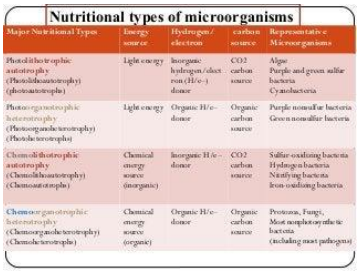
Nutritional Forms of Microorganisms (With Diagram)

. Photolithoautotrophs:

This group includes photosynthetic microalgae, cyanobacteria, and photosynthetic bacteria (purple sulphur bacteria and green sulphur bacteria).

In photosynthetic microalgae and cyanobacteria the external energy-source is light. One or more varieties of chlorophyll are present to trap the solar energy. Such microorganisms are, therefore, largely green. The hydrogen-source of all photoautotrophic microalgae and cyanobacteria is environmental water (H2O) which is split into oxygen and hydrogen with the help of light energy.

The oxygen is released as by-product and the free hydrogen liberated, as a result of photolysis of water reduces carbon dioxide (the inorganic raw material) to carbohydrate (organic metabolite food) which is used as food.



Major Nutritional Types	Energy Source	Hydrogen/Electron Source	Carbon Source
Photolithotrophic autotrophs (algae, cynobacteria, purple and green sulphur bacteria)	Light	Inorganic H/e⁻ donor	CO_2
Photo-organotrophic heterotrophy(purple and green non-sulphur bactria	Light	Organic H/e⁻ donor	Organic carbon (CO_2 may also be used)
Chemolithotrophic autotrophy (sulphur oxidizing bacteria, hydrogen bacteria, nitrifying bacteria, iron bacteria)	Chemical(inorganic)	Inorganic H/e⁻ donor	CO_2
Chemo-organotrophic heterotrophy (protozoa, fungi, most of the non photosynthetic bacteria)	Chemical(inorganic)	Organic H/e⁻ donor	Organic carbon

Chemolithoautotrophs (Chemoautotrophs):

This is a group of non-photosynthetic autotrophic microorganisms consisting entirely of bacteria. They cannot use light and their external energy sources in food manufacture are a variety of inorganic metabolites absorbed from the environment. In the most cases, these metabolites are combined with molecular oxygen in the cells, resulting in release of energy (exothermic reaction) and a variety of inorganic byproducts.

Water and carbon dioxide are the inorganic raw materials in subsequent food manufacture. The concept of chromo-autotrophy (chemolithotrophy) was formulated by Winogradsky. He demonstrated for the first time that a living organism could oxidize H2S to elemental sulphur and then to SO2-4. This process of manufacturing food is called chemosyntliesis.

Photoorganoheterotrophs (Photoheterotrophs):

Some bacteria use light as energy source with organic compounds as the carbon source to grow. These bacteria are called photoorganoheterotrophs (photoheterotrophs) and belong to the group of purple nonsulphur bacteria. The latter have been called "nonsulphur" because it was originally thought that they were unable to use sulphide as an electron donor for the reduction of CO2 to cell material.
Recent studies clarified that most species of these bacteria can use sulphide although the level of sulphide utilised by them is quite low than that by purple sulphur bacteria. Most purple nonsulphur bacteria have additional ability to grow aerobically in darkness utilising organic compounds as electron donor.

It is their great ability to practice photoheterotrophy that likely accounts for their competitive success in nature. Purple non-sulphur bacteria are typically nutritionally diverse with respect to photoheterotrophy as they can use fatty, organic, or amino acids; sugars; alcohols; and even aromatic compounds like benzoate as carbon sources.

Purple non-sulphur bacteria possessing additional ability to photoorganoheterotrophy (e.g., Rhodopseudomonas, Rhodospirillum. Rhodobacter, Rhodovulum, Rhodopila, Rhodobaca, Rhodocyclus, Rhodoferax, Phaeospirillum, Roseospira, Roseospirillum, Rubrivivax, Rhodoplanes, Rhodobium, Rhodomicrobium) are considered to be intermediate between photolithoautotrophs and chemoorganoheterotrophs.

Chemoorganoheterotrophs:
Majority of heterotrophic microorganisms belong to this nutritional category. Since they cannot synthesize their own food (organic substances) they obtain it directly from external environment using chemical energy- source. A clear-cut distinction between the carbon-source and the energy-source, characteristics of the three preceding nutritional forms, loses its clarity in the context of chemoorganoheterotrophs.

In the latter, both carbon and energy can usually be derived from the metabolism of a single organic compound. The chemoorganoheterotrophs include protozoa, fungi (including slime molds), and the great majority of bacteria.

The chemoorganoheterotrophic form of nutrition can further be divided into two categories on the basis of the physical state in which organic nutrients enter the cell. These categories are: holotrophit nutrition and absorptive nutrition.

Nutritional Types of Microorganisms

Microorganisms can be classified into nutritional classes based on how they satisfy the requirements of carbon, energy and electrons for their growth and nutrition.

Based on the carbon source, microorganisms are able to utilize, they are classified into Autotrophs and Heterotrophs.

Autotrophs: These are organisms that utilize CO2 as their sole source of carbon.

Heterotrophs: These are organisms that use preformed organic substances from other organisms as their carbon source.

Based on energy source, microorganisms are classified into Phototrophs and Chemotrophs.

Phototrophs: These are organisms that utilize light (radiant energy) as their energy source.

Chemotrophs: These are organisms that obtain energy by oxidation of organic or inorganic compounds.

Microorganisms are classified into **Lithotrophs** and **Organotrophs** based on the source from which they extract electrons. Lithotrophs are organisms that use reduced inorganic substances as their electron source whereas Organotrophs obtain electrons from organic compounds

All microorganisms fall into any one of the four nutritional classes based on their primary source of carbon, energy and electrons.

1. Photoautotrophs: Eukaryotic algae, Cyanobacteria (Blue Green Algae) (Figure 6.1) and Purple and Green Sulphur bacteria belong to this class. They are capable of using light energy and have carbondioxide as the sole source of carbon.

2. Photoheterotrophs: These organisms make use of light as energy source and organic compounds as electron and carbon source. Example: Purple and Green Non sulphur bacteria

3. Chemoautotrophs: These are ecologically important microorganisms. They oxidize inorganic compounds like nitrate, iron and sulphur to obtain energy and electrons.

4. Chemoheterotrophs: These organisms use organic compounds to satisfy their needs of energy, electron and carbon

Culture media, also known as growth media, are **specific mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi (yeasts and molds)**.

Culture Medium

Culture medium is any solid or liquid material that supports the growth of micro-organisms. The culture medium is composed of beef extract, peptone, yeast extract, agar and distilled water. In addition, it also contains bovin rumen fluid, blood, serum, plant extracts, etc. 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 3 g
Peptone 5 g
Sodium chloride 5 g
Water 1000ml

When agar (15g) is added to the above components, the medium becomes solid and is called nutrient agar.

It is a complex medium for the growth of heterotrophic bacteria.

The culture media are classified variously. Based on consistency, the culture media are classified into the following three types :

1. Liquid medium or broth
2. Semi solid 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 the following types: 1. Selective medium 2. Differential medium 3. Enrichment medium 4. Enriched medium 5. Assay medium 6. Transport medium 7. Maintenance medium 8. Enumeration medium 9. Characterisation medium

1. Liquid Medium or Broth

Broth is a liquid culture medium. It is also called broth. During preparation of the medium, the solidifying agent is not added. This medium is composed of the following components: Beef extract 3 g
Peptone 5 g

Sodium chloride 5 g
Water 1000ml

The broth medium is used to study the growth rate and the sedimentation rate of bacterial cells.

2. Semi - Solid Medium

The semi-solid medium remains in the semi-solid condition and it is prepared by adding small amount of agar (3.75gms) to the broth. It is used to study bacterial motility. 3. Solid Medium

The solid medium is solid in consistency. It is also called agar medium. It is prepared by adding large amount of agar (15gms). It is composed of the following components:

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

It is used for colony characterization, colony identification, isolation of bacterial cells and demonstration of antibiotic sensitivity.

4. Natural or Empirical Medium

When a natural product is used as such for growing bacteria, the medium is called natural medium. Eg. Milk, wine, blood, vegetable juices, yeast extract, coagulated egg, meat extract, etc. This medium is used on the basis of experience and observation and hence the name empirical medium

5. Living Medium

Living medium consists of living cells which are used for the growth of bacteria.

6. Synthetic Medium

Synthetic medium is synthesized by adding inorganic and organic compounds in definite proportions. Hence the chemical composition of this medium is well known. Broth is a synthetic medium.

7. Complex Medium

This medium contains many ingredients of unknown composition. This medium contains, sources for energy, vitamins, nitrogen and carbon. It is composed of sugar, yeast, beef extract vitamins, salts, peptone, etc. It is easy to prepare. It is used for the culture of a wide variety of microorganisms. Eg. Agar medium, broth.

8. Minimal Medium

Minimal medium lacks certain growth factor. This medium is used in genetic experiments

9. Selective Medium

Selective medium is a culture medium which allows the growth of a particular variety of bacteria. Other bacteria cannot grow.

This medium selects a particular bacterium from a mixture and hence the name. It is suitable for the growth of a specific organism.

This medium is best used for isolating a specific organism from a mixed natural population Selective medium contains certain chemicals which suppress or kill unwanted type of microorganisms. For example Mac Conkey's Agar medium contains crystal violet. Crystal violet inhibits Gram positive bacteria. When crystal violet is added to the medium containing both Gram positive and Gram negative bacteria, the Gram positive bacteria are killed and is Gram negative bacteria remain in the culture medium.

10. Differential Medium

A differential medium allows the growth of different kinds of organisms in different ways. This medium allows certain micro-organisms to produce distinct colonies. The different organisms appear differently.Blood agar is the most commonly used differential medium.

Microbiological culture techniques

A microbiological culture, or microbial culture, is **a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions**. Microbial cultures are foundational and basic diagnostic methods used as a research tool in molecular biology. Obtaining a pure culture of bacteria is usually accomplished by **spreading bacteria on the surface of a solid medium so that a single cell occupies an isolated portion of the agar surface**. This single cell will go through repeated multiplication to produce a visible colony of similar cells, or clones.

Streak Plate culture

Streak plate culture is culturing the bacterial sample on an agar plate in the form of thin irregular long lines. It is a technique of isolating bacteria from a mixed culture.It is a pure culture method.

In this method, the agar medium is taken in a petridish. The medium is uniformly distributed by rotating the petridish.

The sample is taken on an inoculation needle. It is streaked on the agar plate. The streaks may be a continuous streak or quadrant streak or radiant streak or T.streak The successive streaks thin out the culture. Streaking isolates the individual cells and are deposited in different regions of the agar medium.

The streaked plates are kept upside down in the incubator for 24-48 hours at 25℃.

The isolated cells will grow into colonies on different regions of the agar medium.

Pour Plate Culture

It is an isolation and purification technique. In pour plate method, the sample is serially diluted in an agar tube and the contents are poured into petridishes and incubated.

Four tubes A,B,C and D are serially arranged. Agar medium is poured into all tubes. One loopful of sample is transferred to tube A. It is vigorously shaken.A loopful of sample is taken from tube A and transferred to tube B. It is vigorously shaken.Now a loopful is taken from tube B and is transferred to tube C and shaken well. Tube D serves as control. It is added with agar medium alone.Now the contents of tubes A,B,C and D are poured into petridishes A,B,C and D.After solidifying, the petridishes are incubated at 25°C for 24-48 hours. The plates are observed for colonies.

Spread Plate Technique

It is an isolation technique. It is a modification of pour plate technique. In this method, the mixed culture is serially diluted in sterile distilled water. A small amount of the diluted mixture is then pour on the surface of an agar plate and it is spread evenly using a sterile bent glass rod called spreader. The isolated cells grow into colonies.

Microbial growth

A microorganism 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 and

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 this method, the microorganism is grown on a limited amount of medium containing all the nutrients at optimum environmental conditions.

The vessel used in the culture is known as fermentor or bioreactor. The vessels used may be a test tube or a petri dish or a conical flask.

In a batch system, the microorganism will pass through four stages, of growth such as, lag phase, log phase, stationary phase and decline phase.

The growth of microorganism continues until either one of the essential nutrients is exhausted or toxic by-products accumulate to inhibit growth.

Growth Curve

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

A typical bacterial growth curve shows four distinct phases viz, lag phase, log phase, stationary phase and decline phase. Lag phase represents an initial period of no growth in terms of increase in cell numbers. In this phase the cells are metabolically active, capable of repairing cell damage and synthesizing enzymes. In the lag phase, there is no increase in the number of cells. However, the cells increase in cell volume. There is no division at this stage. The cells prepare for division. The cells are very active physiologically.

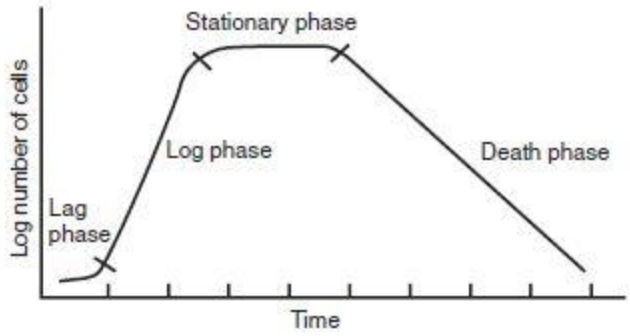
But the population remains stationary. In the log phase, the cells divide rapidly at a constant rate. The cells divide in a geometric progression.

The time required by a cell to divide is called generation time. The generation time varies from species to species. For E.coli, it is 12 minutes in milk at 37°C. In the stationary phase, the growth slows down. The cells begin to die. There is a balance between dead and newly formed cells.

In the decline phase, the cells die continuously. The death is due to the exhaustion of nutrients and the accumulation of toxic byproducts. The growth stages of batch culture can be represented in the form of a curve called Growth curve.

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 a continuous culture. In continuous culture, the bacterial population remains in the log phase and there will be no stationary and decline phase.



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.

The apparatus consists of a culture vessel, a reservoir and a collection vessel. The bacteria grow in the culture vessel. The reservoir contains sterile medium. It supplies medium continuously to the culture vessel.

The used up medium and dead cells are collected into the collection vessel. In continuous culture, the bacterial population is allowed to remain in the log phase of growth. There is no decline phase.

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

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.

Fresh sterile medium from a reservoir is pumped into the culture vessel at a steady rate. At the same time, same level of used up nutrients and bacterial cells are removed from the culture vessel into a spent culture vessel.

Maintenance of Bacterial Culture Storing bacterial culture alive for future use is called maintenance of bacterial culture. It is the preservation of bacterial culture. The preserved culture is called stock culture collection. Some cultures can be preserved for 80 years.

Different methods are employed for maintenance of bacterial cultures. They are

1. Periodic transfer to freshmedia

2. Maintaining with mineral oil

3. Maintenance in formaldehyde

4. Lyophilization

5. Preservation by liquid nitrogen at very low temperature

6. Soredelli's method of preservation

1. Periodic Transfer to Fresh Media

The culture can be stored alive by transferring the culture to fresh medium at regular interval. The transfer can be done once in a month. For better results, the medium should favour slow

rate of growth.

The malum used for this method is called nutrient agar. Nutrient agar is composed of peprore, berterrat Nach agar and stilled water. The culture is maintained in gardant Agar slant is an agar nutrient contaming test toute kopen a siarting position

2. Maintaining with Mineral Oil

Bacional culture can be stored alive for 2 years in mineral oil

The culture is kept in agar slant Mineral oil is poured into the agar slant. The oil led must be above the tip of the slanted surface. The oil covered slant is stored at 5°C.

3. Maintenance in Formaldehyde

Agar plate cultures can be preserved by placing a drop of formaldehyde on the ime sides lid. It is stored in a refrigerator at +C.

4. Lyophilization

Lyophilization or freeze-drying can be used to preserve many kinds of bacteria that werde be killed by ordinary drying. In this process a dense cell suspension is placed in small viak ani frozen at -60°C to -78°C.

The vials are then subjected to rapid dehydration under high vacuum. This results in minimum damage to delicate cell structures.

The vials are then sealed off under a vacuum and stored in a refrigerator. By this methx one can preserve a culture for more than 30 years. This method has the following advantaga

1. It requires minimum storage space.

2. Hundreds of lyophilized cultures can be stored in a small area.

3. Culture vials can be sent conveniently through the mail.

4. The cultures are revived by opening the vials, adding liquid media and transferring rehydrated medium to a suitable growth medium.

5. Preservation by Liquid Nitrogen at very Low Temperatures

A dense cell suspension is taken in a medium containing a cryoprotective agent such as shrerol or dimethyl sulfoxide which prevents cell damage due to ice crystal formation. The cell suspension is sealed into small vials and then frozen at a controlled rate to -50°C. The vials are then stored in liquid nitrogen at -150°C to 196°C. This method is useful for species that cannot be preserved by lyophilization. However, this method is highly expensive.

6. Soredelli's Method of Preservation

In this method, the cultures are incubated on a solid medium for a suitable time. When good growth is obtained, a heavy inoculum from the solid growth is emulsified in a loopful of horse serum and is deposited on the inner wall of a small tube which can be inserted into another tube. A small amount of phosphorus pentoxide is placed at the bottom of the outer tube with the help of a glass rod and a funnel.

Measurement of Bacterial Growth

Growth in bacteria and other unicellular organisms is measured by measuring either increases mass or increases in numbers in relation to time.

1. Measurement of Cell Mass

a. Dry Weight Method

It is an indirect method of measurement of bacterial growth. The cell mass can be measured in the following ways:

In this method, a known volume of culture is removed from the medium, washed effectively, filtered, dried and weighed accurately. Such determinations are time consuming and relatively insensitive.

b. Cell Volume Method

This method involves placing a standard volume, say 10ml, of the liquid culture in a kind of calibrated centrifuge tube called a Hopkin's tube. It is centrifuged at a standard speed. The pellet is measured in volume. From a knowledge of the average volume of the individual cell, the estimation of numbers is possible.

c. Chemical Method

Chemical estimates of cell masses are made by measuring the amount of some chemical component, say protein, DNA, RNA, free amino acid, phosphorus, etc. that are always present in fairly constant amounts in living cells. Such methods are hardly applicable to bacteria but are much used in measuring heavy growth of filamentous microorganisms.

d. Turbidimetric Method

This is a widely used technique. In this, we measure turbidity or scattering of light in the liquid culture due to evenly dispersed cells suspended in it. This technique is based on the fact that small particles scatter light proportionally, to their concentration. When a beam of light is passed through a suspension of bacteria, the reduction in the amount of light transmitted is measured to represent the bacterial mass. Such measurements are usually made in the photoelectric colorimeter or spectrophotometer.

These instruments are convenient for estimating cell concentration. When calibrated against bacterial suspension of known concentration, they provide an accurate and rapid way to estimate the dry weight of bacteria per unit volume of culture.

More sensitive instrument for measuring scattering is called nephelometer. It has a light sensing device kept at right angles to the incident beam of light and hence directly measures the scattered light.

Measurement of Cell Number

Counting the bacterial cells using a microscope is direct count. Total count is made directly with a microscope. This is a direct method and not precise as culture methods. However, it is time consuming and easy to perform.

Generally specimens that contain large numbers of bacteria (more than 10^ cells per ml) are diluted from 1:10 to $1:10^5$ or more depending on sample and counting method, to make the number more manageable and simplify the counting. The following methods are used to measure the cell number.

a. Using Counting Chamber

A number of counting chambers are available for counting the number of cells under the microscope. They are Petroff-Hausser slide, Haemocytometer or special counting chamber In this method, a measured volume of a sample is spread over a measured area of the slide and the cells therein are counted under a microscope. The counted number is multiplied by an appropriate factor to calculate the actual number of cells in the whole volume of culture/sample The counting chambers available have depressions of the known depth and volume, marked off into squared areas. The organisms in an area are counted (say 50 or 100 squares) and the total number of bacteria in the sample can be calculated from the proportion of the total volume. This method is applicable to count upto 1 x 107 cells/ml of suspension.

b. Spread Method or Smear Count

In this method, a known volume of sample is smeared over an exact area on a slide (say 1 square centimetre), dried, fixed and stained with methylene blue or with any other dye. The cells are counted in a known portion of the total area. Knowing the diameter of the microscopic field from previous measurements (by means of stage micrometer), one can calculate the number of organism per ml of culture.

c. Membrane Filter Count

In this method, a measured sample of fluid may be passed through sterile, porous membrane filters and the microorganisms on the filter are then counted directly. The organisms must not be too numerous and must be uniformly distributed. They are stained in situ on the membrane and then counted in calibrated fields. Before counting, the filter is made transparent by saturating it with immersion oil. This gives a total count.

d. Electronic Colony Counter

This method gives an accurate counting of thousands of cells, alive or dead (total count) in a few seconds. This instrument works on the principle of electronic gating or electronic eye. Basically, it depends on interruptions of an electronic beam that traverses a space between two closely adjacent electrodes. Each particle, as it passes between the electrodes, causes an interference with the electron beam due to different conductivities of cells and fluid. The interruptions are taken up by instruments and recorded electronically.

The petriplate in which bacteria to be counted is placed on the machine and each distinct colony that is separated from its neighbours is counted as if it were a single cell. This operation is performed automatically and records the total plate count on the display panel.

e. Plate or Colony Count

The enumeration of unicellular organisms can also be made by plate count, because single viable cells separated from one another in space give rise to separate colonies. The theoretical assumption in this is that one bacterial cell or clump of cells gives rise to one colony. By counting the number of macroscopically visible colonies that develop on the agar plate one can know the bacterial count.

The colony count is widely used for determining appropriate number of microorganisms in mik war and many other materials.

f. Dilution Methods

This method involves making a serial dilution with the given sample of bacterial suspension. ha spical procedure, Iml of the sample diluted to ten-fold, four-fold or two-fold, are placed in mabes of broth. After incubation of the tubes of broth, if presence of growth in the tube that reconed 1:1,000 dilution (1:10-), but no growth in the tube receiving the 1:10,000 (1:104) thation than there were between 1000 and 10,000 organisms per ml of the sample tested.