PAMCB.

4. MANIPULATING CELLS IN CULTURE.

(a) Growth of Microorganism in culture pertaining to Bacteria, Principles of microbial nutrition, physical and chemical environment for microbial growth, Batch, Continuous and Synchronous cultures, Stability and degeneration of Microbial cultures.

MICROBIAL GROWTH.

Microbes that are provided with nutrients and the required environmental factors become metabolically active and grow.

Growth takes place on two levels.

1. A cell Synthesize a new cell Components and increase in size
2. The no. of cells in the population increases

This capacity for multiplication, increasing the size of the population by cell division, has tremendous importance in microbial Control, infectious diseases and biotechnology.

CULTURE MEDIA:

Culture Media may be defined as any Substrate or material that enables to grow and multiply of Microbes.

CHARECTERISATION OF MEDIA:-

≠ All Media Must provide carbon Source, nitrogen Source, minerals and other growth factors.

≠ All Media to be used, must be initially free from Microorganisms. So that it must be sterlized before use.

BASIC CONSTITUENTS OF CULTURE MEDIA:

In Constructing a Culture medium for any Microorganism, the prime goal is to provide a bacterial mixture of required nutrients, at required Concentration That will permit good growth. Tryptic Soy broth, try; Agar are called general purpose media and Support growth of many

① water:-

Source of hydrogen and oxygen. It is used as diluent.

② Electrolyte

Sodium chloride, potassium chloride and other electrolytes

③ Agar:-

It is obtained from sea weed, contains mainly Carbohydrates. a small amount of proteins like materials long chain fatty acids and inorganic salts.

It Melts at 95°c and usually Solidifies at 42°c. Agar Mainly acts as a Solidifying agents of culture Medium and does not provide any nutrition to the bacteria.

④ Peptone:-

It is a Complex mixture of partially digested proteins from animal or vegetable Source by enzymatic action.

⑤ YEAST EXTRACT and Meat Extract (YEME)

YEME Media. Contain protein degradation products, Carbohydrates, inorganic salts and certain other growth factors These are used for enriching culture Media.

⑥ Blood:-

It enriches Media, usually 5-10% defibrinated horse (or) sheep blood is used.

TYPES OF MEDIA:-

Media Can be classified following types:

(a) Based on physical state:-

It may be liquid (or) Solid.

Liquid Medium is called broth, Solid Medium like Agar slant, Agar plate etc.

(b) Based on the presence of oxidizing and reducing Substance in media.

It may be classified into aerobic and anaerobic Media.

(c) Based on chemical components, it may classified as.

(1) Natural Media:-

Here the chemical substances obtained from growth

SIMPLE MEDIA (BASAL MEDIA)

Common media used for the cultivation of most of Microorganisms.

eg. Nutrient broth, Nutrient Agar.

* NUTRIENT BROTH:-

This basic artificial medium is prepared by incorporating the following ingredients:

Peptone - 5 gms
Meat extract - 3 gms
Distilled water - 1000 ml
pH - 7.

peptone, a semi digested protein is a primary nitrogen source. The Meat extract is used as a source of carbon, nitrogen, vitamins and inorganic salts.

* NUTRIENT AGAR:-

When 2-3% of Agar is added to nutrient broth called nutrient Agar.

Peptone - 5 gms.
Meat Extract - 3 gms
Agar - 15 gms
Distilled H_2O - 1000 ml
pH - 7.

(2) SPECIAL MEDIA:-

The actual Media selection for particular bacteria are not completely known.

Certain ingredients are added to a basal medium to study the special characteristics or to provide special nutrients required for the growth of organisms.

It is also called Complex medium.

* Enriched Medium:-

Some Special nutrients Such as Egg, Meat, blood (or) Serum is added to basel Medium.

eg: Loeffler's Medium, blood Agar, Chocolate agar & Dorsett's egg medium used for the cultivation of organisms Such as Streptococci.

* Enrichment Medium:-

This liquid Medium favours the multiplication of a particular Species of bacteria.

eg: Robertson's Cooked Meat Medium is used for the culture of Anaerobes.

(3) SELECTIVE MEDIUM:

In addition to basel Medium they contain Substances Such as bile salt (or) deoxycholate which inhibit the unwanted bacteria and allow growing the Selective bacteria.

eg: 1. MacConkey agar is used to cultivate enterobacteria

2. De oxycholate citrate agar is used as Selective medium for Salmonella and Shigellae

3. Bile salt Agar (BSA) favours the growth of Vibrio cholera.

(4) INDICATOR MEDIUM:

When certain dyes are used as indicator Such as neutral red, bromothymol blue or reducing Substance (potassium cellurite) incorporated in the culture Medium. It is called indicator medium. The bacterial growth changes the Colour of the Medium.

eg:- When Salmonella typhi is grown in wilson and Blair medium Containing Sulphite, the bacterial colonies shows black metallic Colour due to the reduction of Sulphite to Sulphide by the bacteria.

(5) DIFFERENTIAL MEDIUM:-

When a culture Medium Containing certain substances help to distinguish different properties of bacteria, they are called differential Medium.

eg. Blood Agar Medium.

(6) TRANSPORT MEDIUM:-

Specimens like feces, throat swab etc are grown in transport media while transporting for laboratory analysis. It is because the pathogenic bacteria may survive the time taken for transit or may be overgrown by non pathogenic bacteria

eg. Stuart's transport medium for urethial discha

(7) ANAEROBIC CULTURE MEDIA:

These media are employed for cultivation of anaerobic organism.

eg Robertson's cooked meat medium and thioglycollate Medium.

COMMON NUTRIENT REQUIREMENT: Nutritional requirement

Nutrients are substances used in biosynthesis and energy production and required for microbial growth Microbial cell Composition shows that over 95% of cell dry weight is made up of a few major elements.

(I) MACRO NUTRIENTS:

Carbon, Oxygen, Hydrogen, Nitrogen, Sulphur, phosphorus. Potassium, calcium, Magnesium and iron

(II) MICRO NUTRIENTS (TRACE ELEMENTS)

Manganese, zinc, cobalt, Molybdenum, nickel & copper.

MACRO NUTRIENTS:-

These are required by microorganism relatively large amount.

Here C, O, H, N, S & P are the components of carbohydrates, lipids, proteins & nucleic acids.

Remaining four exist in the cell as cations and play a variety of roles.

≠ Potassium (K^+) — required for enzyme activity and protein synthesis

≠ Calcium (Ca^{2+}) - It give heat resistance of bacterial Endospore

≠ Magnesium (Mg^{2+}) = It serves as a co-factor for many Enzymes, complex with ATP and stabilizes ribosomes and cell membranes.

≠ Iron (Fe^{2+} & Fe^{3+}). It is a part of cytochromes and a co-factor for enzymes and electron-carrying proteins.

MICRO NUTRIENTS (TRACE ELEMENTS)

≠ It requires very small quantity and such small amounts contaminates in H_2O, Glass wares & Media.

≠ So, it is very difficult to demonstrate a micronutrient requirement.

≠ They are ubiquitous and probably do not usually limit growth.

≠ It catalysis the enzyme reaction and Maintenance of protein structure.

Zinc (Zn^{2+}) present at the active sites of some enzymes but also involved in regulatory and catalytic subunits in E. coli aspartate carbomoyl transferase.

Manganese (Mn^{2+}) - It aids many enzymes catalysing the transfer of phosphate groups.

Molybdenum (Mo^{2+}) is required for nitrogen fixation

Cobalt (Co^{2+}) is a component of vitamin B_{12}

Basis of macro and trace element, M/o may have particular requirement that reflect the special nature of their morphology (or) Environment.

Most of bacteria do not require large amount of Sodium, but Many bacteria growing in saline lakes and Oceans, depends on the presence of high concentration of Sodium ion (Na)

REQUIREMENTS FOR NUTRIENTS (C, H, O)

Carbon is needed for the skeleton or backbone of all organic molecules and molecules serving as carbon sources normally also contribute both oxygen and hydrogen atoms.

Organic Nutrients :-

- Almost always reduced
- It have electrons that they can donate to other molecules
- It also serves as energy source

More reduced organic molecules are the higher their energy content.

eg. lipids have a higher energy content then Carbohydrates.

Transfer of electron releases energy :-

Here the electrons moves from reduced donors with more negative reduction potentials to oxidized electron acceptors with more positive potentials.

Normally carbon sources frequently serves as energy source.

(1) Carbon Source:

According to the utilization of carbon source they are classified two types

HETEROTROPHS:-

≠ It obtain carbon principally from in the form of organic matter from the bodies of other organisms

eg. In Glycolytic pathway produces carbon he use in biosynthesis and also releases energy.

AUTOTROPHS:

Autotrophs obtain the carbon from inorganic molecules like CO_2.

One important carbon source that does not supply hydrogen (or) energy is CO_2, because it is oxidized not reduced due to lack of hydrogen.

≠ Normally most of microorganisms can fix CO_2 that reduce it and incorporate it in to organic molecules

± Many M/o are Autotrophic, it carry out photosynthesis and use light as the energy source.

~~to donate~~.

Reduction of CO_2 is very energy-expensive process. So many M/o cannot use CO_2 as carbon source. So it rely on more reduced, complex molecules like glucose for supply of carbon.

(2) NITROGEN SOURCE:-

The main reservoir of nitrogen is N_2 gas, it is available 79% in earth atmosphere.

Inorganic nitrogen enters in to the cell, it is converted to NH_3 and it combined with carbon to synthesize amino acids & other compounds.

(3) HYDROGEN SOURCE:-

Hydrogen is a major element in all org. compounds & inorganic compounds including water (H_2O), salt ($Ca(OH)_2$) gas (H_2S, CH_4 & H_2)

Hydrogen performs the following overlapping roles in the biochemistry of cells

1. Maintaining the pH
2. Forming a hydrogen bond between molecules
3. Serves source of free energy in oxidation-reduction reaction of respirations.

(4) PHOSPHOROUS SOURCE:-

Mainly the inorganic sources phosphate salt & phosphoric acid. It is found in nature rocks & oceanic mineral deposites.

phosphate is a key component found in nucleic acids. It also serves in cellular energy transfer.

(5) SULPHUR SOURCE:-

Sulphur is widely distributed in mineral form. Rocks and sediments (gypsum).

eg. $CaSO_4$, FeS, H_2S.

Sulphur is present in certain amino acids vitamins. It give structural stability & shape of protein by forming disulphide bonds.

According to their Nutritional types the bacteria are grouped as

① Autotrophs :-

These bacteria, using CO_2 as prime Source of carbon

eg. Nitrifying bacteria.

There are two types

(i) photo autotrophs (or) photo lithotrophus :-

These bacteria using Sunlight as the energy Source and CO_2 as the prime carbon Source.

They includes most photosynthetic bacteria

ex: Rhodospirillum rubrum, Anabaena variabilis, Synechococcus eximius.

(ii) Chemo autotrophs (or) Chemo litho trophus.

These bacteria using a chemical energy Source and CO_2 as the prime carbon Source. (Agan bacterium).

eg. Iron bacteria, H_2 bacteria. , N_2 bacteria

Nitrosomonas,
Nitroso coccus.

2. HETEROTROPHES :-

These bacteria using one or more ~~carbon~~ organic Compounds as carbon Source

eg. Most Gram negative bacteria.

There are 2 types

(i) PHOTO heterotrophes :

These bacteria using Sunlight as a ~~carbon~~ energy Source and organic Compound as the carbon Source.

eg. purple and green bacteria. , Rhodospirillum spp.
Rhodomicrobium spp.

(ii) chemo heterotrophes :-

These bacteria using a chemical energy Source and organic substances as a prime carbon Source. (Nitro bacter)

eg. Most bacteria.