

**Nutrients** – are substances used in biosynthesis and energy production.

### **Nutrient Requirements:**

Microbial cell composition shows that 95% of cell dry weight is made up of a few major elements: Carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorous, potassium, calcium, magnesium and iron.

### **Macronutrients or macro elements:**

These are required by microorganisms in relatively large amounts. Carbon, oxygen, hydrogen, nitrogen, sulfur and phosphorous are components of carbohydrates, lipids, proteins and nucleic acids. The remaining four macro elements (K, Ca, Mg and Fe) exist in the cell as cations.

$K^+$  - is required for the activity by a number of enzymes, including those involved in protein synthesis.

$Ca^{2+}$  - contributes to the heat resistance of bacterial endospores. 15% of spore contains dipicolinic acid and calcium.

$Mg^{2+}$  - serves as a cofactor for many enzymes, complexes with ATP and stabilizes ribosomes and cell membranes.

$Fe^{2+}$  and  $Fe^{3+}$  - part of cytochromes and a cofactor for enzymes and electron-carrying proteins.

### **Micronutrients or Trace elements:**

These are manganese, zinc, cobalt, molybdenum, nickel and copper. These are normally part of enzymes and cofactors, and they aid in the catalysis of reactions and maintenance of protein structure.

$Zn^{2+}$  - is present at the active site of some enzymes but is also involved in the association of regulatory and catalytic subunits in *E.coli* aspartate carbomoyl transferase.

$Mn^{2+}$  - aids many enzymes catalyzing the transfer of phosphate groups.

$Mo^{2+}$  - required for nitrogen fixation.

$Co^{2+}$  - is a component of Vitamin B12.

Besides macro and micro nutrients, some microorganisms may have particular requirements that reflect the special nature of their morphology or environment. Diatoms need silicic acid to construct their beautiful cell walls of silica. Bacteria growing in saline lakes and oceans depend on the presence of high concentrations of sodium ion. Microorganisms require a balanced mixture of all the above nutrients for proper growth.

### **Requirements for carbon, hydrogen and oxygen:**

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. One important carbon source that does not supply hydrogen or energy is  $CO_2$ . **Autotrophs** – can use  $CO_2$  as their sole or principal source of carbon. Many microorganisms are autotrophic, and most of these carry out photosynthesis and use light as their energy source. Some autotrophs oxidize inorganic molecules

and derive energy from electron transfer. **Heterotrophs** – are organisms that use reduced pre-formed organic molecules as carbon sources. Ex. Glycolytic pathway produces carbon skeleton for use in biosynthesis and also releases energy as ATP and NADH. Actinomycetes will degrade amyl alcohol, paraffin and even rubber. *Burkholderia cepacia* can use over 100 different carbon compounds. Some microorganisms can metabolize even relatively indigestible human-made substances such as pesticides. Indigestible molecules can be oxidized and degraded in the presence of a growth promoting nutrient that is metabolized at the same time, a process called Co-metabolism. The products of this breakdown can then be used as nutrients by other microorganisms.

### **Nutritional types of microorganisms:**

In addition to Carbon, hydrogen and oxygen all organisms require sources of energy and electrons for growth.

#### *Carbon sources:*

Autotrophs - CO<sub>2</sub> sole or principal biosynthetic carbon source

Heterotrophs – reduced, preformed organic molecules from other organisms.

#### *Energy sources:*

Phototrophs – use light as their energy source.

Chemotrophs – obtain energy from the oxidation of chemical compounds (either organic or inorganic)

#### *Electron sources:*

Lithotrophs – use reduced inorganic substances as their electron source.

Organotrophs – extract electrons from organic compounds.

Four major nutritional classes based on their primary sources of carbon, energy and electrons is known.

### **Photolithotrophic autotrophs or photoautotrophs or photolithoautotrophs:**

Source of energy – light energy

Source of electrons – Inorganic hydrogen/ electron

Carbon source - CO<sub>2</sub>

Example: Algae, purple and green sulfur bacteria and cyanobacteria.

### **Photoorganotrophic heterotrophy or photoorganoheterotrophy:**

Source of energy – light energy

Source of electrons – organic hydrogen/ electron

Carbon source – organic carbon sources (CO<sub>2</sub> may also be used)

Example: Purple and green nonsulfur bacteria (common inhabitants of lakes and streams)

**Chemolithotrophic autotrophs or chemolithoautotrophy:**

Source of energy – Chemical energy source (inorganic)

Source of electrons – Inorganic hydrogen/ electron donor

Carbon source - CO<sub>2</sub>

Example: Sulfur-oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron-oxidizing bacteria.

**Chemoorganotrophic heterotrophs or chemoorganoheterotrophy:**

Source of energy – Chemical energy source (organic)

Source of electrons – Inorganic hydrogen/ electron donor

Carbon source – organic carbon source

Example: Protozoan, fungi, most non-photosynthetic bacteria (including most pathogens)

The most common nutritional types are photolithoautotrophs and chemoorganoheterotrophs. Bacteria *Beggiatoa* rely on inorganic energy sources and organic (or sometimes CO<sub>2</sub>) carbon sources. These microbes are sometimes called Mixotrophic because they combine chemolithoautotrophic and heterotrophic metabolic processes.

**Requirements for nitrogen, phosphorous and sulfur:**

Nitrogen is needed for the synthesis of amino acids, purines, pyrimidines, some carbohydrates and lipids, enzyme cofactors and other substances. Most phototrophs and many nonphotosynthetic microorganisms reduce nitrate to ammonia and incorporate the ammonia in assimilatory nitrate reduction. A variety of bacteria like many Cyanobacteria and *Rhizobium* can reduce and assimilate atmospheric nitrogen using the nitrogenase systems. Phosphorous is present in nucleic acids, phospholipids, ATP, several cofactors, some proteins and other cell components. All microorganisms use inorganic phosphate as their phosphorous source and incorporate it directly. *E.coli* can use both organic and inorganic phosphate. Organophosphates such as hexose 6- phosphate can be taken up directly by transport proteins. Other organophosphates are often hydrolyzed in the periplasm by the enzyme alkaline phosphatase to produce inorganic phosphate which is then transported across the plasma membrane. When inorganic phosphate is outside the bacterium, it crosses the outer membrane by the use of a porin protein channel. Sulfur is needed for the synthesis of substances like the amino acids cysteine and methionine, some carbohydrates biotin and thiamine. Most of them use sulfate as a source of sulfur and reduce it by assimilatory sulfate reduction; a few require a reduced form of sulfur such as cysteine.

**Growth factors:**

Many microorganisms have the enzymes and pathways necessary to synthesize all cell components. Many lack one or more enzymes and hence require organic compounds because they are essential cell components or precursors of such components and cannot be synthesized by the organisms are called – growth factors. There are three major classes of growth factors:

Amino acids – needed for protein synthesis.

Purines and Pyrimidines – for nucleic acid synthesis

Vitamins – small organic molecules that usually make up all or part of enzyme cofactors, and only very small amounts sustain growth.

Knowledge of the specific growth factor requirements of many microorganisms makes possible quantitative growth response assays for a variety of substances. The observation that many microorganisms can synthesize large quantities of vitamins has led to their use in industry. Several water-soluble and fat-soluble vitamins are produced using industrial fermentations.

Riboflavin – *Clostridium*, *Candida*, *Ashbya*, *Eremothecium*

Coenzyme A – *Brevibacterium*

Vitamin B<sub>12</sub> – *Streptomyces*, *Propionibacterium*, *Pseudomonas*

Vitamin C – *Gluconobacter*, *Erwinia*, *Corynebacterium*

β- Carotene – *Dunaliella*

Vitamin D - *Saccharomyces*

In the laboratory, bacteria are normally grown or cultured in either liquid medium, in flasks, bottles or large culture vessels called fermenters or solid medium in Petri dishes which are round, normally plastic or glass dishes. Introduction of microbes into or onto these media is called **inoculation**. The nature of the medium depends on the microorganism's natural environment because its nutrient requirements reflect its natural surroundings. The culture medium must contain all the nutrients that the microorganism requires for growth like water, a source of energy, carbon, nitrogen, essential inorganic ions and a number of trace elements. Bacteria that can synthesize all they require from the basic ingredients are called **Prototrophs** – and most microorganisms that survive in the outside environment can do this. Microbes that have become adapted to life in a situation rich with nutrients such as human body may require other growth factors like vitamins, amino acids or nitrogenous bases to be provided. These are called **auxotrophs**.

The media that are used in microbiology laboratories to culture bacteria are referred to as artificial media or synthetic media, because they do not occur naturally; rather they are prepared in the laboratory. There are number of ways of categorizing the media, one way is to classify based on whether the exact contents of the media are known or not.

**Chemically defined media** : Is one in which all the ingredients are known; and was prepared in the laboratory by adding a certain number of grams of each of the components (Carbohydrates, amino acids, salts etc). Particularly photolithoautotrophs such as Cyanobacteria and eukaryotic algae can be grown on relatively simple media containing CO<sub>2</sub> as a carbon source (often added as sodium carbonate or bicarbonate), nitrate or ammonia as a nitrogen source, sulfate, phosphate and a variety of minerals. Chemoorganoheterotrophs can be grown in a defined media with glucose as a carbon source and an ammonium salt as a nitrogen source.

**Complex media** : contains undefined ingredients and the exact contents are not known. Complex medium may be sufficiently rich and complete to meet the nutritional requirements of many different microorganisms. They contain undefined components like peptones, meat extract and

yeast extract. Peptones – are proteins hydrolysates prepared by partial proteolytic digestion of meat, casein, soyameal, gelatin and other protein sources (Carbon, energy and nitrogen). Beef extract – aqueous extracts from lean beef and contain amino acids, peptides, nucleotides, organic acids and minerals and vitamins. Yeast extract – from brewer's yeast and contain an excellent source of B vitamins, nitrogen and carbon compounds. Three commonly used complex media are

1. Nutrient broth – Peptone (5.0g/L), Beef Extract (3.0g/L)
2. Tryptic soya broth – Tryptone (enzyme digest of casein), Peptone (enzyme digest of soybean meal), glucose, NaCl and  $K_2HPO_4$
3. MacConkey Agar – Pancreatic digest of gelatin, casein, peptic digest of animal tissue, bile salts mixture, NaCl, neutral red, crystal red and agar.

These media are routinely used for cultivation of bacteria in the laboratory and particularly useful for cultivation of bacteria whose growth requirements have not been defined.

Culture media can also be categorized as liquid or solid. Liquid media (also called broths) are contained in tubes, flasks and fermenters. Solid media are prepared by adding agar to liquid medium and then pouring the media into tubes or Petridishes where the media will solidify. Agar is a complex polysaccharide that is obtained from red marine algae. Other solidifying agents are gelatin, silica. A 1% or 2% agar can be used to solidify, but 1.5% is the most commonly used.

**Enriched Medium** : Is a broth or solid medium containing a rich supply of special nutrients that promotes the growth of fastidious organisms (that have complex nutrition and environmental requirements). It is usually prepared by adding extra nutrients to a medium called nutrient agar. Blood agar (nutrient agar + 5% sheep red blood cells): It is bright red in color and distinguishes between the hemolytic and non-hemolytic bacteria ( *Streptococci* and other pathogens)

**Chocolate agar (nutrient agar + powdered hemoglobin)**: It is brown in color and is considered more enriched than blood agar as hemoglobin is more readily accessible in chocolate agar. Pathogens like *Neisseria gonorrhoeae* and *Haemophilus influenza*, which will not grow on blood agar, can be cultured.

### Types of Media:

*Selective Media*: Are designed to suppress the growth of unwanted bacteria and encourage the growth of the desired microbes.

Examples: MacConkey agar inhibits growth of gram positive bacteria and thus is selective for gram-negative bacteria. Phenylethyl alcohol (PEA) agar and Colistinidixic acid (CAN) agar are selective for gram positive bacteria.

Manitol salt agar contains a concentration of 7.5% NaCl that is quite inhibitory to most human pathogens. Bile salts, a component of fecus, inhibit most gram positive bacteria while permitting many gram negative rods to grow. This media is used for selecting intestinal pathogens which contain bile salts. Dyes such as methylene blue and crystal violet also inhibit certain gram positive bacteria (Staphylococcus can produce acid from mannitol and turn the phenol red dye to bright yellow.

A medium containing acetate as a carbon source would be selective for organisms that grow on acetate. Sabaraud's dextrose agar is used to isolate fungi and cellulose for cellulose digesting bacteria.

*Differential media* : Makes it easier to distinguish colonies of the desired organism from other colonies growing on the same plate.

MacConkey agar is also a differential and selective medium to distinguish between lactose fermenting organisms (e.g, *E.coli*) and other non-fermenters (e.g, *Shigella* sp.). Lactose in the medium is fermented by *E.coli* producing acid which causes an indicator dye to change color to red and colonies that do not ferment lactose are white. Dyes can be used as differential agents because many of them are pH indicators that change color in response to the production of an acid or base. MacConkey agar contains neutral red, a dye that is yellow when neutral and pink or red when acidic.

Mannitol salt agar is used to screen for *Staphylococcus aureus*, and it turns the originally pink medium to yellow due to its ability to ferment mannitol. In a sense, blood agar is also differential because it is used to determine the hemolytic and non-hemolytic bacteria.

Blood agar is both differential and an enriched one. It distinguishes between hemolytic and non-hemolytic bacteria. Hemolytic bacteria (eg., many *Streptococci* and *Staphylococci*) produce clear zones around their colonies because of red blood cell destruction.

### **Isolation of pure cultures:**

In natural habitats, microorganisms usually grow in complex, mixed populations containing several species. One needs a pure culture, a population of cells arising from a single cell, to characterize an individual species. Robert Koch introduced pure culture techniques.

#### *Spread plate and streak plate:*

*Spread plate* - Mixture of cells is spread out on an agar surface so that every cell grows into a completely separate colony, each colony representing a pure culture. A small volume of around 30 to 300 cells (mixed) is transferred to the center of the plate and spread evenly over the surface with a sterile bent-glass rod.

*Streak plate* – the microbial mixture is transferred to the edge of an agar plate with an inoculating loop or swab and then streaked out over the surface in one of several patterns. In both these techniques, successful isolation depends on spatial separation of single cells.

*The pour plate* : Extensively used with bacteria and fungi. The original sample is diluted several times to reduce the microbial population sufficiently to obtain separate colonies when plating. The microbes are mixed with molten agar which has been cooled to 45° C and then poured into petridishes. All these techniques require petridishes (special culture dishes) after their inventor Julius Richard Petri (1887). They consist of two round halves, the top half overlapping the bottom. Each bacterium replicates to form a colony that is visible to the naked eye. A colony contains up to 10<sup>9</sup> copies of the original bacterium.

### **Colony morphology and growth:**

Colony development on agar surfaces aids the microbiologist in identifying bacteria because individual species often form colonies of characteristic size and appearance. Structure of bacterial colonies also has been examined with the scanning electron microscope. Generally the most rapid cell growth occurs at the colony edges. Growth is much slower in the center and cell autolysis takes place in the older central portion of some colonies. These differences in growth appear due

to gradients of oxygen, nutrients and toxic products within the colony. At the colony edge, oxygen and nutrients are plentiful. Bacteria growing on solid surfaces such as agar can form complex and intricate colony shapes. Nutrient diffusion and availability, bacterial chemotaxis and the presence of liquid on the surface all appear to play a role in pattern formation. These patterns vary with nutrient availability and the hardness of the agar surface.

**1. 1. Name the different nutritional types of microorganisms?**

**2. Write short notes on the nutritional requirements of microorganisms**

- a. Macronutrients
- b. Micronutrients
- c. Requirement of carbon, hydrogen and oxygen
- d. Requirement of nitrogen, sulfur and phosphorus

**3. What are growth factors and how are they important for microbial nutrition**

**4. Write a short note on the following:**

- a. Photolithoautotrophs.
- b. Photoorganoheterotrophs.
- c. Chemolithoautotrophs.
- d. Chemoorganoheterotrophs.

**5. State the role of following micro and macronutrients:**

- a.  $K^+$
- b.  $Ca^{2+}$
- c.  $Fe^{2+}$  and  $Fe^{3+}$
- d.  $Zn^{2+}$
- e.  $Mo^{2+}$