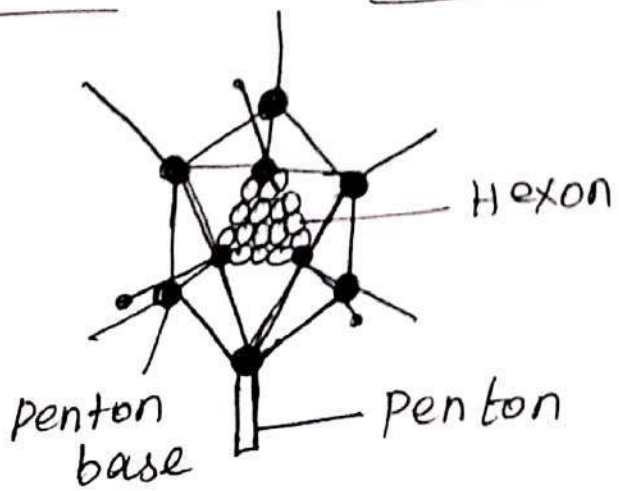

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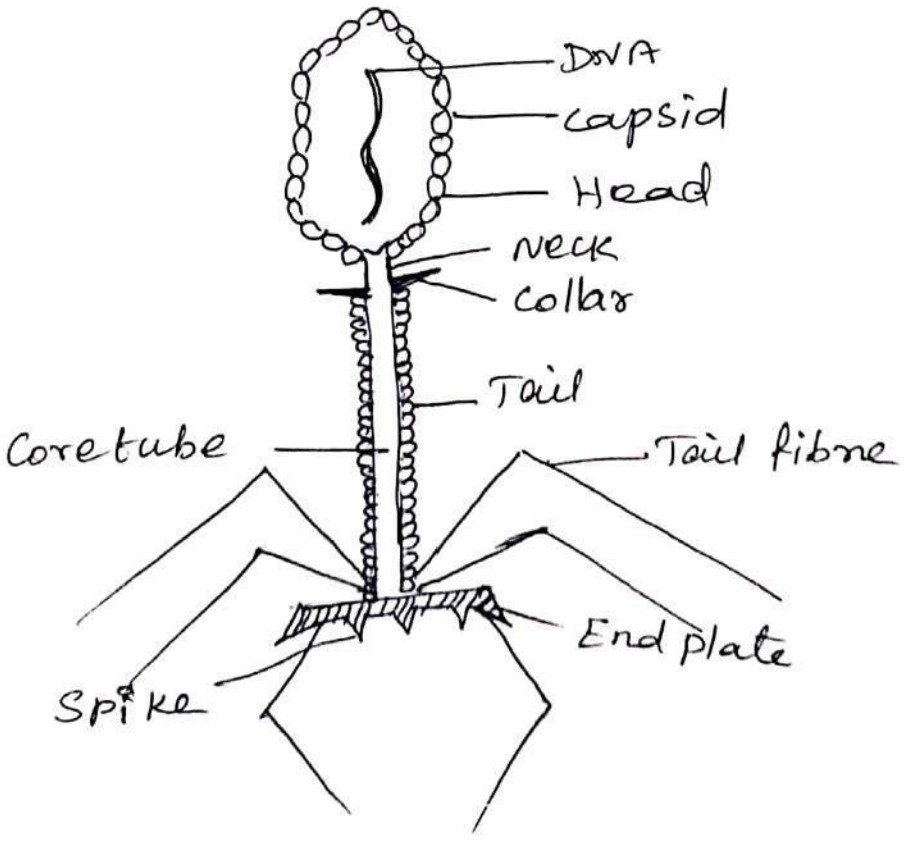


Unit - II

Sterilization Techniques:

Sterilization is the destruction and removal of living organisms. The culture vessels equipments tools and media are sterilized before starting the culture of bacteria

Sterilization is of six types: 1) Heat sterilization 2. Chemical sterilization 3. Filteration 4) Sterilization by UV radiation 5. Sterilization by aldehydes 6) Sterilization by gases.

Heat sterilization:-

Heat is an effective agent of sterilization. Two types of heat are used for sterilization. They are dry heat, and moist heat.

a) Dry heat: i) Flaming is used to sterilize needles, scalpels forceps, glass slides etc;

b) Red heating: Inoculation needles, platinum loops and needles are heated red hot in the flame of a bunsen burner to kill microbes.

c) Hot air oven: used for sterilization by dry heat.

Moist heat: when moist heat is used, the sterilization is called wet sterilization. They are Pasteurization, Boiling, & Autoclaving.

Chemical sterilization: Alcohol, chloroform and Formalin.

Filteration: membrane filters

sterilization by u-v radiation - sterilization by gases.

Culture Medium

Culture medium is any solid or liquid material that supports the growth of microorganisms. The culture medium is composed of beef Extract, peptone, Yeast extract, agar, and distilled water. A typical culture medium is prepared by mixing beef extract Peptone, Sodium chloride and water. This medium is liquid in nature and it is called nutrient broth.

Beef extract- 3g
Peptone - 5g
Sodium chloride 5g
Water 1000ml.
Agar 15g

It is a complex medium for the growth of heterotrophic bacteria

Based on the consistency the culture media is classified into Liquid medium or broth (ii) Semisolid medium (3) Solid medium.

Based on composition, the culture medium is classified into five types (1) Natural or Empirical medium (2) Living medium (3) synthetic medium 4. Complex medium 5. Minimal medium.

Based on the uses the culture medium is classified into (i) Selective medium (ii) Differential medium 3) Enrichment medium (4) Enriched medium 5) Assay medium 6) Transport medium (7) Maintenance medium (8) Enumeration medium 9) characterization medium.

Bacterial culture

Bacterial culture refers to the growth of a particular type of bacterium in a culture medium. They are divided into Broth culture (ii) Plate culture and Differential culture

Broth culture

Culture of bacteria in a liquid medium is called broth culture. The broth consists of following components Beef Extract, Peptone, Sodium chloride, Distilled water.

Plate culture

Growing of bacteria in a solid medium is called plate culture. The medium used for plate culture is agar medium

Differential culture

MacConkey agar medium is used as differential medium in the clinical lab. It is made up of the following components. Eosin Methylene blue agar medium is another differential medium used for E. coli culture.

Culture Techniques

The micro organisms can be cultured in a culture medium. The culture techniques are of different types. They are 1. Batch culture 2) continuous culture 3) Synchronous culture 4) Fed batch culture

1. Batch culture:

Growth of microorganisms in a limited volume of liquid medium is called batch culture. As only one batch of bacteria is cultured in the medium. It is called batch culture. This is the simplest method of culture of microorganisms. In the batch culture system, the culture will pass through four stages namely lag phase, log phase, stationary phase and decline phase.

2. Continuous culture:

Continuous culture refers to the growth of the microorganisms in a medium at a constant

rate continuously. continuous culture is possible when the nutrients is supplied continuously and the toxic by products and dead cells are removed continuously

For continuous culture two types of apparatus are used. They are the chemostat and turbidostat.

Chemostat :- Chemostat is a device for continuous culture. It keeps the bacterial culture in the log phase of growth.

Turbidostat : Turbidostat is a device for continuous culture of bacteria. The turbidostat consists of a reservoir, culture vessel and a collection vessel. Continuous culture provides a constant supply of bacteria

3. Synchronous culture: The division of all the cells at the same time in a culture is called synchronous culture Synchronous growth allows the researchers to study microbial growth.

4. Fed batch culture :

The term fed batch culture is used to describe batch cultures, which are fed continuously or sequentially with fresh medium without the removal of culture fluid. In this culture volume increases with time.

Isolation and Maintenance of Pure culture

Natural environments such as soil, water, air and human body contain many different species of microbes. They are mixed populations. The isolation of one kind of microorganisms from a mixture is called Isolation and purification technique or pure culture technique. A pure culture is obtained by any one of the following methods.

1. ~~Stock~~ serial dilution (II) Pour plate method (III) Spread plate method (IV) Streak plate method (V) Enrichment culture (VI) Selective medium culture (VII) Differential medium culture (VIII) single cell isolation

1. Serial dilution Technique

The dilution of sample in successive stages is called serial dilution. The mixture of micro organisms is serially diluted in test tubes of sterile medium until the last tube contains only a single organisms. In this technique the dilution factor increases in a regular fashion.

In this method 1ml of sample is mixed to 9 ml of sterile water in a test tube. This gives 10 fold dilution factor represented as 1/10 or $(10^{-1}$. Now from the 10 fold dilution. 1ml of sample is added to 9ml of sterile water taken in a second test tube. Now the second tube contains a 100 fold dilution and the dilution factor is represented as 1/100 or 10^{-2}.

Likewise from the tube two, 1 ml of sample is taken and is added to 9 ml of sterile water taken in a third test tube. Now the third tube contains a 1000 fold dilution and the dilution factor is represented as 1/1000 or 10^{-3}.

Likewise test tubes 4 and 5 are prepared. Test tube 4 provides 10^{-4} dilution. and the test tube 5 provides 10^{-5} dilution. A 6th tube is prepared as a control containing 10 ml of sterile water only. From each tube 1 ml of diluted sample is taken and is added to an agar plate. The agar plates are incubated at 30°C for 48 hrs.

The agar plate containing 30-300 colonies are taken as pure culture of that organism.

Streak plate method:

In this technique for isolating bacteria from a mixed culture. In a streak plate method mixed culture is taken on a sterile inoculation loop and is drawn back and forth on the solid agar medium.

Pour plate method:

It is an isolation and purification technique. In this method the mixed culture is diluted in sterile medium and the diluted mixture (1ml) is added to test tubes containing melted agar medium. The content of the tubes are then poured into a sterile petridish and allowed to solidify. The plates are then incubated. The isolated cells develop into colonies here and there.

Spread plate Technique:

It is an isolated technique. In this method the mixed culture is serially diluted in sterile distilled water. A small amount of the diluted mixture is poured on the surface of an agar plate and it is spread evenly

using a sterile L rod. The isolated cells grow into colonies

Maintenance of Bacterial culture

Storing bacterial culture alive for future use is called maintenance of pure culture. The preserved culture is called as the stock culture. Some cultures can be preserved for 80 yrs. Different methods are employed for maintenance of bacterial culture. They are

1. Periodic transfer to fresh media
2. Maintaining with mineral oil
3. Maintaening in formaldehyde.
4. Lyophilization
5. Preservation by liquid nitrogen
6. Soredelli's method of preservation
7. Storage in sterile soil
8. Storage in silica gel.

(i) Periodic transfer to fresh media

The culture can be stored in alive condition by transferring the culture to fresh medium at ~~regular~~ regular intervals. The transfer can be done in once a month. The culture is maintained in agar slants.

2) Maintaining with Mineral oil:

Bacterial cultures can be stored

alive for 2 years in mineral oil. The culture is kept in agar slant and mineral oil is poured into the agar slants.

3. Maintenance in Formaldehyde.

Agar plate cultures can be preserved by placing a drop of formaldehyde on the inner side of lid. It is stored in a refrigerator at 4°C.

4. Lyophilization

Lyophilization is used to preserve many kinds of bacteria that would be killed by ordinary drying. In this process a dense cell suspension is placed in small vials and frozen at -60°C to -78°C

5. Preservation by liquid Nitrogen.

A dense cell suspension is taken in a medium containing cryoprotective agent such as glycerol or dimethyl sulphoxide which prevents cell damage due to ice crystal formation. The vials are stored in liquid Nitrogen at -150 to -196°C.

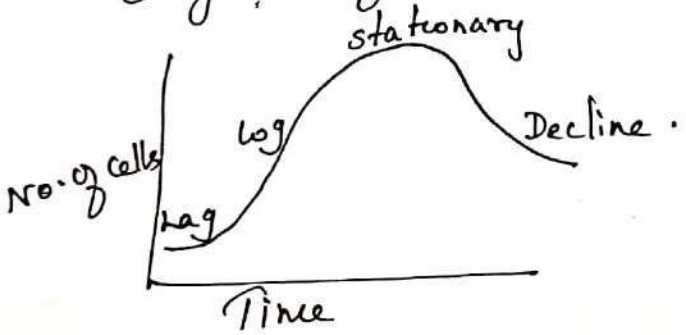
Significance of preservation

1. Used for laboratory experiments
2. Research work.
3. test agent 4. reference strain for taxonomic studies

Bacterial Growth curve.

Growth curve is a graph obtained by plotting the number of cells against time factor. A typical growth curve is obtained when known concentration of bacteria is inoculated into a suitable culture medium. The bacteria grow by dividing binary fission. The bacterial cells are counted at regular intervals of one hour.

A typical bacterial growth curve shows four distinct phases namely lag phase, log phase, stationary phase and decline phase. Lag phase represents an initial period of no growth interms of increase in cell numbers. In this phase the cells are metabolically active capable of repairing cell damage and synthesizing enzymes.



Log phase is a period of rapid growth. In this phase the bacterial population increases exponentially and is also called growth phase

Log phase is followed by a stationary phase during which no new growth occurs.

Lastly, there is decline in the viable population in which all microbial cells die. This phase is called decline phase or death phase

When nutrient is regularly added to the culture the bacteria grow continuously and we get continuous culture. In continuous culture, the bacterial population remains in the log phase and there will be no stationary and decline phases.

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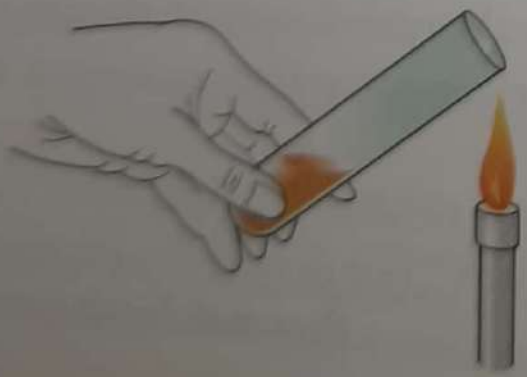

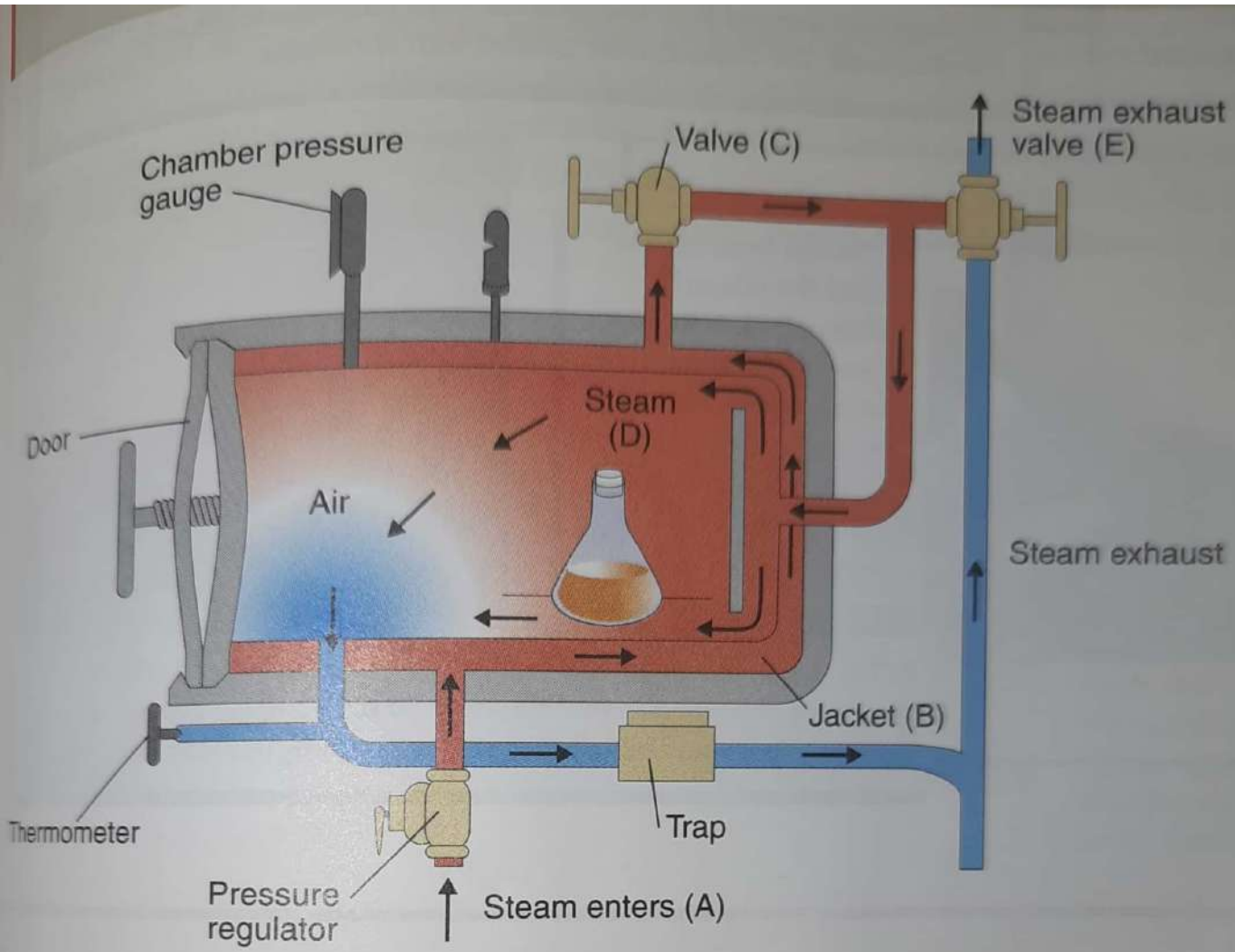

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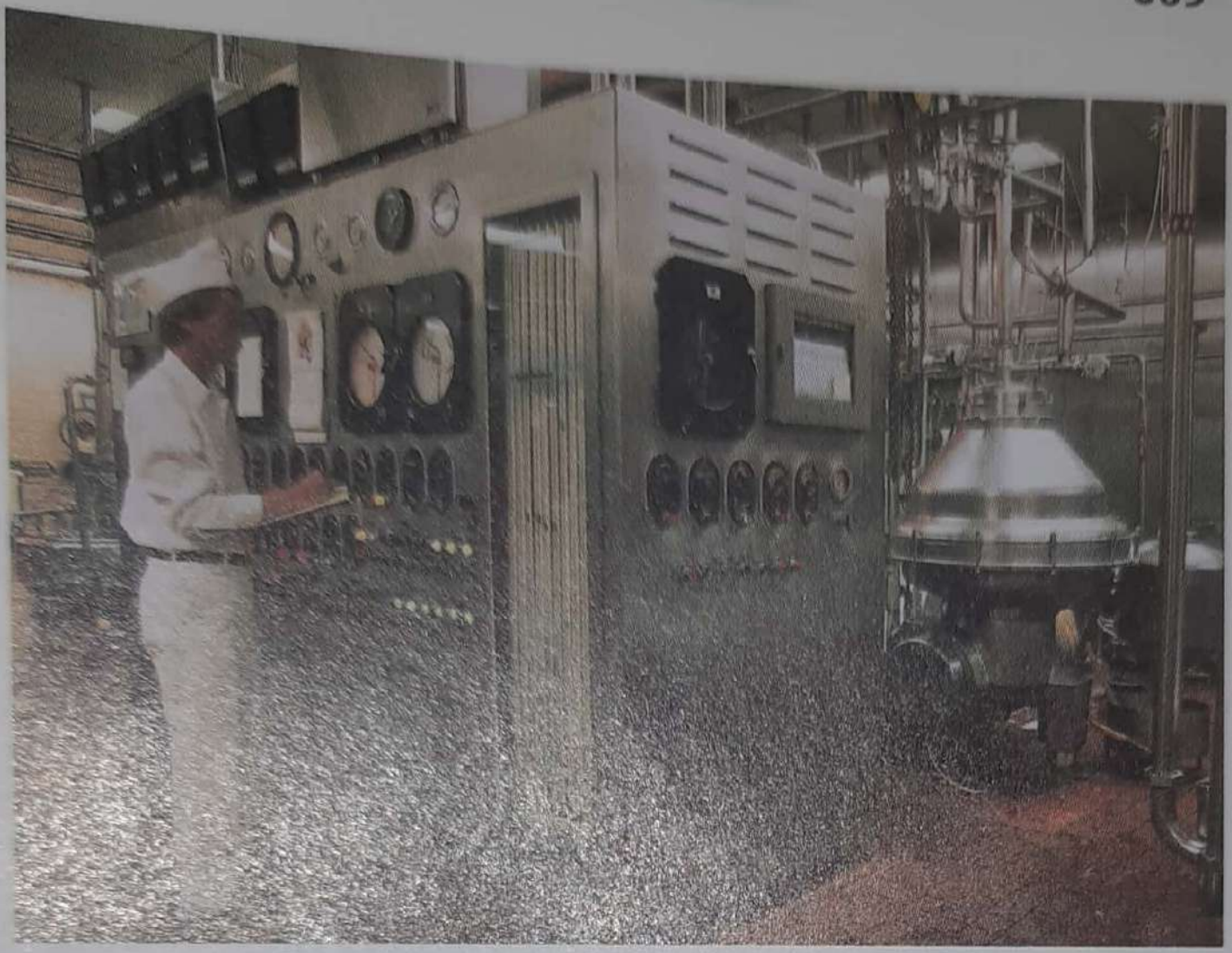
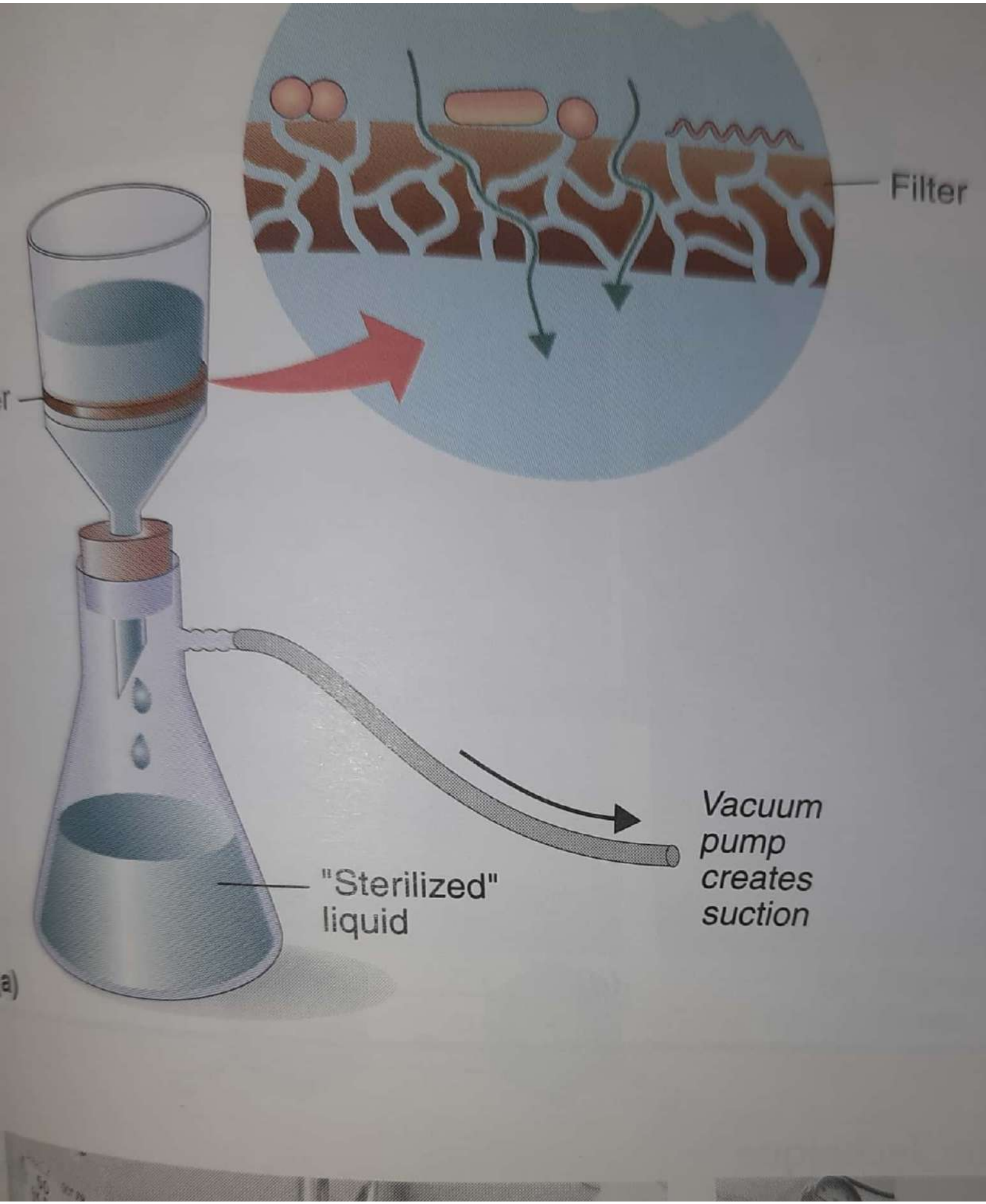


FIGURE 21.9

The Pasteurization of Milk


Filter
"Sterilized"
liquid
Vacuum
pump
creates
suction

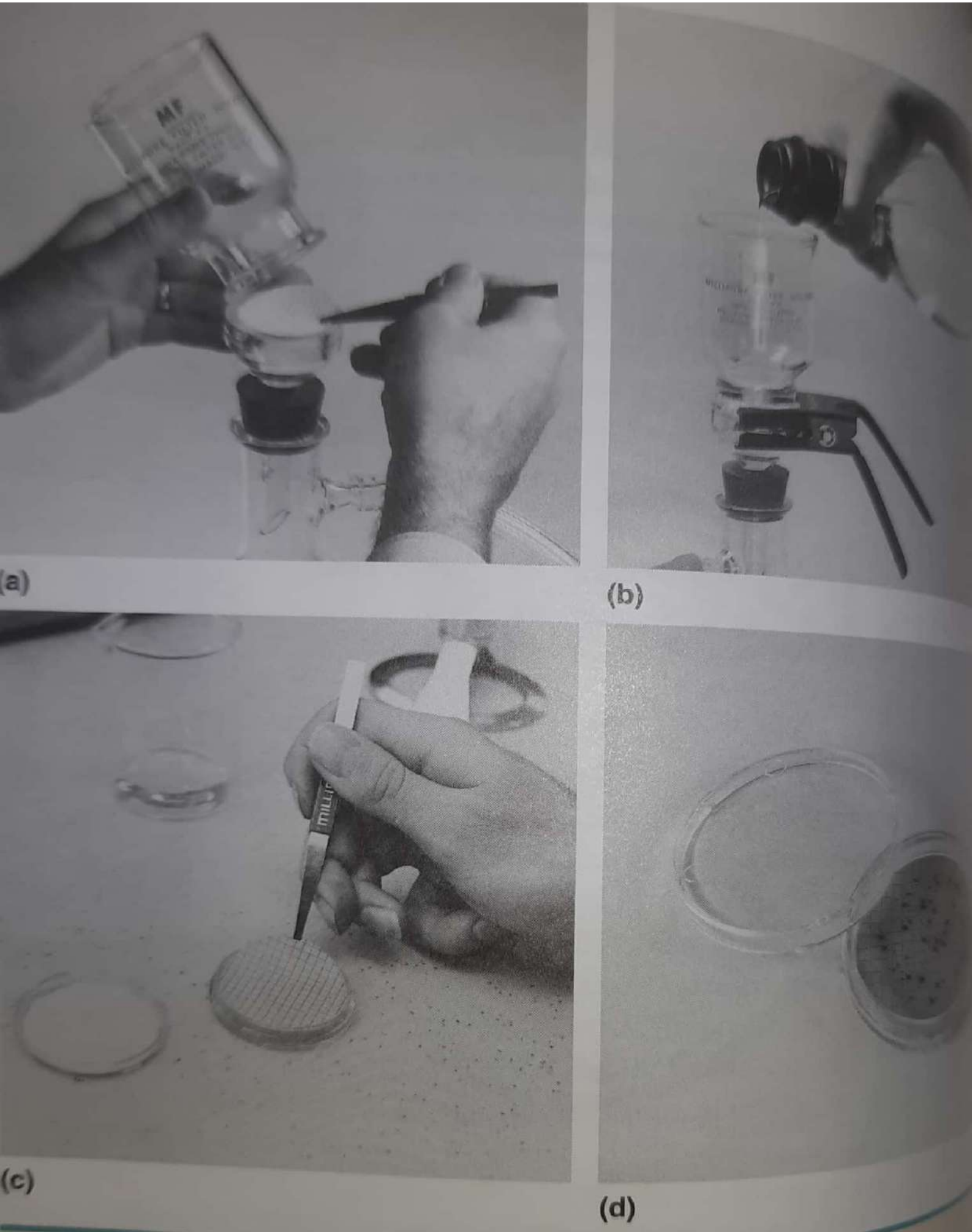


FIGURE 21.12

The Membrane Filter Technique

(a) The mem... mounted in a ... filtering ...

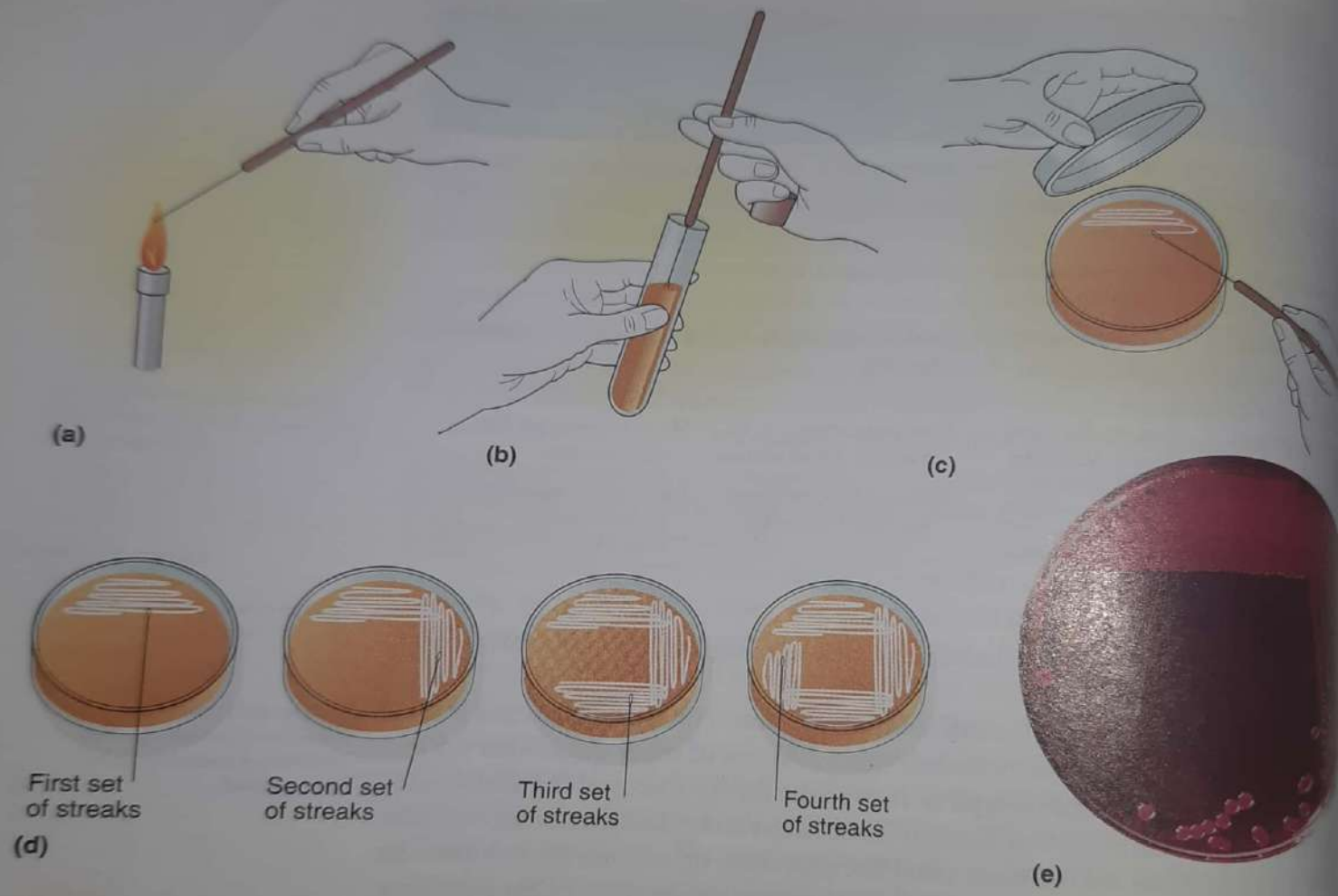


FIGURE 4.20

The Streak Plate Isolation Method

A loop is sterilized, and a sample of bacteria is obtained and streaked along one edge of the plate of medium. Successive streaks are formed, and the plate is incubated. Well-isolated and defined colonies illustrate a successful isolati

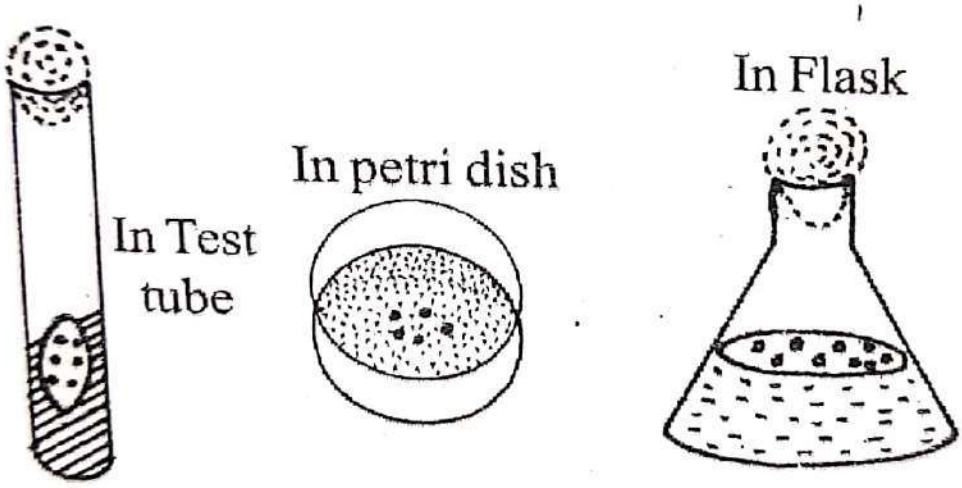


Fig.38.1: Batch culture.

growth curve.

2. Continuous Culture

Continuous culture refers to the *growth of the microorganism in a medium at a constant rate continuously.*

Continuous culture is possible when the nutrient is supplied continuously and the toxic by products and dead cells are removed regularly.

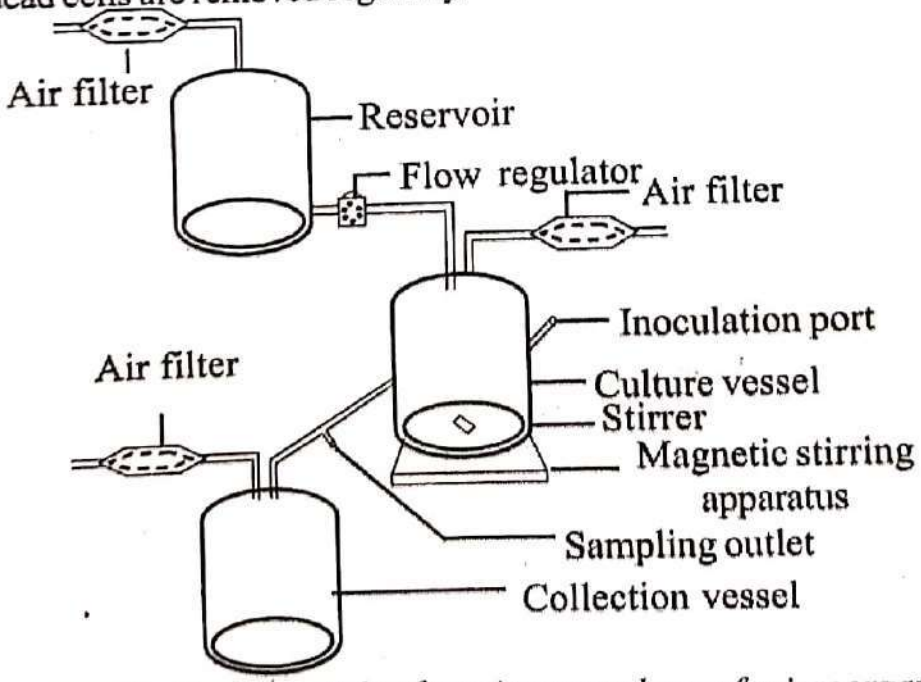


Fig.38.3: Working principle of continuous culture of microorganisms.

1. Chemostat

is a device for continuous culture. It keeps the bacterial culture in the log phase of growth.

The chemostat consists of a ***reservoir***, a ***culture vessel***, and a ***collection vessel***. The ***culture vessel*** is equipped with an out flow siphon and a mechanism for dripping in fresh medium from the ***reservoir*** at a regulated rate.

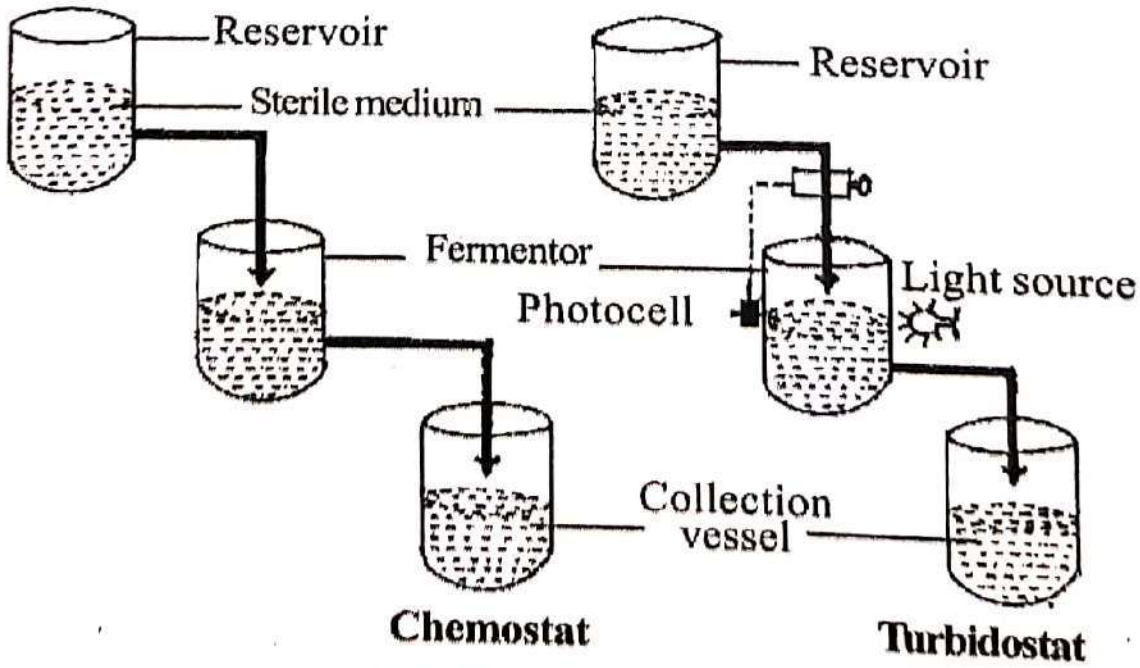


Fig.38.5: Continuous culture.

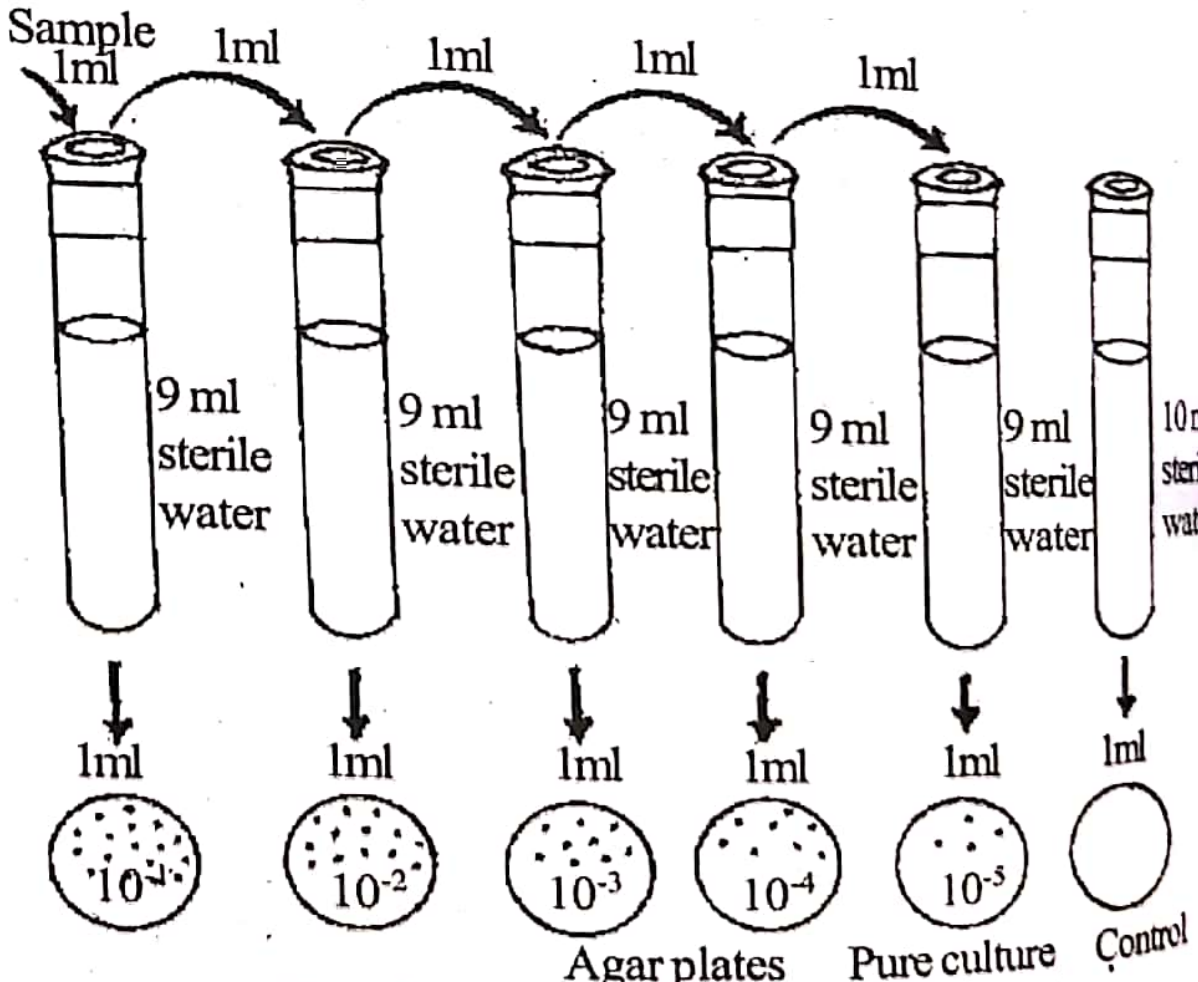


Fig.39.5: Serial dilution technique.

this method, the agar

ring the petridish.

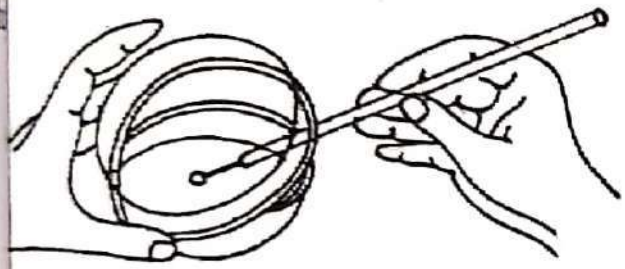


Fig.39.6: Streaking an agar plate.

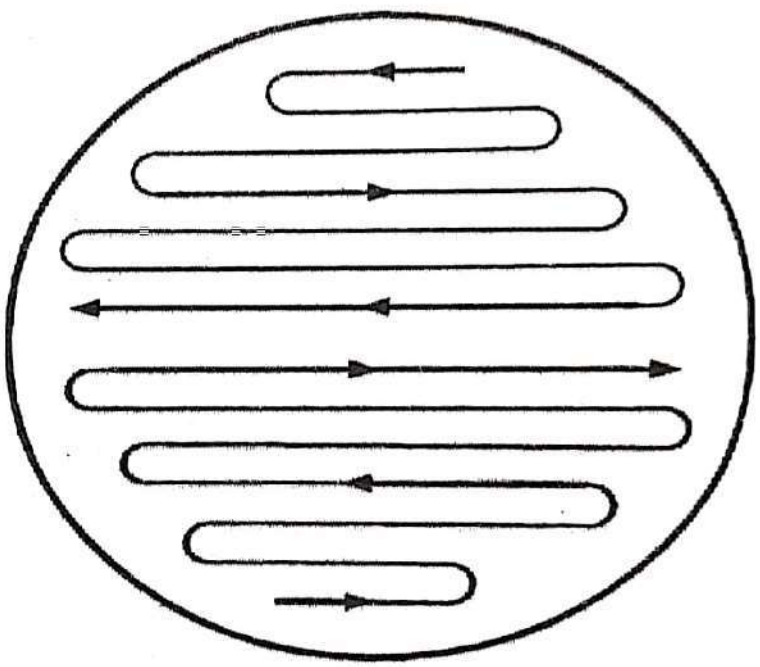


Fig.39.7: Streak plate method

One loopful of sample is transferred to tube A. It is vigorously shaken.

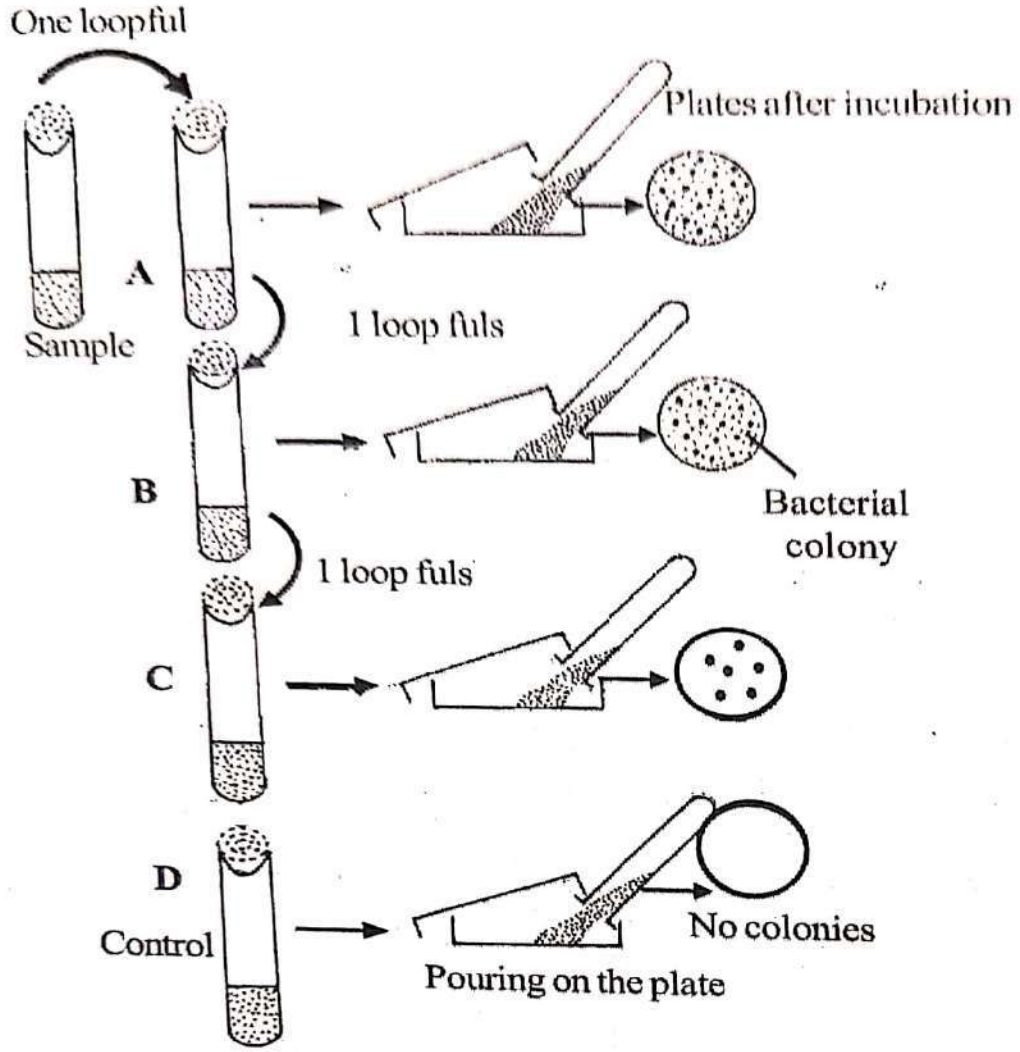


Fig.39.8: Steps in pour plate method.

A loopful of sample is taken from tube A and

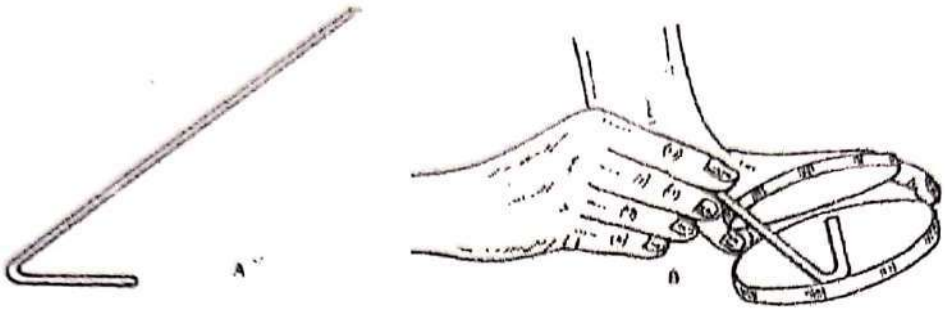


Fig. 39.9: A. Glass spreader and B. Method of spreading.

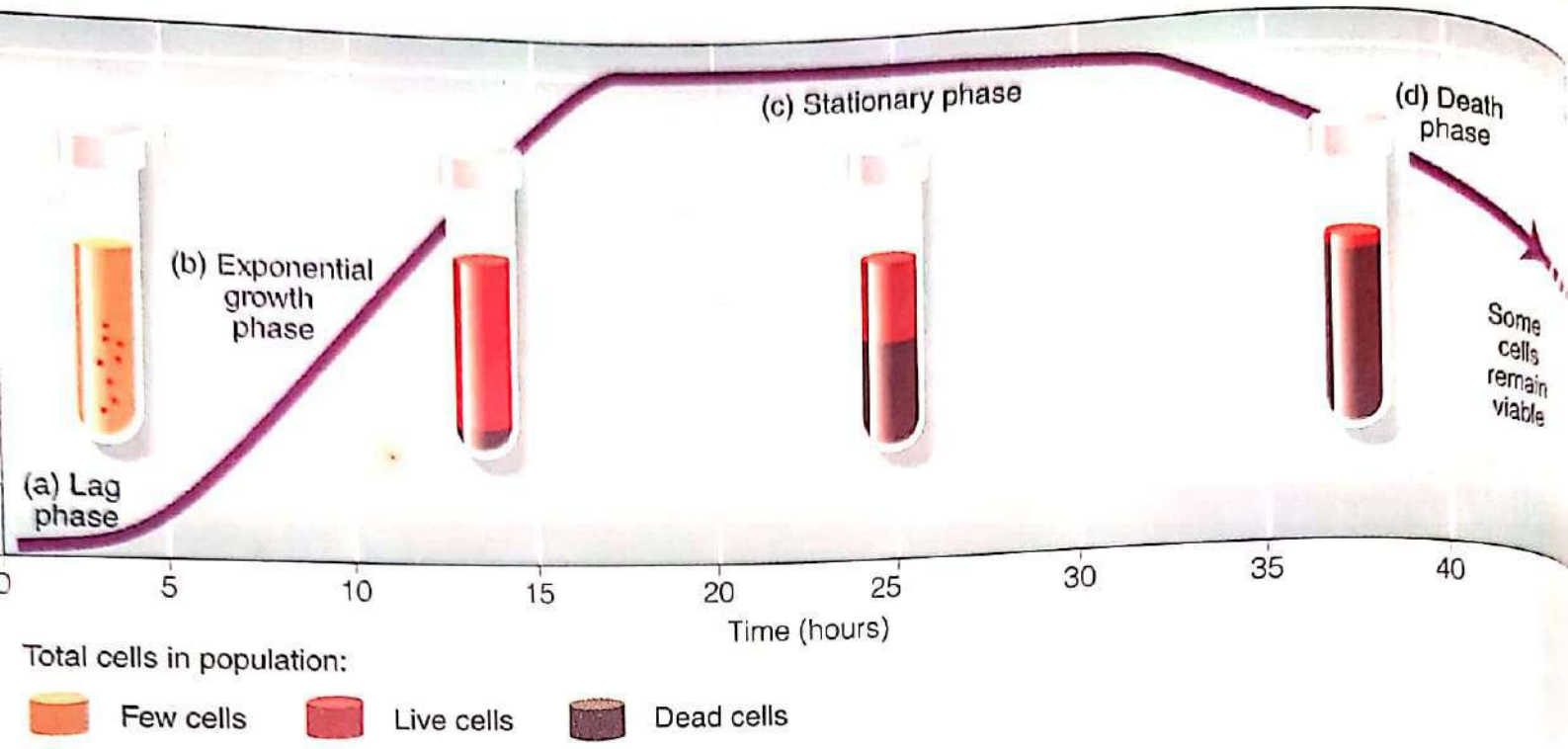
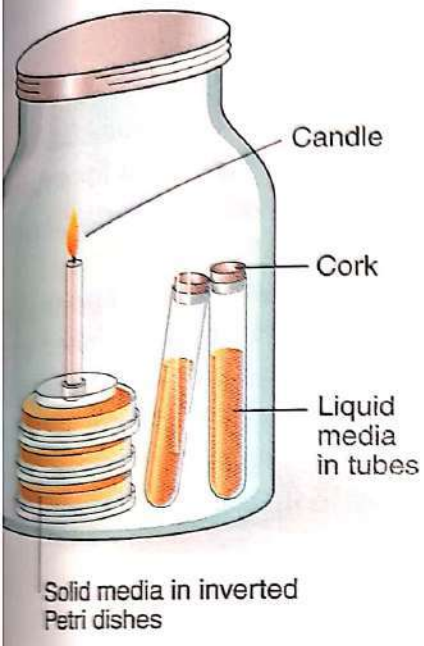
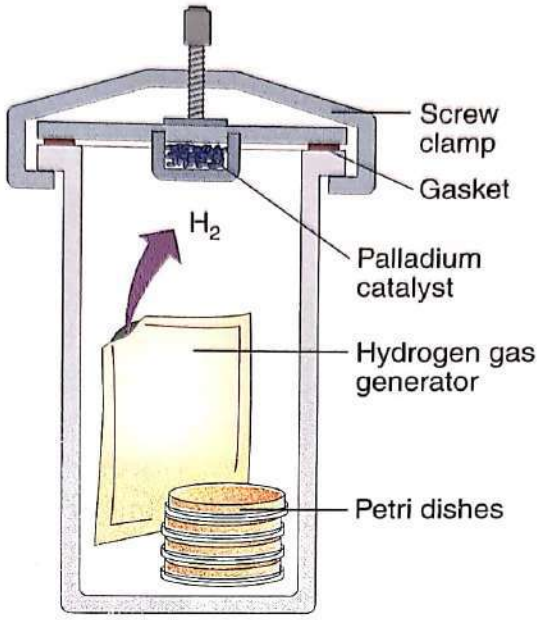

(a) Lag phase
(b) Exponential growth phase
(c) Stationary phase
(d) Death phase
Some cells remain viable
0
5
10
15
20
25
30
35
40
Time (hours)
Total cells in population:
Few cells
Live cells
Dead cells

FIGURE 4.14


Candle
Cork
Liquid media in tubes
Solid media in inverted Petri dishes


Screw clamp
Gasket
H_2
Palladium catalyst
Hydrogen gas generator
Petri dishes

(b)


GasPak