SYLLABUS

B.Sc IV SEM

SUBJECT: MICROBIAL BIOTECHNOLOGY

Unit I	**Topic** **Microbial classification- 3 kingdom, 5 kingdom and 3 domain** **Basic nutrition and** **nutritional classes of bacteria,** **Microbial media and its typea** **Different methods of cultivation of microorganisms** **Aerobic and anaerobic**
Unit II	**Microbial growth , methemetical expression of growth,** **Factors affecting growth , growth curve,** **Quantification of growth,** **Batch , continuous , synchronous, diauxic growth,** **Control of microorganisms- Physical,chemical methods of control,** **Evalution of chemical disinfectans,** **Tube dilution test, agar dilution test,** **phenol-coefficient**
Unit III	**Microbial strain improvement,** **Normal flora of the body, antiviral agents** **Infection of different system** **Chemotheraphy- use of antibiotics** **Operon concept with example(lac,trp, ara)** **Microbial and viral diseases**

	bacterial genetics **Transformation, conjugation, transduction**
Unit IV	**Types of fermentations-batch, continuous , fed batch , submerged and solid state fermentation basic design of fermenters and factors types of Fermentation – fluidized packed bed, air lift fermenter, try fermenter, tower fermenter**
Unit V	**Industrial production of ethyl alcohol, penicillin, cynocobalamine, citric acid, amylase, protease**

UNIT-1

1. Q.1 Microbial classification- Taxonomy is the science dealing with the description, identification, naming, and classification of organisms. Classification is the 'grouping' of organisms based on particular characters and is not arranged in hierarchical order.

(1) 3 KINGDOM CLASSIFICATION: - Three kingdom system is given by Ernest Haeckel-1866. This classification is as follows-

KINGDOM PROTISTA: - It includes unicellular and colonial eukaryotes such as bacteria, algae, fungi and protozoans.

- KINGDOM PLANTAE:-It includes multicellular photosynthetic plants.
- KINGDOM ANIMALIA: - It includes multicellular animals.

(2) 5 KINGDOM CLASSIFICATION:-

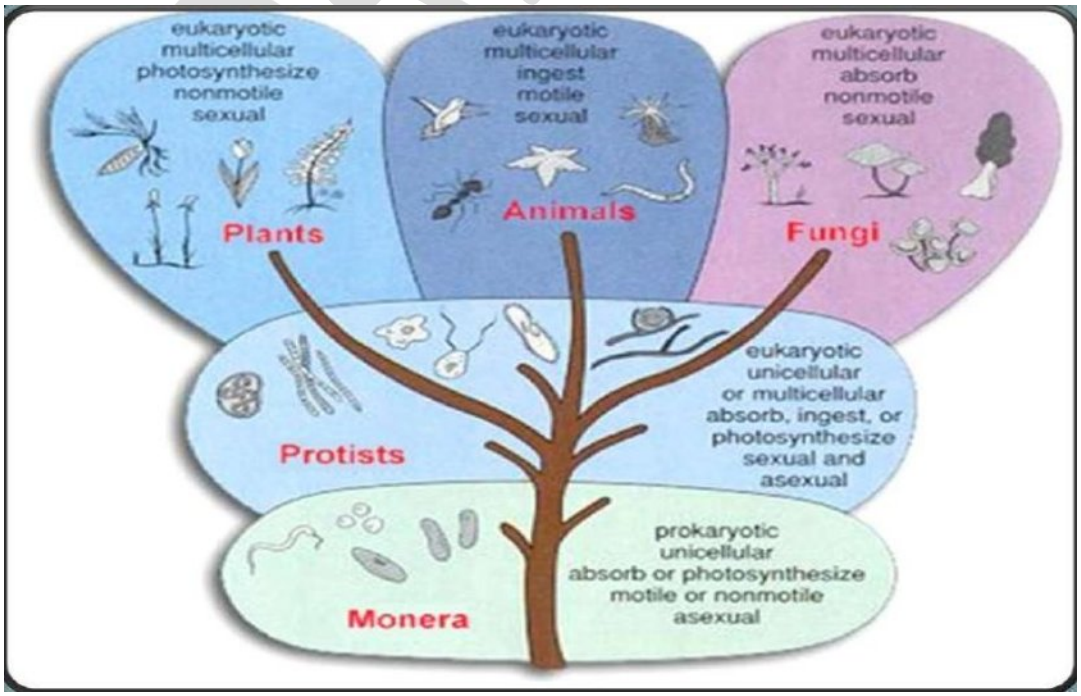


Fig.:- 5 kingdom system

(3) 3 DOMAINS CLASSIFICATION:-

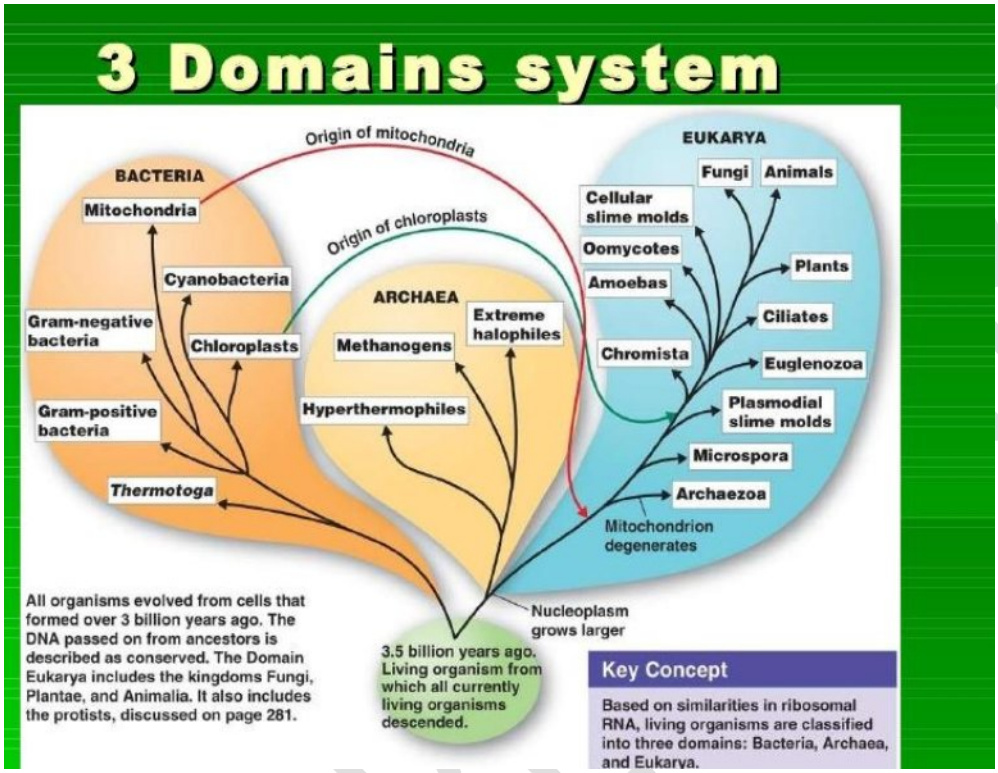
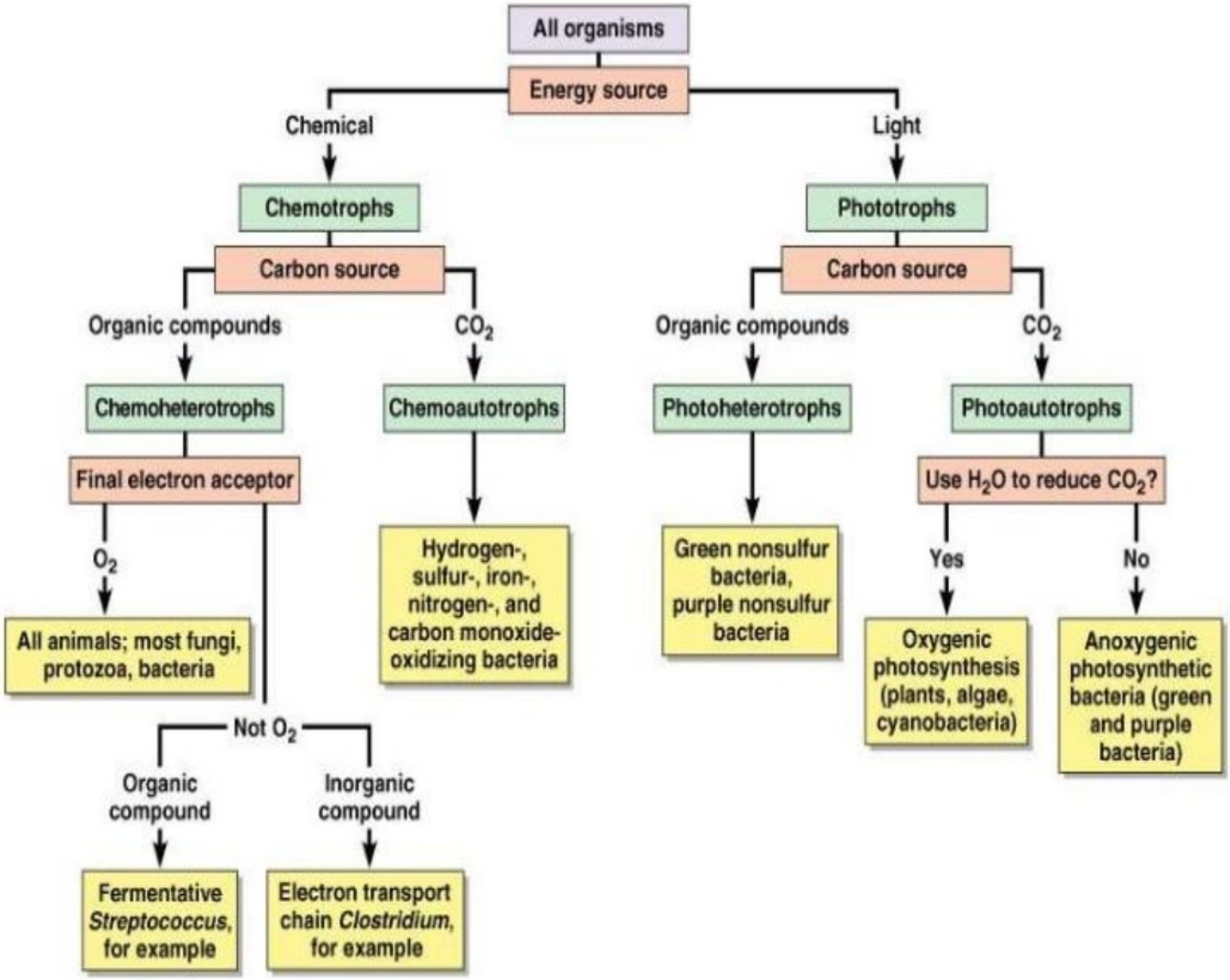


Fig.:- 3 Domains System

Q.2 BACTERIAL NUTRITION:-

Basic Nutritional Requirements of Bacteria

- For growth and multiplication of bacteria, the minimum nutritional requirements are water, a source of carbon, nitrogen and some in organic rates.
- At least ten elements are considered essential for growth which are usually supplied from the environment (Tube from sots).
- Amino acids, pur, pyramidines and vitamin are also required for cell growth.
- AA are required for the synthesis of cellular protein purinices and pyramides are needed for synthesis of nucleic acid biosynthesis as well as participating as activating cervical molecule in a biosynthesis reaction (e.g. peptidoglycan, LPS, polysaccharide synthesis). The vitamins function primarily as components of co enzymes in the numerous enzyme driven chemical reactions that occur in cells.



UNIT-II

Q.1. Explain growth kinetics or growth curve of micro –organisms. (m.o)

Ans: Microbial Growth:- Growth can be defined as an orderly increase of all chemical compounds in a cell/population resulting in an increase in mars of the cell/population.

Growth is followed by cell division resulting in cell number. Under ordinary conditions of growth, all actively growing cells multiply by a sexual process of cell division, and this process can continue indefinitely, provided food and energy are available and environmental conditions remain favourable,.

When a bacterium grows, it ultimately increase in the cell number, but their size remains relatively unchanged.

Generation Time of Bacterial: Most bacterial resproduce by binary fusion, which results in doubling of the number of viable cells. Therefore, during active bacterial growth, the number of bacterial cells and hence their population, continuously doubles at specific time intervals, because each binary bission takes a specific duration of time. This 'specific time intervals' between two subsequent binary fissions is known as 'generation time or doubling time'.

1 ----2----2------2------2-----2-------→2

Geometric Progression. n={the no. of generations}

Growth Curve:-

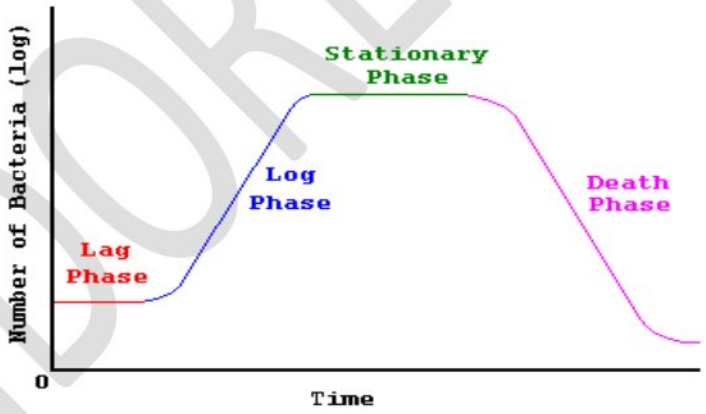


Fig: characteristic growth curve showing lag, log and stationary, death phase.

1) Lag Phase: Lag phase represent a period of active growth during which bacteria prepare for reproduction, synthesizing DNA. Various inducible enzymes, and other macromolecules needed for cell division.

   Therefore, during this phase, there may be increase in size, (volume) but no increase in cell number. The lag phase may last for an hour or more, and near the end of this phase some cells may double or triple in size.

2) Log Phase: Bacterial cells prepared for cell division during lag phase now enter into the log phase during which the cells divide at a maximum rate and their generation time reaches a minimum and remains constant.

   This phase is called log phase because the logarithm ef the bacterial mass increases linearly with time, and exponential growth phase because the number of cells increases as an exponential function of 2 n (2, 2, 2 …….). The Log phase also represents the time when bacterial cells are most active metabolically, and in industrial production, this is the period of peak activity and effieciency.

3) Stationary Phase: Since the bacteria are growing in a constant volume of medium of batch culture and no fresh nutrients are added, the growth of bacterial population eventually ceases and the growth curve becomes horizontal. The ceasation of growth may be because of the exhaustion of available nutrients or by the accumulation of inhibitory end product of metabolism. The bacterial cells start dying and the number of such cells balances the number of new born cells, and the bacterial population stabilizes. This state of growth, during which the total number of viable cells remains constant because of no further net increase in cell number and the growth rate is exactly equal to the death rate is called stationary phase.
4) Death Phase: After a while, lthe number of dying cells begins to exceed the number of new born cells and thus the number of viable cells present in a batch culture starts declining. Thiscondition represents the death phase.

Q.2. Cell constituents:-

1. CAPSULE: Most of the bacterial contain some sort of polysaccharide layer out side the cell wall or outer membrane; this layer is called a capsule. Capsule is found in many species of bacteria like Diplococcus , Pneumococcus etc. It is a viscous or gelatinous loose aggregates of substance secreted around the cell wall.

2. Cell Wall: The cell wall of bacteria is the wall that gives a rigid structure that gives shape to the cell. The main function of cell wall is to prevent the cell from expanding and bursting. Tell wall also protects the cell from antibodies and harmful chemicals.

The cell wall of gram, negative is generally thinner (10-15 mm) and gram positive species (20-25 mm). Bacterial cell wall is usually essential for bacterial growth and division

(A) Gram positive Bacterial, showing thick wall consisting of peplidoglycan.
(B) Gram Negative Bacteria showing outer membrance and thin peptidogly – can layer.

3) Cell Membrance: The plasma membrane or cytoplasma cell membrance is present internal into cell wall. It is nearly 7.5 mm thick made up of proteins (20-70%), lipids (20-80%) and oligosacclerides (1-5%) and water (20%).

The structure of cell membrane was explained by 'Fluid Mosaic Model' of singer and Nicolson (1972). According to this model, the plasma membrance is a quasifluid structure, which consists et a

continuous bilayer of phospholipid molecule in which globular proteins are embedded in a manner,. The globular proteins are of two types extrinsic and intrinsic protein.

4. Cytoplasm: The cytoplasm is viscous and semitransparent. The cytoplasm is viscous and semitransparent. The prokaryotic cytoplasm consists of water (80%), proteins, carbohydrates, lipids, inorganic ims and certain low molecule compounds, organelles like ribosome, DNA, mole, inclusion bodies.

5. Nuclear Material: The bacterial nucleus material do not possess the nuclear membrane and nucleolus, it is called nucleoid. Usually the bacterial nucleoid consists of a circular double standard DNA . Vibrio cholera and Borrelia bergdorferi have a linear.

6. Plasmid: These are small circular, self replicating and ds DNA molecules present in bacterial cell, in addition to bacterial chromosome. Some plasmids are able to integrate into bacterial chromosome. Thus they replicate with the bacterial chromosome. Such plasmids are called episomes.

7/ Ribosomes: Ribosome's are the site of protein synthesis. No. of ribosome's increase during the protein synthesis. The prokaryotic ribosome is free in the cytoplasm unlike the eukaryotic ribosome, which are found to be attached to the cell membrane. The prokaryotic ribosome is small and dense, often called 70s ribosome which is made up of two subunits – 50s &30s. Each subunit composed et r RNA and protein.

8. Mesosomes: The cell membrane of some bacterial invaginates and forms vesicles or pocket like structure called Mesosomes. It is commonly found in gram positive bacteria.

9. Extra Cellular structure: A large number of bacteria posses' extracellular structures such as flagella, Pilli and Fimbrae

Flegella: Bacterial flagella are hair like,. Helical appendages that protrude through the cell wall. It is 20-30 nm, in diameter and 15 um long. It is responsible for swimming motility of the bacterial cell. The flagella of prokaryotes are several times tinier than those of eukaryotes. It may be polar or literal.

"Pilli and Fimbrial: Fimbriae and pilli are short time and hair like structure found on surface of all wall in many gram negative bacteria such as Entero bacteriaceae, Pseudomonadeaceae.

The number of fimbriae is around 1,000 and number of pilli varies from 3-5. These are thin, shorter, straight and less rigid than flagella.

Some pilli which are longer than remaining pilli, are called sex pilli , they help in formation of conjugation tube during gene transfer.

Q. 3 Quantification of Growth:-

Growth leads to increase both the number and the mass of the population, both are counted for quantification of growth.

(A) Measurement of cell numbers:-

(B) 1. Breed Method:

A know volume of microbial cell suspension (0.01 ml) is spread over a clean glass slide. This smear is fixed by heating, stained and examined under oil immersion lens and cells are counted.

The total number of cells can be counted with the help of following calculation.

a) Area of microscopic field =

R (oil immersion lens) + 0.08 mm

Area of microscopic field uner the oil – immersion lens =

=3.14 x (0.0 8 mm) 2

= 0.02 sq. mm.

B) Area of smear one sq. cm = 100 sq. mm

Then the number of microscopic field =1oo/0.02=5000

C. No. of cells 1 sq. cm = Average number of microbes

Microscopic field x 5000

2. Counting chamber Technique:

The number of cells in a population can be measured by taking direct microscopic count using Petroff – Hausser counting chamber (for prokaryotes) and Hemocytometer (for Eukaryotes). Prokaryotes are more easily counted if they are stained or not, phase contrast of florescence microscope is used.

3. Viable count: A bacterial culture need not contain all lining cells. The culture when grown in proper medium and under standard set or growth conditions, only living cells grow and form colony. This fact is used to estimate number of living bacterial cells, the estimation et. Number of living cells is called 'viable count'

Standard plate count (SPC) method is most commonly used lab technique for viable count of bacterial cells in milk, food, water.

4. Coulter counter: Coulter counter is an electronic device used to count number of bacterial and other m.o. such as protozoa, microalgae and yeasts. This device is provided with a tiny orifice 10-30 um in diameter. This orifice connects the 2 compartments et the counter which contain an electrically conductive set (electrodes).

In this method, the sample of bacterial cells is forced through the small orifice. On the both sides, electrodes are present to measure the electric resistance when electric current is passed through the orifice. Each time a bacterial cell passed through the orifice, electrical resistance, between the 2 compartments increases momentarily or the conductivity drops. This generates an electrical signal represent the counting of one bacterial cell. It gives accurate results.

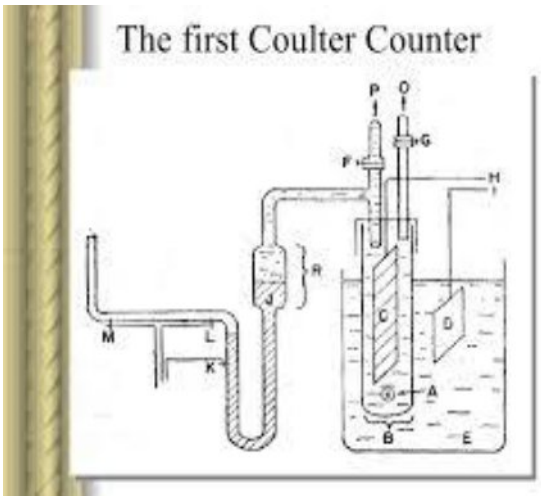


Fig: The coulter counter

5. Membrance – Filter Technique: Microbial cell no. are frequently determined using special membrane filters process milipores small enough to trap bacteria. In this technique a water sample containing cells is passed through the filter. The fitter is then placed on solid agar medium and incubated until each cell develops into a separate colony. A colony count gives the number of cells m.o. in the filtered sample, and the specific media can be used to select for specific m.os. This technique is especially useful in analyzing aquatic samples.

B Measurement of cell Mass:

1) Dry Weight Technique: The cell mass of a very dense cell suspension can be determined by this technique. In this technique, the m.os are removed from the medium by filtration and the m.o.on filters are washed to remove all extraneous matter, and dried in dessicator by putting in weighing bottle. The dried microbial content is then weighed accurately. This method is generally used for mi9crofungi.
2) Measurement of Nitrogen Content: As the microbes (bacteria) grow, there is an increase in the protein cone, (i.e. nitrogen conc.) in the cell. Thus cell mass can be subjected to quantitative chemical analysis methods to determine total nitrogen that can be correlated with growth.

   This method is useful in determining the effect of nutrients or antinitabolites upon the protein syntheses of growing culture.
3) Turbidometric Estimation: Rapid cell masss determination is possible using turbidometry method. Turbidometry is based on the fact that microbial cells scatter light6 striking them. Since the microbial cells in a population are of regularly constant size, the amount of scattering is directly proportional to the biomass of cells present and indirectly related to cell number. One visible character of growing bacterial culture is the increase in turbidity. When the conc. Of bacterial culture reaches 10 million cells (10%) per mil, the medium appears slightly cloudy or turbid. Further increase in conc, results in greater turbidity. When a beam of light is passed through a turbid culture, the amount of light transmitted is measured. Greater the turbidity, lesser would be the transmission of light through medium. Thus, light will be transmitted is inverse

preparation to the no. of bacteria. Turbidity can be measured using instruments like spectro photometer and nepuelometer.

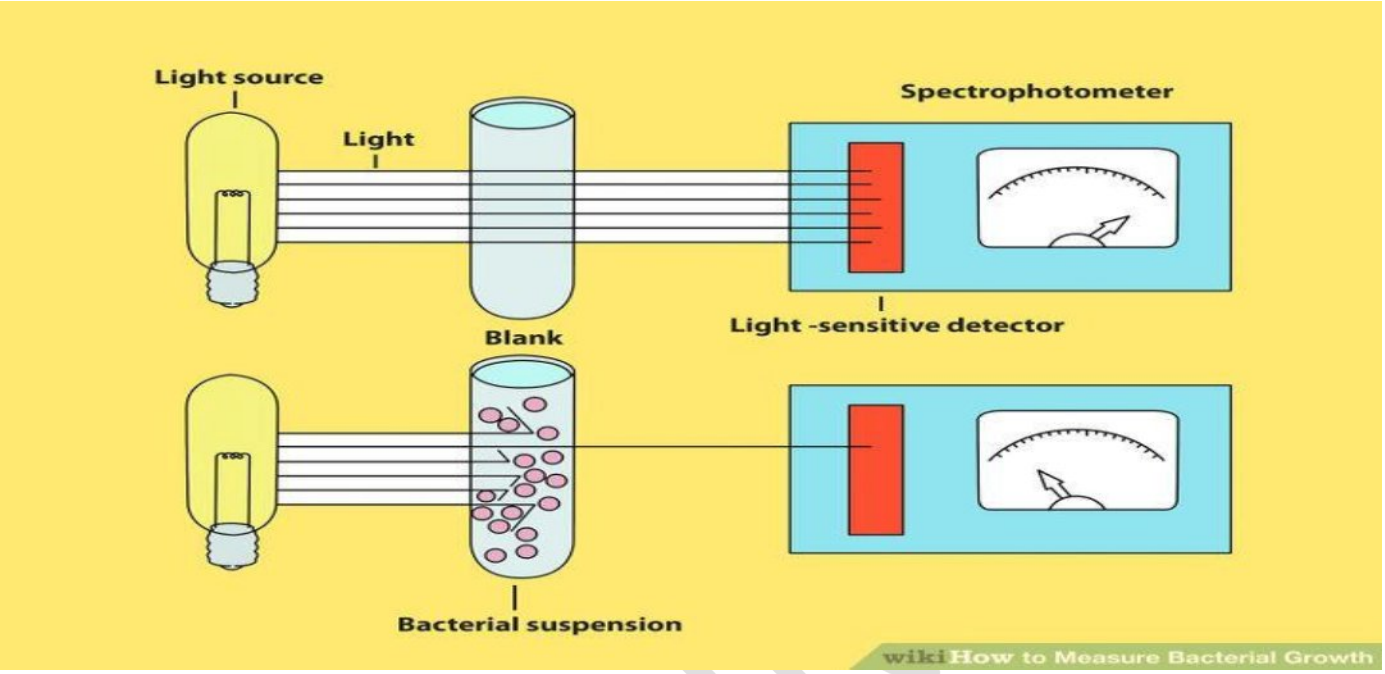


Fig: Deterination of cell mass using turbiodometry method.

Q.4 Physical and chemical control of micro-organisms: The term 'control' with respect to micro-organisms refers to the reduction in their number and/or activity.

(A) Physical Methods of Control: sterilization:

1. **HEAT**: Temperature) Sterilization ---Fire and boiling water have been used for sterilization. Heating is one of the most popular ways to kill m.o. Microorganisms grow over a wide range of temp., and every type of them has an optimum, minimum and maximum growth temperature. Temperature above the maximum generally kill the m.o.
2. **Moist Heat Sterilization:** Moist heat sterilization i.e. sterilization by heat combined with moisture is one of the most effective method of killing m.o.

   Moist heat kills m.o. by coagulating their proteins and sterilizes the equipments etc. quite rapidly. However the moist heat sterilization can be done at temp. below 100.c, at 100.c, and above 100.c under increase pressure at saturated steam (eg.autodone). The first two (below 100 c and at 100c) are used as disinfection devices and only the third (above 100 c)one is suitable for sterilization and killing of bacterial endospores.
3. Dry Heat Sterilization: Dry heat (not air) sterilized is recommended where it is either undesirable or unlikely that steam under pressure will make direct and complete contact with the objects to be sterilized contain items of lab glassware (eg. Petridish.Pipetles) and objects like oil, powders and similar substances and surface of some articles like inoculating needle, scalpel, month of culture tubes are contaminated with the m.o. Direct heating or flaming of such object can be one et the method of dry heat sterilization. Ex – Hot Oven – Detail.

4. Incineration : Destruction of m.o. by burning is practiced routinely in the lab when the inoculation needle is introduced into the flame. Ex –Bunsen burner. Incineration is used for the destruction of carcasses, infected lab animals, and other infected materials to be disposed of.
5. Filteration: Many of the biological fluids (liquids) or gases that need to be sterilized can not be done so by the application of heat. In this care the sterilization is achieved by filtration. Some ingredients of a culture medium are thermo-labile i.e destroyed by heat, the use of heat sterilization is not applicable. For instance, biological fluids such as solution of antibiotics, vitamins, tissue extracts, animal serum come under this category. In this case filtration methods are used. The filters suitable for this purpose are seize filter (Asbestos filter), chamber land Pasteur filter (porcelain filter), Berkefeld filter (Diatomaceous earth filter) and Membrane filter. The first 3 are biological filter i.e. they allow to pass liquid but retain bacteria. Membrane filters retains all forms of organisms (even viruses).
6. Radiations: Radiation refers to the transmission of energy in a variety of forms through space or medium. The most effective type of radiation to sterilize or reduce the microbial burden in almost any substance is through the use of electromagnetic radiation. The radiations of shorter mavelengths are more damaging to m.o. The 2 types of radiation are electromagnetic waves and streams of minute particles.

Electromagnetic waves: Infrared, ultra –biolet light, x –rays and gamma rays.

Streams of minute particles of matter are alpha and beta radiation

Radiation

Ionizing Radiation — Non-ionizing Radiation

Ultraviolet radiation

Gamma rays

x-rays

Electron beam radiation

(cathode rays)

(B) Chemical Methods of control: Disinfection:

Varieties of chemical agents processing antimicrobial activities are now available for use as disinfectants and each has its own advantages:

1. Phenol and phenol derivatives:

Phenol is obtained by distillation of coal air. It is credited to be the first disinfectant used in the form of carbolic acid by 'Joseph Lister' (1867) to create antiseptic condition during surgery.

Today phenol and phenol derivatives such as cresol, xylenols, orthrophenylphenol are used as disinfectant in laboratories and hospitals. Phenol is very effective as 5% aqueous solution of this chemical rapidly destroys the vegetative cells of m.o. endospores show much more resistance and are not affected.

2. Alcohols: The two most popular alcohols used in sterilization are ehanol and isopropanol. Methanol is also germicidal but is not generally used as it is highly poisonous and may harm the user.
   Ethenol is effective in conc. Between 50-90%; the ideal conc. Is 70% which kills all the vegetative cells but not spores. Alcohols are effectively used to reduce the microb8ial flora of the skin and for the disinfectant of clinical oral thermometers.

3. Hologens: A halogen is any of the five elements (fluorine, chlorine, bromine, iodine and astatine) in group VII A of the periodic table. Chlorine or iodine are the important halogens used as potent antimicrobial agents either in their free form or in the form of their compounds.
4. Heavy Metals and Their compounds:
   1) Mercury: Inorganic compounds of mercury kills bacterial when used in dilution 1:1000. Ex – Marcuric chloride, mercurous chloride, mercuric oxide, ammoniated mercury.
   2) Silver: These compounds are bacteriostatic or bacteriocidal and their effects are exerted by the free silver ions released from the compound. Collidal silver tested as antiseptics, ex-silvernitrate, silver lactate and silver picrate.
   3) Copper: Copper sulfate is much more effective as algicide and fungicide than as bactericide.
   4) Aldehydes: Several of 10 m molecular weight aldehyal are used as antimicrobial. Two aldehydes, for maldehyde asid glutaraldehyde are the most effective and are most commonly used to kill spores hence are sporicidal.
   5) Gaseous Agents: Ethylene oxide, betapropio/actone, forme /dehyde
   6) Dyes: Two groups of dyes, aniline (triphenlmethane) dyes and acridine dyes, that possess antimicrobial properties are often used by microbiologists.

DYES

Aniline Dyes	Acridine –Dyes
ex	ex
Brilliant green	acriflavine
Malachite green	Proflavine
	Euflavine

Crystal violet Aminoacrine

8 Detergents:

Ex –Quaternary ammonium compounds –act as disinfectant

(cationic)

Sodium lauryl sulfate (Anionic)

Ceepryn (cationic)

Q.5 Fermentation –

The anaerobic oxidation of compounds by the enzyme action of microorganisms; neither gaseous oxygen nor a respiratory chain is involved in this energy-yielding process. An organic compound is the electron acceptor.

Types of Fermentation:-

Fermentation processes generally is of 2 types : -

1. Anaerobic fermentation :-

In this a provision for aeration is usually not needed.

But in some cases, aeration may be needed initially for inoculum buildup.

Once fermenta begins, the gas produced in the process generates sufficient mixing.

The air present in the headspace of the fermentor should be replaced by carbon dioxide **(CO2),** hydrogen (H2), nitrogen (N2,). or a suitable mixture of these. This is particularly important for obligate anaerobes like *Clostridium.*

The fermenta; usually liberates CO2, and H2, which are collected and used. For example. CO2, for making? dry ice and methanol and for bubbling into freshly inoculated fermenters.

Recovery of products from anaerobic ferementers does not require anaerobic conditions.

But, many enzymes or such organisms are highly O2 sensitive: when recovery of such enzymes is the objective, cells must be harvested under strictly anaerobic conditions.

2. Aerobic Fermenta : -

Main feature in this is the provision for adequate aeration; in some cases the amt, of air needed per hour is about 60 times the medium volume.

bioreactors used for aerobic fermentation have a provision for adequate supply of sterile air, which is generally sparged into the medium.

Aerobic fermenters may be either of the :-

(i) stirred-tank type in which mechanical motor-driven stirrers are provided or
(ii) (ii) of air-lift type in which no mechanical stirrers are used and the agitation is achieved by the air bubbles generated by the air supply.

1.Batch fermentor (BF):

Batch fermentors are used to carry out micro biological processed on a batch basis.

These are associated with the development of m.o. form a stock culture, and include agar slope and shake flask states.

Thereafter, this is followed by 'seed' and production stages.

Size – Batch fermentors are available with varying capacities.

The capacity of the tank may range from a few hundred to several thousand gallons.

The capacity of the fermentor is usually stated on the basis of the total volume capacity of the same.

Thus, small laboratory fermentors, pilot-plant fermentors and larger or production fermentors may be available small laboratory fermentors, plot-fermentors and larger or production fermentors may be available.

The small laboratory fermentor ranges from 1-2 liters with a maximum up to of 12 –15 liters.
Pilot plant fermentors have a total volume of 25 –100 gallons upto 2000 gallons total volume.
Larger fermentors range form 5,000 or 10,000 gallons total volume to approximately 1,00,000 gallons.

pH Control:
pH control is achieved by acid or alkali addition, which is controlled by an auto- titrator. The autotitrator in turn is connected to a pH probe.
Temperature control:
Temperature control is achieved by a water jacket around the vessel. This is often supplemented by the use of internal coils, in order to provide sufficient heat-transfer surface.
Agitation:
The agitating device consists of a strong and straight shaft to which impellors are fitted. An impeller, in turn consists of a circular disc to which blades are fitted with bolts. Different types of blades are available and are used according to the requirements.

Aeration:
Usually, the aerating device consists of a pipe with minute holes, through which pressurized air escapes into the

aqueous medium in the form of tiny air bubbles. This aeration device is called a "SPARGER". The size of the holes in a sparger ranges from 1/64 to 1/32 of an inch or larger.

There are various ways for introducing air into the fermentation vessel, one is impeller air injecn. Shown above in which air is feed to the impeller by means of a hollow drive shaft, and injected into the medium through holes in the impeller. Time – the time required for a batch fermentation, varies from hours to week depending upon the conversion being attempted, and the conditions used.

Designing _ the design problem associate with a peep tank fermentor lies in the specification of the size of the vessel, the process time, the initial reactant (substrate). Concn required, the hold up volume of microbial mass per unit volume of a fermentor, of m.o. the power & aeration, requirements & the area of heat – transfer surface.

The process of 'Sludge Digestion' in the waste water treatment industry is an eg. Of a batch fermentation.

In such bioreactors, the medium and inoculum are loaded in the beginning, and the cells are allowed to grow. There is no addition/replacement of medium, and the entire cell mass is harvested at the end of incubation period.

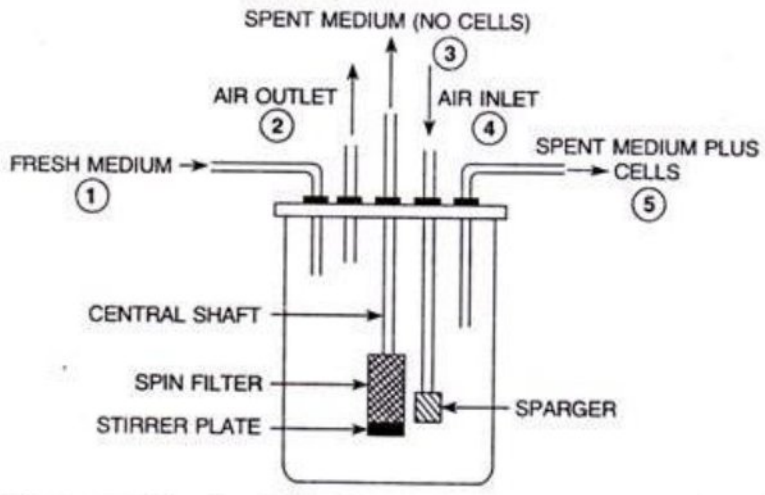
The characteristic features of such bioreactor systems are as follows:

(i) Continuous depletion of medium,

(ii) Accumulation of cellular wastes,

(iii) Alterations in growth rate, and

(iv) Continuous change in the composition of cells.



A schematic representation of a spin-filter bioreactor. The bioreactor has ports for (1) addition of fresh medium, (2) air escape, (3) removal of only spent medium (minus cells; they are separated by the spin-filter) through the hollow central shaft, (4) entry of air to the sparger, and (5) removal of spent medium plus cells. When all the 5 ports are in operation, the bioreactor is of continuous flow type. But when only ports (2) and (4) (air outlet and inlet, respectively) are open [the remaining ports, *viz.*, (1), (3) and (5) are closed], the bioreactor becomes a batch type.

The spin filter bioreactor can be used as a batch bioreactor by closing the inlet for medium and the outlets for medium/medium plus cells.

2.Fed batch Culture and bioreactor

The term fed batch culture is uded to describe batch cultures which are fed, continuously or sequentially, with fresh medium without the removal of culture fluid.

Thus, the volume of a fed-batch culture increases with time.

An enhancement of the closed batch process in the feed – batch fermentation, which is used in the production of substances such as penicillin.

In the fed-batch process, substrate is added in increments as the fermentation progresses.

Typically feed is added at a slow rate to a batch culture but unlike CSTR no culture medium is removed.

Filled up , or the desired product/biomass conc. Achieved

- The reactor may be partially emptied so that the residual culture serves as a starting inoculum and the feed started once again in the case of repeated fed-batch culture.
- The slow feed has many advantages chief among them being the ability to control the residual substrate conc. In the reactor and hence the specific growth rate of the cells.
- Also the rate of feed can be adjusted so as to get the desired specific growth rate which is best studied for product formation

3.Continuous Bioreactors:

- A continuous stirred-tank fermenters (CSTF) need not to be basically different
  From the batch ferment or except in so far as feed and overflow devices added
- The fundamental difference lies in the fact that the contents of the vessel are at a steady state.
- In other words, contents of the vessel no longer vary with time , this applies to the hold-up of m.o. and the conc. Of the components of medium in the fermenters
- Steady state conditions can be achieved by operations on either ‘chemostatic‘ or ‘turbidostatic’ principles.
- In both the types, cultures begin as a batch culture
- Chemostat- inoculated cells grow to the maximum density when somwnutrients , e.g. a vitamin, becomes growth limiting.
- Fresh medium is added after 24-48 hrs of growth , at a const. rate and at an equal rate the culture is withdrawn
- When the rate of growth equals the rate of cell withdrawl , the cultures are in a steady state and both the cell density and medium composition remain constant.
- Turbidostat- cells grow to achieve a predecided density(measured as turbidy using a photoelectric cell)
- At this point , a fixed volume of culture is withdrawn and the same volume of fresh normal medium is added. This lowers the cell density or turbidity of the culture

- Cells keep growing keep growing and once the culture reaches the preset density the fixed volume of culture is replaced by fresh mediumconntinous flow cultures provide a continuous source of cells, and are suitable for product generation e.g. for the production of viruses and infersons
- A spin filter bioreactors is a good e.g. of continuous flow bioreactor
- It has the following features:-
  1) The central shaft of bioreactor houses a spinning filter which enables the removal of used medium, free of cells , through the shaft.
  2) A stirrer plate magnetically coupled to the central shaft provides continuous stirring the spinning filter also stirs the culture.
  3) The culture is aerated by a sparger which allows a wider rate of aeration rates
  4) A port is provided for addition of fresh medium
  5) Another port enables removal of the culture (used medium+ cells) as per need
- A continuous flow bioreactor is used to grow cells at a specified cells density in an active growth phase. Such culture may either provide inoculum for further culture or may serve as a continuous source of biomass yields

4.Specialized bioreactors:-

- There are various types of alternative vessel design commonly found for the fermentation processes
- Usually , alternative vessel designs are tried when the standard vessel configuration does not allow adequate growth of the organism
- There are also used when the scale up criteria require a different design of bioreactor.
- For e.g. production of large quantities of single cell protein is cheaper on a large scale if airlift fermenters are used to eliminate energy costs associated with a drive system
- A no. of these special designs are available at the bench/pilot scale of operation to allow small scale research into the suitability of a particular method.

1) Fluidized Bed:-

- The microbes / cells are trapped in a physical medium (eg. Alginate beads) and held in the vessel by a mesh
- Medium is recirculated through a pump and this can be easily adapted to give a continuous /semi-continuous flow to allow the trapped cells to effect the changes on constituents of the medium without being washed out along with spent medium
- This system is well suited to growth of animal cells on the smaller scale and has large-scale applications in effluent / decontamination treatment plants
- Fluidized bed bioreactors are suited to reactions involving a fluid suspended particulate biocatalyst such as the immobilized enzymes as cell particles or microbialsflocs
- An up-flowing stream of liquid is used to suspend or fluidise the solids as in given fig.
- Geometically the reactors is similar to a bubble column except that the top section is expanded to reduce the superficial velocity of the fluidizing liquid to a level below that need to keep the solid in suspension
- Consequently the solids sediment in the expanded zone and drop back into the narrower reactor column below hence the solids are retained in the reactor whereas.

Above fig. is photobioreactor monoculture

(a) Continuous run tubular loop (d) a solo Receiver made of multiple parallel tubes(b)

Helical wound tubular loop (d) flat panel configuration

Configuration (a) and (d) may be mounted vertically , or parallel to the ground

- A continuous sing le run tubular loop configure is also used ,or the tube may be wound helically around a vertical cylindrical support
- in addition to the tubes ,flat or inclined thin panels may be employed in relatively small-scale operations
- an array of tubes or flat Panel constitutes solar receiver
- the culture is circulated through the solar receiver by a variety of methods including centrifugal pumps, positive displacement mono pumps, Archimedean screws and airlift devices
- the flow in a solar receiver tube or panel should be turbulent enough to air periodic movement of cells from the deeper poorly lit interior to the regions nearer the walls,
- generally, a minimum reynolds no. value of lo^4 is recommended
- in addition to light, photosynthesizing algal cells need a source of carbon, usually carbon dioxide
- part of the carbon may be derived from dissolved bicarbonate species cells convert co_2 to carbohydrate and other cellular components
- too high a concentration of co2 can rebuke photosynthetic productivity
- closed photobioreactor for monoculture consists of arrays of transparent tubes that may be made of glass or more commonly, a clear plastic the tubes may be laid horizontally, OR ARRANGED AS LONG RUNGS ON AN UPRIGHT LADDER

2) Photobioreactors:

- certain micro-algae and cyanobacteria provide imp. chemicals, such as asta -xanthin and beta- carotene, being used as aquaculture feed fish hatchlings

- cyanobacteria, such as spirulina, are also grown as human health foods

- photosynthetic culture require sunlight or artificial illuminations

- although some algae may be grown heterotrophically , i.e without sunlight this type of growth requires an alternative organic energy source, usually glucose

- heterotrophically grown cultures often lack photosynthetic pigments and may not yield the same products as a photosynthesis population

- artificial illumination is impracticably expensive , only outdoor photobioreactors appear to be promising for large scale production

- photobioreactors needs light in which photosynthesis occur only at relatively shallow depths

- however , too much light causes photoinhibition; a situation in which slightly reducing the light intensity will actually improve the rate of photosynthesis the liquid flow out.

- a liquid fluidise bed may be sparged with air or some other gas to produce a gas-liquid-solid fluid bed.

- the condition shown in given fig. represents 'particulates' fluidization. This is a characteristics of beds of regular particles suspended in an upflowing liquid stream.

- if an additional gas phase is involved there is a tendency for the particles in the bed to become less evenly distributed

- there are 2 imp. Features of the beds of mixed particles sizes:

1) the increase in porosity from the bottom to the top of the bed.

2) the decreased particle movement when compared with beds containing particles of constant size.