**University of Technology**

**Department of Applied Science**

**Biotechnology Division**

# Microbial physiology

*3rd class*

*Produced by*

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# Microbial physiology

The study of how the microbial cell functions biochemically. Includes the study of **microbial cell structure**, **microbial growth** and **microbial metabolism**.

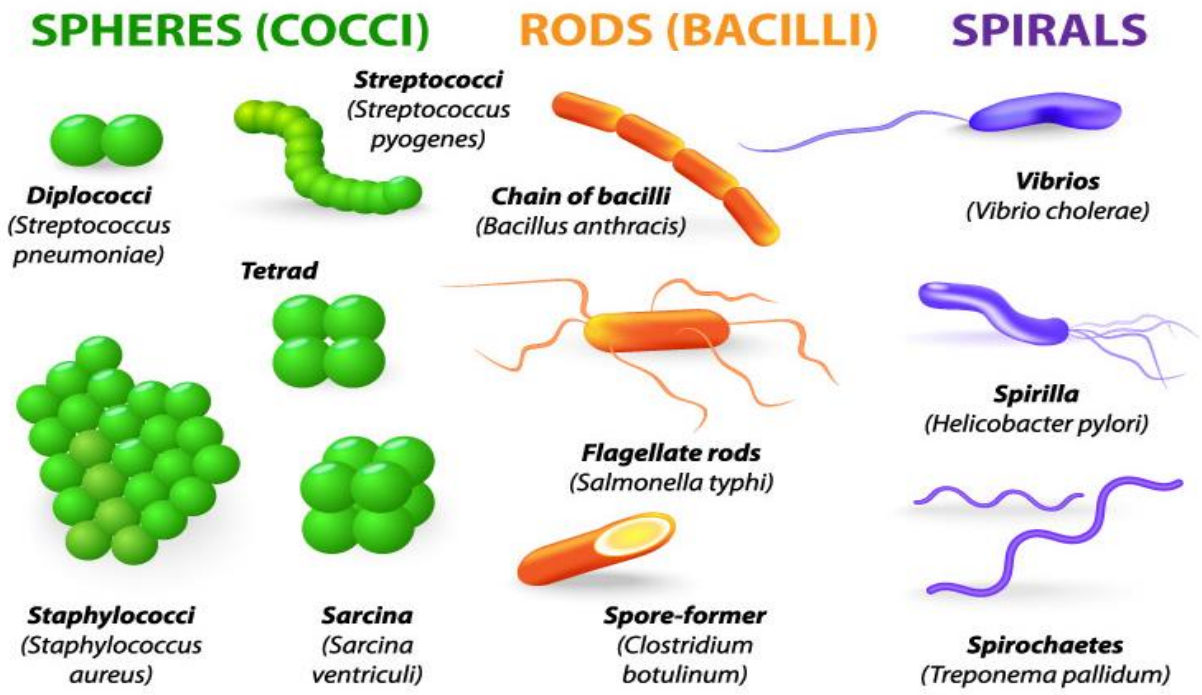
## 1. Microbial Cell Morphology and Fine structure

### A- Morphology of microbial cells:

Morphology includes the size, shape and arrangement of microbial cell. However, these features vary with species of microorganisms.

1- **Size and shape:** The size, shape and arrangement of microbial cells vary with species to which they belong. Bacteria are of about 0.1 to 60 x 6 µm in size. However, there is variation in dimension of bacilli (5 x 0.4 -0.7 µm), pseudomonads (0.4-0.7 µm diameter, 2-3 µm length) and microccoci (about 0.5 µm diameter).

The shape of bacterial cell determines by its rigid cell wall. Generally, the bacterial cells are spherical (coccus, plural cocci which mean berries), elongated rods (bacillus, plural bacilli), helical rods (Spirillum, plural spirilli), pear-shaped (Pasteuria), lobed spheres (Sulfolobus), rods with squared ends (Bacillus anthracis), rods with helically sculptured surface (Seliberia) and of changing shape (pleomorphic), etc. The unicellular cyanobacterial cells are usually spherical, some are elongated and multicellular (figure 1).



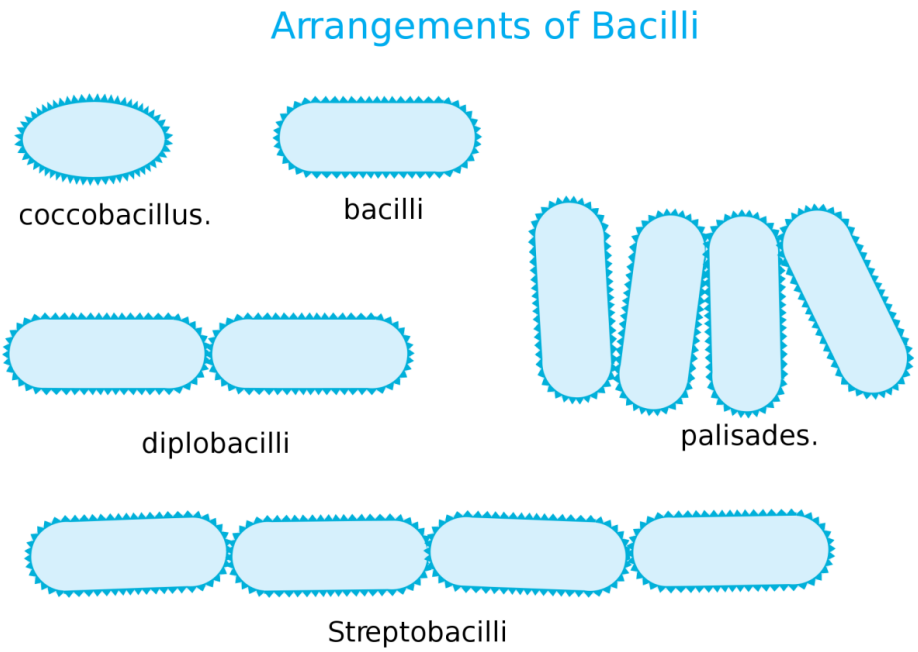


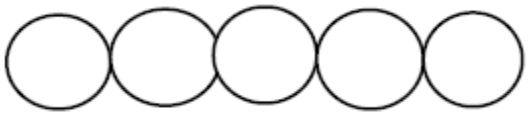

Figure 1: Bacteria come in a wide variety of shape.

**2- Arrangement**: The arrangements of cells are more complex in cocci than bacilli. The arrangement of cells depends upon adherence of cells together after the cell division (figure 1). Different forms of arrangement are given below:

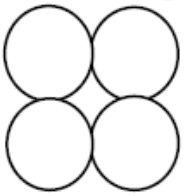
**i. Coccus forms:** there are several groups of cocci based on the number and arrangement of cells.

a. Diplococcus: Cells divide in one plane and get attached permanently in pairs.

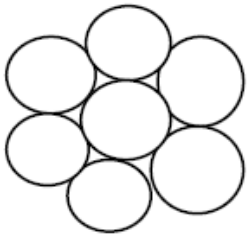
b. Streptococcus: Cells divide in one plane and remain attached to forms a linear chain of cells.



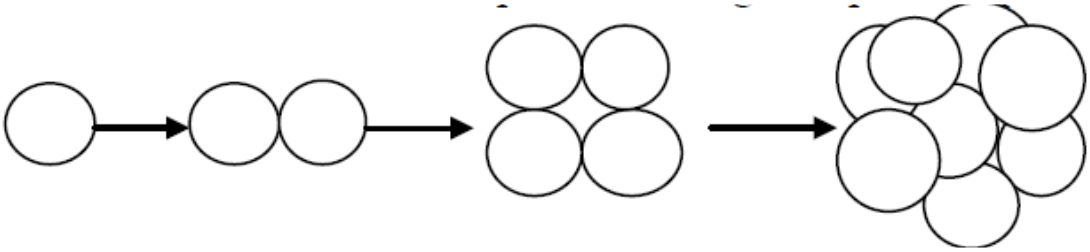
c. Tetracocci: Cells divide in two planes and form group of four cells.



d. Staphylococci: Cells divide in three planes in an irregular pattern producing bunches of cocci.



e. Sarcinae: Cells divide in three planes in regular pattern producing bunches of cocci.



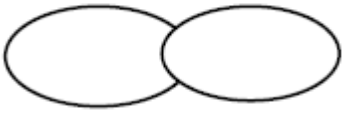
**ii. Bacillus forms:** There are a few groups of bacilli unlike cocci as the former divide across their short arise.

a. Monobacillus: The single elongated cells freely present in nature are monobacillus.

b. Diplobacillus: After division the cells remain adhered and appear in paired form.

c. Streptobacillus: After division the cells remain attached in chain appearing like straws.

d. Coccobacillus: The oval cells looking like cocci are called coccobacilli.



There is two meaning of bacillus, one is the form and the second is the genus. For example the bacterium *Bacillus anthracis* causes anthrax disease.

**iii. Spirilli forms:**

a. Vibrioid: bacterial cells having less than one complete twist from vibrioid shape e.g. *Vibrio cholerae*.

b. Helical: Cells that have more than one twist form a distinct helical shape e.g. *Spirillum* ( with flagella).

iv. Other forms:

a. Pleomorphic: of changing forms e.g. *Rhizobium, Mycoplasma*, etc.

b. Trichomes : Cells divide in one plane forming a chain which has much larger area of contact between the adjacent cells e.g. *Baggiatoa, Saprospria*.

c. Palisade: The cells are arranged laterally (side by side) to form a match sticks like structure and at angles to one another e.g. *Corynebacterium diptheriae*.

**B- Structure of microbial cell**

The structure of microbial cells reveals the composition and chemical constituents building the cell wall, the components outside and internal to the cell walls. Some of these structures are found only in certain species of bacteria or fungi. Out of all these only cell wall is common to all microbial cells. However, the cell wall structure differs in different microbial groups; for example the prokaryotic cell wall differs from the eukaryotic cell wall and Gram- positive bacteria from Gram-negative bacteria.

**- Structure of prokaryotic bacterial cell:**

Upon observation under microscope there reveal several structural components outside and inside the cell wall (figure 2) which is explained below:

1- **Capsule**: Some of bacterial cells are surrounded by the extracellular polymeric substances which are commonly called capsule. It forms an envelope around the cell wall and can be observed under light microscope after special staining technique. The capsule is gelatinous polymer made up of either polysaccharide (*Klebsiella pneumoniae*) polypeptide (*B. anthracis*) or both. The polysaccharides may be of a single type of sugars (homopolysaccharide) or several types of sugars (heteropolysaccharides). The bacterial capsule is species specific and, therefore, can be used for immunological differentiation of related species. Amount of these polymers vary with bacterial species. It is sticky in nature and secreted from the inner side of cell which gets firmly attached to the surface of cell wall. If the substances are unorganized and loosely attached to cell wall, the capsule is

called slime layer. The fresh water and marine bacteria form trichomes which are enclosed inside the gelatinous matrix called sheath. Sheath is also found in cyanobacteria and algae.

**Function of Capsule**: Capsule may have a number of functions according to bacterial species.

1- The capsule may prevent the attachment of bacteriophages.

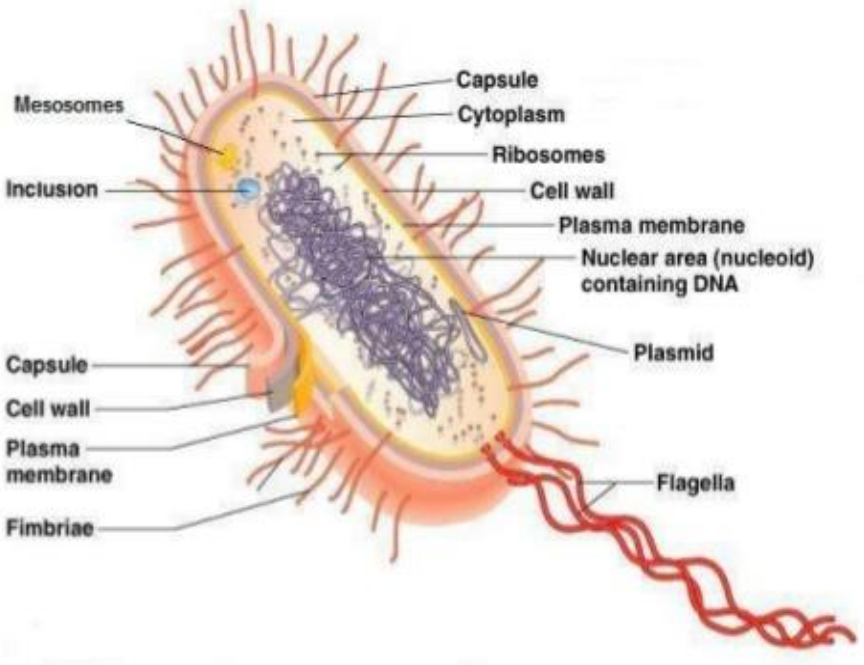
2- It protects the bacterial cells against desiccation as it is hygroscopic and contains water molecules.

3- It may survive in natural environment due to its sticky property. After attachment they can grow on divers surfaces e.g. plant root surfaces, human teeth and tissues (dental carries, respiratory tract), rocks in fast flowing streams, etc.

4- They may inhibit the engulfment by WBCs (antiphagocytic feature) and, therefore, contribute to virulence. Capsule protects from phagocytosis for example the capsulated strains of *Streptococcus pneumonia* causes pneumonia and uncapsulated strain is phagocytized.

5- *S. mutans* uses its capsules as a source of energy. Its breaks down the sugars of capsule when stored energy is in low amount.

6- Capsule maintains the viscosity and inhibits the movement of nutrients from the bacterial cell.



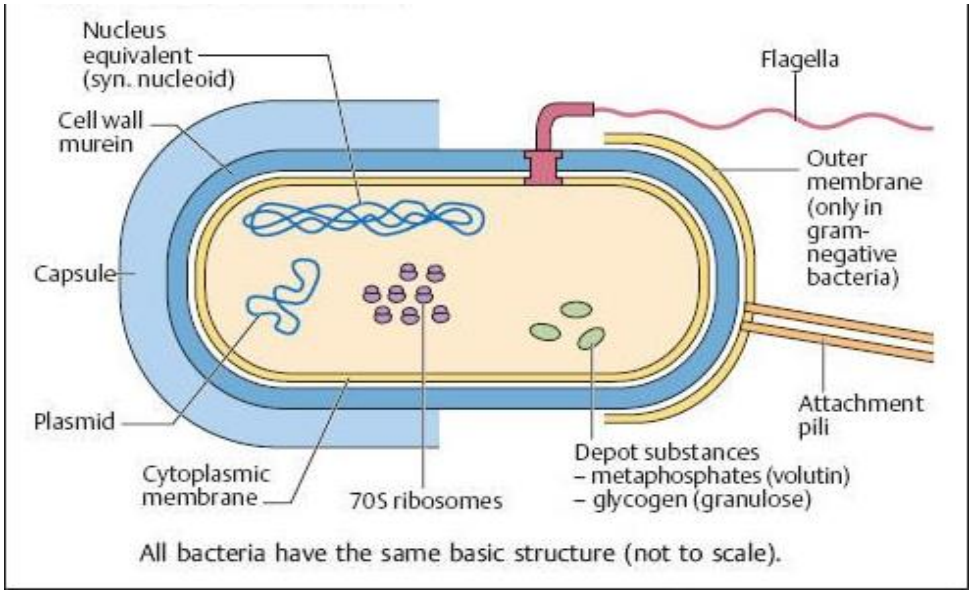



Figure 2: cross – section of a bacterium cell

**2- Flagella:** The motile bacterium may possess a flagellum (plural flagella). The flagellum is hair like, helical and surface appendages emerging from the cell wall. It is of 20-30 nm in diameter and 15 μm long. It provides various types of motility to the bacterial cell. The flagella of prokaryotes are several times thinner than that of eukaryotes.

In addition, the number and position of flagella vary (figure 3). The arrangement may be monotrichous (a single polar flagellum e.g. *V. cholera*), lophotrichous (a clusture of polar flagella e.g. *Spirillum*), amphitrichous (flagella at both the end either singly or in clusture), cephalotrichous (two or more flagella at one end of bacterial cell e.g. *Pseudomonas*), peritrichous (cell surface evenly surrounded by several lateral flagella e.g. *Proteus vulgaris*) or atrichous (cells devoid of flagella e.g. *Lactobacillus*).

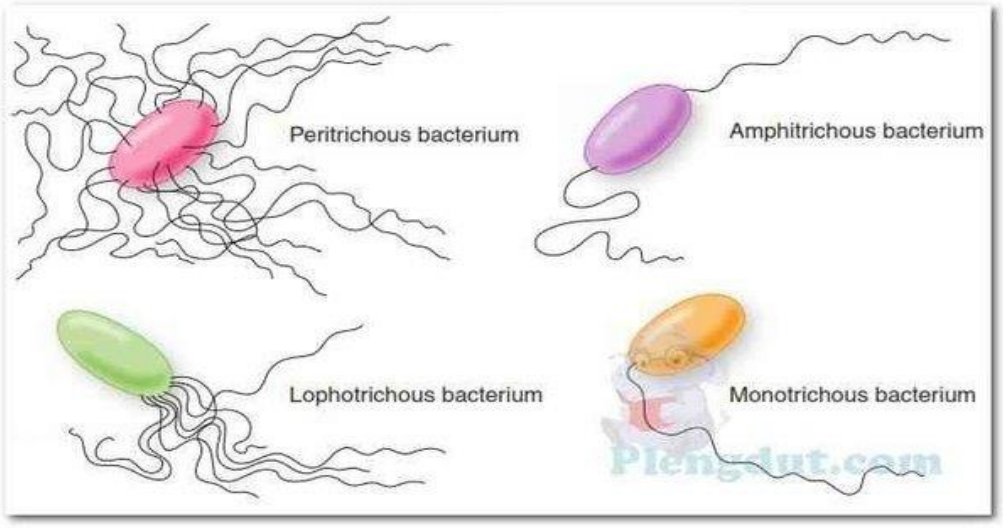



Figure 3: Bacterial flagella.

**Structure of flagella:** A flagellum consists of three basic parts, the basal body, hook and filament (figure 4).

a- **Basal body**: Isolated the basil body of a flagellum of *E. coli* and *B. subtilis* and studied its fine structure and arrangement of rings. The basal body attaches the flagellum to the cell wall and plasma membrane. It is composed of a small central rod inserted into a series of rings. In Gram- negative bacteria two pairs of rings, the proximal ring and the distal ring are connected by a central rod. These two pairs of rings i.e. four rings are L-(lipopolysaccharide) ring P- (peptidoglycan) ring, S- (super membrane) ring, and M- (membrane) ring.

The outer pair of rings, L-ring and P-ring, are attached to respective polysaccharide and peptidoglycan layer of cell wall, and the inner pair of rings i.e. S-ring and M-ring are attached with cell membrane. The outer rings form a bearing for the rod to pass through it. In Gram-positive bacteria only the distal (inner) pair of rings is present. The S-ring is attached to inside thick layer of peptidoglycan and M-ring is attached to cell membrane.

b. **Hook:** The hook is present outside the cell wall and connects filament to the basal body. It consists of different proteins. The hook in Gram-positive bacteria is slightly longer than the Gram-negative bacteria.

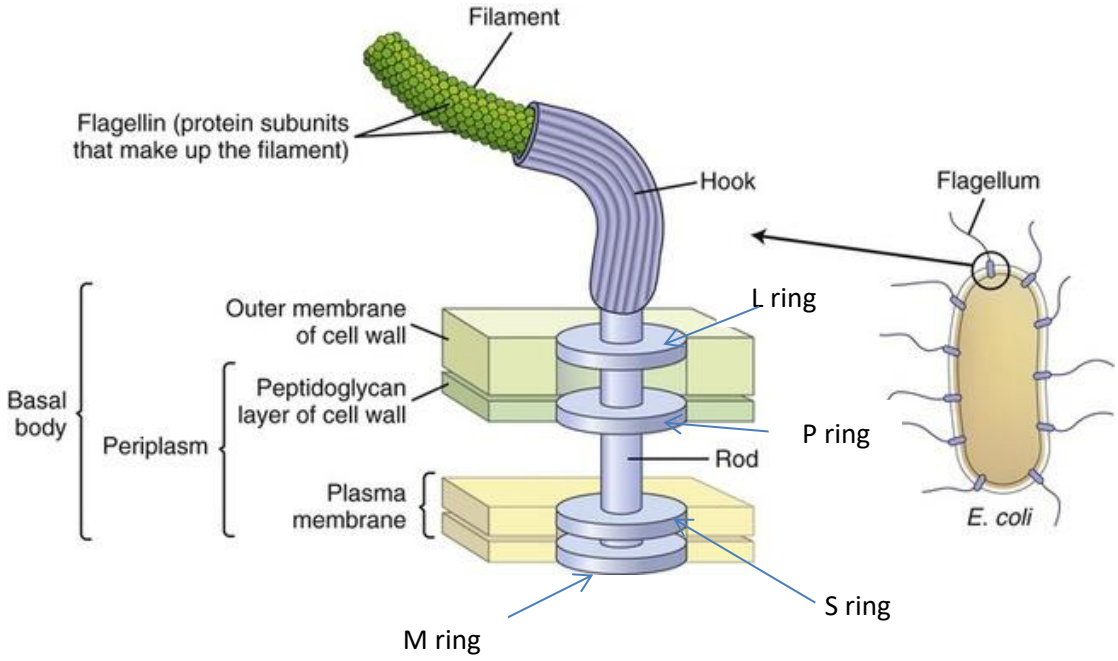



Figure 4: Flagellum structure in Gram- negative bacteria.

c. **Filament or shaft**: The outermost long region of the flagellum is called filament or shaft. It has a constant diameter and is made up of globular proteins, the flagellin. The flagellins are arranged in several chains that intertwine and form helix around a hollow core. The proteins of flagella act to

identify certain pathogenic bacteria unlike eukaryotes, the filaments are not covered by a membrane or sheath.

**- Locomotion:** There are four types of movement in bacteria. a. **flagerllar movement** : bacterial flagella are motile and help in locomotion of bacterial cells . Prokaryotic flagellum is semi rigid, helical rotor that moves the cell by rotating from the basal body either clockwise or counter clockwise around its axis. The helical waves are generated from the base to the tip of flagellum. The rotating the flagellum forms a bundle that pushes against water and propel the bacterium. The basal body acts as motor and cause rotation. Suggested that a turning motion is generated between S-ring and M- ring, where the former acts as a starter and the later acts as rotor. The P-ring and L-ring are just bashings. The basal body gives a universal joint to the cell and allows complete rotation of the hook and shaft both clockwise and counter clockwise.

The polar flagellum rotates anticlockwise but the cell rotates clockwise when moving normally. Rotation of flagellum in anticlockwise direction results in movement of bacterial cell in opposite direction. The peritrichous flagella as trailing bundle also rotate in anticlockwise direction. Cells of spirilla possess a non- helical tuft of polar flagella. The flagella rotate either at one end or both and result in cell movement. There is coordination between the flagella of both ends for rotation and movement of cell.

Energy is required for the movement of flagella. In eukaryotes, energy is generated by ATP. The mechanism of energy generation for the movement of prokaryotes flagella is not known. However, the basal body requires energy to causes motion. Movement of ions between M-ring and S-ring possibly energizes the flagellar motor.

b. **Spirochaetial movement**: The spirochaetes show several types of movements such as flexing, spinning, free swimming and creeping as they are flexible and helical bacteria lack flagella. Just within the cell envelope they have flagella like structure which are known as periplasmic flagella or axial fibrils or endoflagella. The axial fibrils are present in the space between inner and outer membrane of cell envelope. The mechanism of motility is not known. The axial fibrils rotate in periplasmic space and cause the rotation of the opposite direction.

c. **Gliding movement**: some bacteria such as the species of *cyanobactertia* (e.g. *cytophaga)* and mycoplasma show gliding movement when come in contact with a solid surface. However, no organelles are associated with the movement. Except mycoplasma, in others two gram-negative type cell walls are present.

d**. Chemotaxis**

Chemotaxis is the movement of bacteria towards chemical attraction and away from chemical repellants. Bacteria are attracted towards the nutrients such as sugar and amino acids, and are repelled by harmful substances and bacterial wastes. They also respond to other environmental fluctuations such as temperature, light, gravity, etc.

**3. Pili and fimbriae**

Pili and fimbriae are hair like appendages found on surface of cell wall in gram-negative bacteria (e. g, *Enterbacteriaceae*, *pseudomodaceae* and *caulobacter*). Eukaryotic cells lack pili. The term fimbriae are used for all hair like structure covering the surface of the cell. Pili are genetically governed by plasmids, the number of which varies from 3 to 5. The number of fimbriae is around 1,000. However, a similar structure has also been observed only in *Corynebacterium renale*, gram-positive bacterium. Pili differ from flagella in being shorter and thinner, straight and less rigid. But they are in large number. They occur either at poles of bacterial cell or evenly distributed over the enteric surface of the cell. The pili are 0.2-20 um long with diameter of about 250 A°.

**- Classes of Pili:** According to the function pili are of two types:

a. Common pili which act to adhere the cell to surfaces , and b. Sex pili which join the other bacterial cell for transfer of genome.

**- Structure of Pili :** both fimbriae and pili are like flagella as both are the appendages on bacterial cell wall. They originate from cytoplasm that protrudes outside after penetrating the peptidoglycan layer of cell wall. fimbirae are made up of 100% protein called fimbrilin or pilin which consists of about 163 amino acids. Fimbrilin has a molecular weight of about 16,000 Daltons. In addition, the sex pili are helical tubules consisting of a hollow core (25-30A°). The sex pili are the cylinder of repeating protein units. Its filamentous structure is governed by the sex factor (plasmid) of the bacterium for example F Factor. Col I factor and R factor. As compared to fimbriae the sex pili have greater diameter (65-135 A° diameter length up to 20 μm), and a terminal knob of 150-800 A° diameter. There are two types of pili in *E. coli* for example F-pili (determined by F factor) and I-pili (determined by Col I factor) .

**- Function of Pili :** there are several functions of fimbirae and pili as given below :

a. Bacteria containing fimbriae called fimbriate bacteria. Fimbriae have the adhesive properties which attach the organism to the natural substrate or to the

other organism. Fimbriea agglutinate the blood cells such as erythrocytes, leucocyte, epithelial cells, etc.

b. Fimbriae are equipped with antigenic properties as they act as thermolabile nonspecific agglutinogen.

c. Fimbriae affect the metabolic activity. The fim+ cells (cells containing fimbriae) possess higher rate of metabolic activity than the Fim- cells (cells devoid of fimbriae). Moreover, they function as aggregation organelles i.e. they can form stellate aggregation on a static liquid medium.

d. The sex pili make contact between two cells, Since they possess hollow core, they act as conjugation tube. The tip of pilus recognizes the female ($F^-$) cell through which the genetic material of donor ($F^+$) cell passes to the recipient (female) cell. Only F-pili (not I-pili) contain axial hole.

## 4- The cell Wall (Outer Membrane)

The cell wall of bacteria is a semi rigid and complex structure present under capsule and external to the plasma membrane. It is responsible for shape of the cell. The cell wall protects the plasma membrane and the other cytoplasmic inclusions from outer environment. It also protects the bacterial cell from bursting when the osmotic pressure of cytoplasm is higher than that of outside of cell wall. It provides support for attachment to the flagella. It rescues the cell from antibodies and harmful chemicals.

The cell wall of gram-negative bacteria is comparatively thinner than the cell wall of gram-positive bacteria. In addition, Chemical composition of cell wall differs. Also cell walls of eukaryotic microorganisms (e.g. algae. fungi) differ chemically from those of prokaryotes.

### Chemical composition and cell wall characteristics

The cell wall of bacteria is made up of network of peptidoglycan. It is present almost on all bacterial cell wall except *halobacterium* and *halococcus*. Because these bacteria live in marine water which contains high salt concentration the osmotic pressure of cytoplasm is more or less similar to outside the cell environment.

Peptidoglycan determines the shape of the cell. It accounts for 40-80% of total dry weight of cell. Its thickness is about 30-80 nm. It is insoluble and porous polymer that provides rigidity. It is a mucopolysaccharide. However, its chemical composition differs from species to species. It consists of repeating disaccharide attached to chains of four or five amino acids. The monosaccharide, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) are linked by β-1, 4-glycosidic bond. These are related to glucose

attached with amino acid groups. The structural formulae of NAG and NAM are shown in figure 5.

A tetrapeptide side chain containing four amino acid (L-alanine, D-glutamate, L-lysine and D-alanine) was attached to each NAM. The third amino acid varies with different bacteria and may be lysine, diaminopametic acid or threonine. For example in *E. coli* instead of L-lysine (the third amino acid) there is meso diaminopimelic acid. Due to extensive cross linking the peptidoglycan becomes a rigid macromolecule of the cell wall.

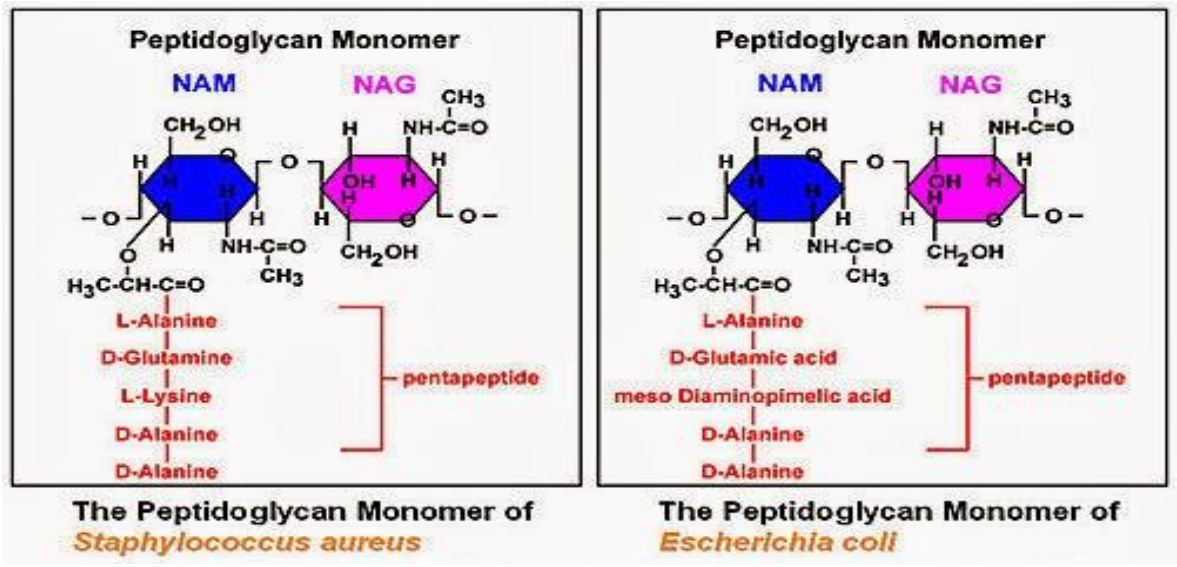



Figure 5: Chemical structure of N-acetylglucosamine (NAG) and N- acetylmuramic acid (NAM) linked together by β-1, 4 linkage.

i. **Archaeobacteria**: Structure and chemical composition of cell wall of archaeobacteria differ from that of eubacteria. Many archaea have a well with a single, thick homogeneous layer. Archaeal cell walls lack peptidoglycan and posses no common cell wall polymer. Usually cell wall is composed of proteins, glycoproteins or polysaccharides. Due to unusual chemical composition the cell envelope show a high degree of resistance against cell wall antibiotics and lytic agents.

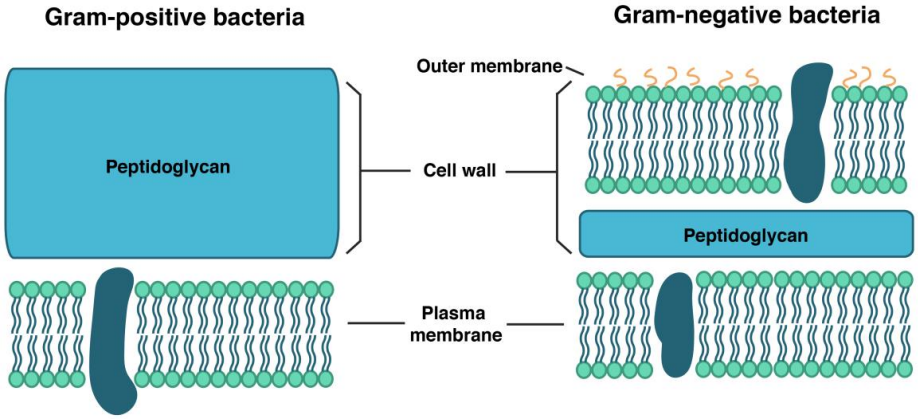
Their walls chemistry varies from species to species but usually consists of complex heteropolysaccharides; in Gram-positive archaeobacteria wells consists of pseudomurein, methanochondroitin , or heteroplysaccharide and in all Gram-negative archaeobacteria have cell envelopes which are composed of crystalline protein or glycoprotein subunits. In most organism of methanogenic branch and extreme thermophilic sulphur metabolizers, single S-layers is found in cell envelope which provides remarkable resistance ( tolerate the extremes of environmental conditions such as high salt, low pH and high temperature).

ii. **Gram-positive bacteria**: In most of Gram-positive bacteria, the cell wall contains several layers of peptidoglycan which is inter-connected by side chains and cross bridges. Peptidoglycan accounts for 40-90% of total dry weight of cell wall. However, the thickness may vary with types of species and provides rigidity to cell wall. The layers of peptidoglycan are thicker in Gram-positive bacteria than that in Gram-negative bacteria (Table 1). In most of Gram-positive bacteria peptidoglycan is associated with acidic polymers containing phosphorus called teichoic acid or acidic polysaccharides. Teichoic acids are hydrophilic, flexible and linear molecules. The presence of teichoic acid makes easy to diagnose the bacteria serologically.

Teichoic acids are mainly of three types (a) **ribitol teichoic acids** (b) **glycerol teichoic acid**, and (c) **glucosylglycerol phosphate teichoic acid**. The acids are linked to layers of peptidoglycan of plasma membrane. The phosphate groups provide negative charge which in **turn controls the movement of cation** i.e. positive ions across the cells. Teichoic acids **possibly play a role in growth of bacterial cell by regulating the activity of an enzyme autolysin**. **The acids prevent the extensive break down and possibly the lysis of cell wall. They also store phosphorus**.

The wall of most eubacteria contains very low amount of lipid except *mycobacterium* and *corynebacterium.*

iii. **Gram-negative bacteria:** The cell envelope of gram-negative bacteria consists of two unit membranes of 75 A° wide, separated by 100 A° wide periplasmic space. Peptidoglycan is present in periplasmic space in Gram-negative bacteria but in very low amount. They totally lack teichoic acids. Peptidoglycan is situated in periplasmic space and covalently linked to lipoproteins in the outer membrane. The periplasmic space is a space between the outer membrane and plasma membrane which appears like gel and contains a high amount of enzymes and transport proteins. Due to the presence of low amount of peptidoglycan, the cell wall of Gram-negative bacteria can easily be disintegrated (figure 6).



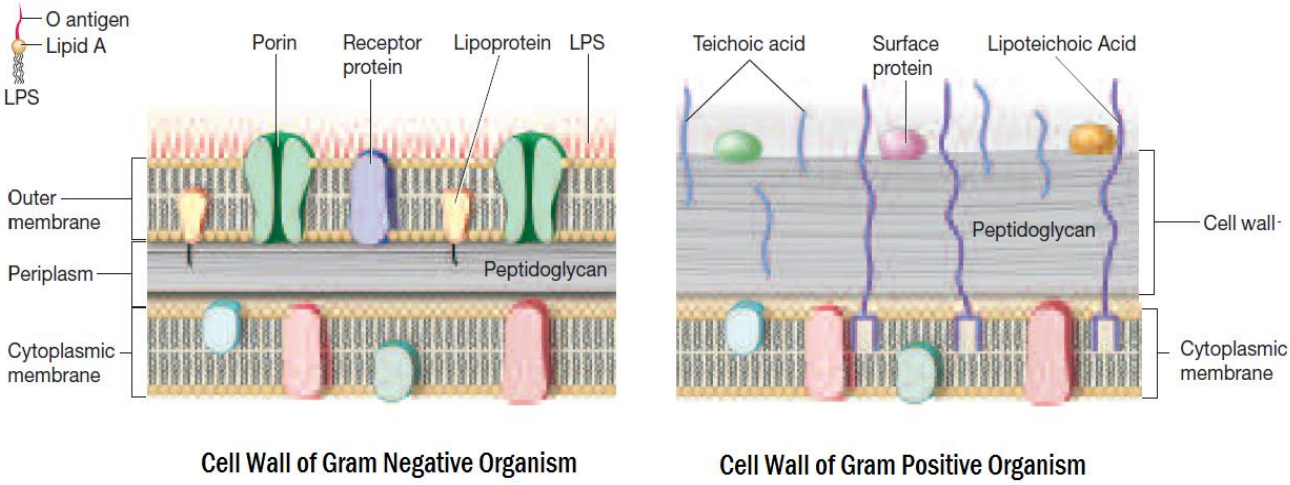



Figure 6: Structure and chemical composition and cell walls of Gram –negative and gram positive bacteria.

The cell envelope of Gram-negative bacteria is a bilayer structure consisting of mainly Apo proteins, lipopolysaccharides (LPS) and phospholipids.

The chemical constituents and arrangement are described in details as below:

**a**. Lipoproteins: Lipoproteins occur freely and in bound forms as well. In lipoproteins of outer membrane, proteins bind to lipid non-covalently; whereas in lipoproteins of plasma membrane, proteins bind to lipid covalently. Lipoproteins together with matrix proteins form a complex which contains diffusion channels. A diffusion channel is enclosed by three molecules of matrix protein leaving a diameter of about 1.5-2nm.

**b.** Lipopolysaccharides (LPS): The outer membrane of Gram-negative bacteria is covered by LPS which is made up of polysaccharides covalently linked to lipid A. Lipid A: it consists of **glucosamine, phosphate** and **fatty acids**.

**c**. Polysaccharide: the polysaccharide portion of LPS of *salmonella* cell wall is composed of three important components, the inner core, the outer core and the O-antigen side chain. Although the polysaccharide of LPS is also known as O- polysaccharide. The O-antigen side chains in rough strains of Gram-negative bacteria are absent; whereas the smooth strains of *salmonella* have O-antigen side chains which may extend out the wall surface and attain about 30 nm length. These chains have antigenic property and, therefore, can be distinguished serologically (e.g. species of *salmonella*). Its role is comparable to that of teichoic acids in Gram-positive bacteria cell wall.

**d.** Matrix Proteins: the outer membrane of cell envelope doesn't warrant the entry of all substances since the nutrients are to pass across the membrane. It is impermeable only to macromolecules such as proteins, lipids, etc. The permeability of outer membrane is due to the presence of proteins called

porins that from channels. The pornis are not specific and allow the small molecules. Certain porins are specific and permit only the specific substances such as vitamin $B_{12}$ nucleotides. Porins also act as receptor sites for bacteriophages and bacteriocins (the proteins produced by certain bacteria that inhibit or kill the related species).

**- Function of G –ve cell wall**: following are the functions of the cell wall (the outer membrane):

(a) Peptidoglycan provides structural integrity of cell by forming a rigid layer in outer membrane. The matrix proteins to some extent also contribute to structure with peptidoglycan.

(b) The cell envelope acts as barrier for diffusion to certain molecules across the envelope.

(c) The matrix proteins act as receptor sites for bacteriophage and bacteriocins.

(d) The O-antigen side chain of polysaccharide of LPS determines the antigen specificity of Gram-negative bacteria.

Differences between cell walls of gram-positive and gram-negative bacteria are given in table 1.

**Table 1:** Differences between cell walls of gram-positive and gram-negative bacteria. Character

**A comparison between Gram positive and Gram negative cell wall**

| Item | Gram positive | Gram negative |
|---|---|---|
| Peptidoglycan layer | Thick (multilayered) | Thin (single-layered) |
| Teichoic acids | Present | Absent |
| Periplasmic space | Absent | present |
| Lipopolysaccharide (LPS) content | Virtually none | High |
| Lipid and lipoprotein content | Low | High |
| Resistance to physical disruption | Low | High |
| Inhibition by basic dyes | Low | High |
| Susceptibility to anionic detergents | Low | High |
| Resistance to drying | Low | High |
| Gram reaction | Retain crystal violet dye and stain dark violet | Can be decolorized to accept counter stain |

## A typical cell wall

There are a few groups of microorganisms that have no cell walls. The protoplasts are surrounded by only a cytoplasmic membrane, for example the members of mollicutes (*Mycoplasma* and *Ureoplasma*). Due to the lack of rigid cell wall the cell can take any shape cocci, filamentous, discs. However, mycoplasmas are the smallest known microorganism. Like viruses they can pass through the bacterial filters. Their plasma membrane is unique in having lipids called sterols. Sterols possibly protect the cell from osmotic lysis.

The other example of a typical cell wall is the L-form of bacteria. The L-forms are named after the Lister institute where they were discovered for the first time. The L-forms are the **small mutant bacteria containing defective cell walls**. This form can be produced by inducing the bacteria with certain chemicals and antibiotics. Some L-forms after giving proper nutrition revert to the original forms and the others are stable. The L-forms attain irregular shape. It has been found when the protoplast of *B. subtilis* is placed in a 25% gelatin medium and incubated at 26 C°; it reverts back to the normal walled form.

The cell wall of Gram-positive bacteria is degraded when treated with lysozyme. The cellular material is surrounded by plasma membrane. If osmotic lysis does not occur the plasma membrane remains intact. These cells devoid of cell wall are known as protoplast. In contrast the lysozymes destroy the cell wall of Gram-negative bacteria only to some extent. Some of outer membrane remains intact. Thus the cellular contact, plasma membrane and remaining outer cell wall layer are jointly called spheroplast.

## 5. Plasma membrane (cell or cytoplasmic membrane):

The plasma membrane also called cell membrane or cytoplasmic membrane is a structure situated just beneath the cell wall. However, no cell could be alive without a plasma membrane. It consists of proteins (20-70%), lipids (28-80%), oligosaccharides (1-5%) and water (20%). The plasma membrane consists of a continuous bilayer of phospholipid molecules in which globular proteins are embedded.

The plasma membrane is semifluid structure in which lipids and proteins are arranged in a mosaic manner. The globular proteins are of two types; extrinsic (peripheral) proteins and intrinsic (integral) proteins. The extrinsic protein is soluble and, therefore. Dissociate from the membrane, while the intrinsic protein is insoluble and could not (or rarely) dissociate. The intrinsic proteins are partially embedded either on outer surface or on inner surface of the bilayer and take part in lateral diffusion in lipid bilayer (figure 7).

The lipid matrix of membrane has fluidity that permits the membrane components to move laterally. The membrane fluidity is due to the hydrophobic interactions of lipids and proteins. The fluidity is important for a number of membrane functions.

The presence of complex lipids becomes a key character of certain microorganisms on the basis of which they can be identified. For example, the cell wall of mycobacterium contains high amount of lipids such as waxes and glycolipids which gives the bacterium a distinctive staining characteristic.

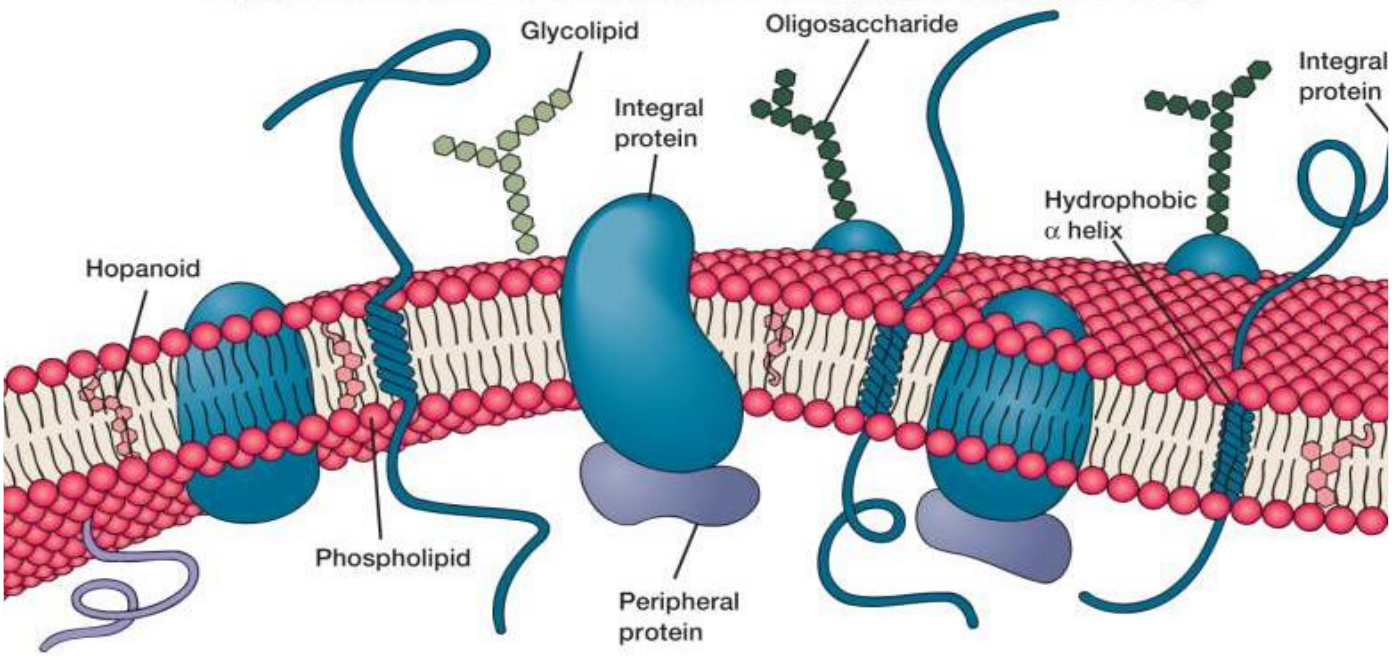



Figure 7: bacterial plasma membrane.

**Function of Plasma Membrane**:

The cytoplasmic membrane is the site of many metabolic activities as given below:

a. The organic and inorganic nutrients are transported by permeases through plasma membrane.

b. It consists of enzymes of biosynthetic pathways that synthesize different components of the cell wall such as peptidoglycan, teichoic acids, polysaccharides, lipopolysaccharides and phospholipids.

c. It possesses the attachment site for bacterial chromosome and plasmid DNA.

d. The inner membrane invaginates to form mesosomes, a site for respiratory activity. The plasma membrane contains about 200 respiratory proteins that have been found to be responsible for the transport of H + ions.

e. It provides permeability barrier and thus prevent the escape of cellular materials outside the cell, show selective permeability.

**6- Mesosomes:**

Mesosomes are the invaginated structures formed by the localized infoldings of the plasma membrane. The invaginted structures comprise of vesicles, Tubules of lamellar whorls. Generally mesosomes are found in association with nuclear area or near the site of cell division. They are absent in eukaryotes. The lamellae are formed by flat vesicles when arranged in parallel. Some of the lamellae are connected to the cell membrane. The lamellar whorl can be observed in *Nitrobacter*, *Nitromonas* and *Nitrococcus*.

The constriction does not cause the complete separation of tubules. However, they are the special cell membrane components, the proteins of which differ from the cell membrane.

The exact structure and function of mesosomes are not known. However, it has been suggest that these are artifacts (figure 8).

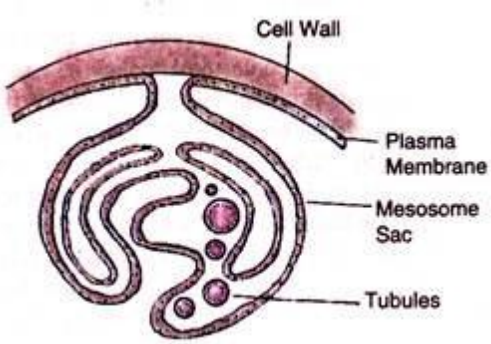



Figure 8: The bacterial mesosomes.

Moreover, mesosomes **are supposed to take part in respiration but they are not analogous to mitochondria** because they lack outer membrane. Respiratory enzymes have been found to be present in cell membrane. In the vesicle of mesosomes the respiratory enzymes and the components of electron transport such as ATPase, dehydrogenases, cytochrome are either absent or present in low amount. In addition, mesosomes are supposed as **a site for synthesis of some of wall membranes**.

Mesosomes might **play a role in reproduction** also. During binary fission a cross wall is formed resulting in formation of two cells. Mesosomes begin the formation of septum and attach bacterial DNA to the cell membrane. It separates the bacterial DNA into each daughter cell. In addition, the infoldings of mesosomes **increase the surface area of plasma membrane that in turn increases the absorption of nutrients**.

**7- Cytoplasm**:

Cytoplasm of prokaryotes refers to the internal matrix of cell inside the plasma membrane. Cytoplasm consists of water (80%), proteins, carbohydrates, lipids, inorganic ions and certain low molecular components. Cytoplasm is thick and semitransparent. The DNA molecules, ribosomes and the other inclusions are the structure of cytoplasm. In certain cyanobacteria gas vacuoles are found. The prokaryotic cytoplasm differs from the eukaryotic cytoplasm.

**i. Ribosomes**: All living cell contains ribosomes which vary complex structures made of protein and ribonucleic acid (RNA), act as site of protein synthesis. Number of ribosomes represents high rate of protein synthesis and vice versa. Cytoplasm of a prokaryotic cell contains about 10000 ribosomes which account up to 30% of total dry weight of the cell. Presence of ribosomes in high number gives the cytoplasm a granular appearance. The eukaryotic ribosomes are found attached to cell membrane, whereas the prokaryotic ribosomes are free in cytoplasm or loosely attached to the plasma membrane. Prokaryotic ribosomes are smaller and less dense than eukaryotic ribosomes. Ribosomes of prokaryotes are often called 70S ribosomes and that of eukaryotes as 80S ribosomes. The letter S refers to Svedberg unit which indicates the relative rate of sedimentation during ultracentrifugation. Sedimentation rate depends on size, shape and weight of particles.

**ii. Nucleoids** (the bacterial chromosome): As in eukaryotes, in prokaryotes also the basic dye stains the nuclear material and reveals as dense and centrally located bodies of irregular outline. Upon observation with electron microscope it was found that this central region is not separated from the cytoplasm by a membrane and consists of nuclear structure besides; the DNA fibrils. The eukaryotes contain a well-organized nucleus in which the genetic material is enclosed by a nuclear membrane, whereas, the DNA material of prokaryotes is not enclosed by any covering. Hence the bacterial chromosome is known as chromatin bodies or nucleoids. The nucleoid is a single long circular double stranded DNA molecule free from histone protein. The histone is present in eukaryotes, therefore, results the eukaryotic DNA into the beaded structures i.e. nucleosomes.

**iii. Plasmids**: during 1950s, working on conjugation process it was found that maleness in bacteria is determined by a transmissible genetic element. When male and female bacteria conjugate, every female is converted into a male. This inherited property of male is called the F (fertility) factor which is transmitted by cell to cell contact. Therefore, F is a separate genetic element. In 1952, J. Lederberg coined the term plasmid as a genetic name for this element. Hence the plasmids may be defined **as a small circular, self-**

**replicating and double stranded DNA molecule present in bacterial cell, in addition to bacterial chromosome**. It replicates independently during cell division and inherited by both of daughter cells. Therefore, its function is not governed by the bacterial chromosome. Some important types of plasmid are F factor, R plasmids, Col plasmids, virulence plasmids, metabolic plasmids.

**vi. Granules or inclusions**: this granules or inclusions also called storage bodies. Bacteria store nutrients intra cellular in inclusions bodies during periods of nutrient abundance, these bodies varying in size, number and content.

Some types of inclusions bodies:

**-Glycogen bodies (granules) and poly β hydroxyl – butyrate (PHB**), this granules source of Carbone (rich organic substance) and energy, it is have single –layered membrane.

**-Inorganic compound granules**, this contains crystal of inorganic compound are not enclosed by membrane, like:

a- Sulfur granules: of photosynthesis bacteria.

b-Meta chromatic granules: or poly phosphate granules, volutin granules, in Corynebacterium and mycobacterium, an important source of building blocks for nucleic acid and ATP, the stained redopurple by methylene blue so that have been termed methachromatic granules.

**-Lipid inclusions**: Lipids are found in a high amount as source for energy, in several species of *Bacillus, Mycobacterium, and Spirillum*.

**-Magnetosomes**: Magnetosomes are the intracellular chains of 40-50 magnetite ($Fe_3O_4$) Particles found in magnetotactic bacteria. Each iron particle is a tiny magnet. Hence, the bacteria employ their magnetosomes to determine northward and downward directions, also help them to swim to nutrient rich sediments or locate the optimum depth in fresh water and marine habitats.

**-Gas vesicles:** Found in floating forms prokaryotic Microorganisms in lakes and sea. These vesicles not found in flagellar microorganisms, they are gas-filled structure which contains the same gas in which the organisms are suspended. Gas vesicles provide buoyancy and keep the cell floating form.

**8- Endospores:**

Endospores are bacterial survival structures that are highly resistant to many different types of chemical and environmental stresses and therefore enable **the survival of bacteria in environments that would be lethal for these cells in their normal form**. It has been proposed that endospore formation

has allowed for the survival of some bacteria for hundreds or millions of years (e.g. in salt crystals).

Endospore formation (figure 9) is limited to several genera of Gram-positive bacteria such as Bacillus and Clostridium. It's called endospore because it's produced inside a cell and differs from reproductive spores in that only one spore is formed per cell resulting in no net gain in cell number upon endospore germination. The location of an endospore within a cell is species-specific and can be used to determine the identity of a bacterium. Dipicolinic acid is a chemical compound which composes 5% to 15% of the dry weight of bacterial spores. It is implicated as responsible for the heat resistance of the endospore.

The process of endospore formation is called sporulation or sporogenesis. It occurs normally when growth of bacterium stop due to lack of nutrients, sporulation is a complex process and occurs in several stages.

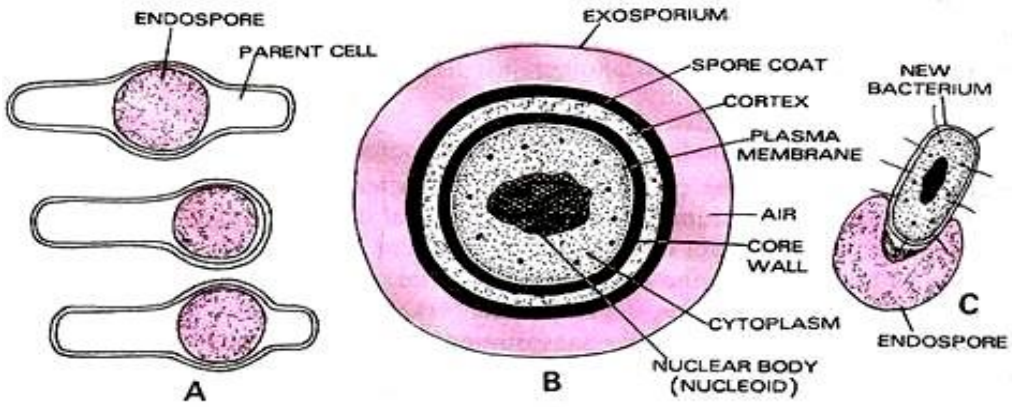
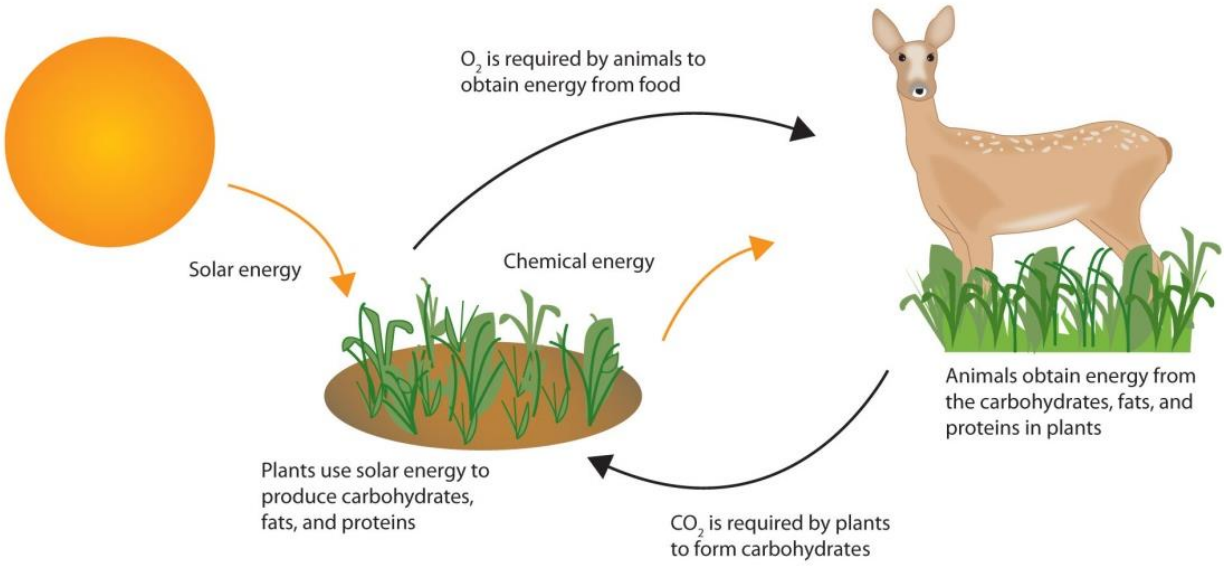



Fig. 2.12. Endospores. A, types of endospores according to their position in parent cells. B, structure of an endospore. C, germination of endospore.

Figure 9: bacterial endospore.

## Microbial Nutrition

All organisms extract energy from their environment. Producers (also called autotrophs) extract energy from the nonliving environment, such as plants capturing light energy from the sun or bacteria deriving chemical energy from rocks. Consumers (also called heterotrophs), in contrast, obtain energy by eating nutrients made by other organisms. Decomposers are consumers that obtain nutrients from dead organisms. Fungi such as mushrooms are decomposers. Like all other living things, microorganisms need to acquire energy in order to survive figure 10.




Energy is required:
1) To maintain the structural integrity of the cell by repairing any damage to its constituents.
2) To synthesis new cellular components such as nucleic acids, polysaccharides and enzymes.
3) To transport certain substances into the cell from its surroundings.
4) For the cell to grow and multiply.
5) For cellular movement.

Microbial cell are structurally complex and carry out numerous functions. In order to construct new cellular components and do cellular work, organisms must have a supply of raw materials or nutrients and a source of energy. In nature, particularly in a given environment, the distribution of microbes is partly determined by the availability of specific nutrients required to support their growth. **Nutrients** are substances used in biosynthesis and energy release, therefore are required for microbial growth. Or all chemicals required by microorganisms (and all other organisms) as raw material for their metabolism and reproduction are called **Nutrients**. Nutrients can be classified as **mineral nutrients** and **growth factors**.

## A. The Common Nutrient requirements:

**1- Mineral Nutrients** The microbial mineral nutrients can be classified as macro (major) nutrients, and micro (minor) nutrients or trace elements on the basis of their amount required.

### a. Macro or Major Mineral Nutrients

These are called **macro mineral nutrients** because they are required by microorganisms in relatively large amount, the lack of which can limit growth. The microbial cells contain water accounting for some 80-90% of their total weight and, therefore, the water is always the major essential nutrient in quantitative terms. The solid matter of cells contain, in addition to oxygen and

hydrogen (derivable metabolically from water), the other macro (major) elements, namely, **carbon, nitrogen, phosphorus, Sulphur, potassium, magnesium, sodium, calcium and iron** in order of decreasing abundance. About 95% of cellular dry weight of microbial cells is accounted for only six macro (major) elements (O, H, C, N, P and S). However, approximate percentage of dry weight and general physiological functions of major mineral nutrients are given in the table 2.

**Table 2: Approximate percentage of dry weight and general physiological functions of mineral nutrients**

| Element (Nutrient) | % of dry weight | Physiological functions |
|---|---|---|
| Carbon (C) | 50 | Constituent of all organic cell materials |
| Oxygen(O) | 20 | Constituent of cellular water and most organic cell materials; oxygen serves as an electron receptor in aerobic respiration |
| Nitrogen (N) | 14 | Constituent of proteins, nucleic acids, coenzymes |
| Hydrogen (H) | 8 | Constituent of cellular water, organic cell materials |
| Sulphur (S) | 1 | Constituent of some amino acids (cysteine and methionine), of some coenzymes (e.g., CoA, Co carboxylase) |
| Potassium (K) | 1 | Important inorganic cation in cells, cofactor for some enzymatic reactions |
| Sodium (Na) | 1 | Important inorganic cations in cells, important in membrane transport |
| Calcium (Ca) | 0.5 | Important inorganic cation in cells, cofactor for some enzymatic reactions (e.g., reactions by proteinases). It is essential component of endospores. Calcium concentrations affect membrane permeability and play a critical role in movement of flagella and cilia |
| Magnesium (Mg) | 0.5 | Important inorganic cation in cells, cofactor for some enzymatic reactions sometimes replacing Mg. Magnesium plays important role in protein synthesis; without the ribosomal subunits do not associate and translation of nucleic acids into protein is not possible |
| Iron (Fe) | 0.2 | Constituent of cytochromes and other haeme or non-haeme proteins, cofactor for a number of enzymatic reactions |

**- Requirement for Carbon, hydrogen, Oxygen, and Electron**

All organisms need C, H, O, and source of electrons. C is needed for backbones of all organic molecules from which organisms are building. H and O are also important elements found in organic molecules. Electrons are needed for two reasons, the movement of electrons through electron transport chains and during oxidation – reduction reactions can provide energy for in cellular work also needed to reduce molecules during biosynthesis.

Organic compounds serve microorganisms (heterotrophs) as a source for:

- The required element of C, H, and O
- Energy

- Electrons for oxidation-reduction reactions or biosynthetic processes.

However, not all carbon sources provide energy or H, e.g., carbon dioxide ($CO_2$) as in Autotrophs use $CO_2$ as carbon source, but this molecule does not provide H or energy, many autotrophs use sunlight for energy via photosynthesis, some autotrophs oxidize inorganic molecule to derive electrons and energy.
Remarkably, microorganisms are extraordinary flexible with regard to their carbon source, some will utilize a diverse array of carbon source, including waxes, rubber, oil, etc.

**- Requirement of Nitrogen, Phosphorus, and sulfur**
To grow, microorganisms must be able to incorporate large quantities of nitrogen, phosphorus and sulfur. Although these elements may be acquired from organic molecules, microorganisms usually provided by inorganic sources as well.
**Nitrogen** is needed for the synthesis of amino acid, purines, pyrimidines, some carbohydrates and lipids, enzymes cofactors, and others. Most phototrophs and many chemotrophs microorganisms reduce nitrate ($NO_3$) to ammonia ($NH_3$) and incorporate the ammonia in a process known as assimilatory nitrate reduction. A variety of bacteria (e.g. many cyanobacteria and the symbiotic bacterium *Rhizobium*) can assimilate atmospheric nitrogen ($N_2$) by reducing it to ammonium ($NH_4^+$). This is called nitrogen fixation.
**Phosphorus** is present in nucleic acid, phospholipids, nucleotide like ATP, several cofactors, some proteins, and other cell components. Almost all microorganisms use inorganic phosphate as their phosphorus source and incorporate it directly. Some Microorganisms utilize organophosphate. Low phosphate levels actually limit microbial growth in many aquatic environments. Some microbes, such as *Escherichia coli*, can use both organic and inorganic phosphate.
**Sulfur** is needed for the synthesis of substances like the amino acid cysteine and methionine, some carbohydrates, biotin, and thiamine. Most microorganisms use sulfate ($SO_4$) as a source of sulfur and reduce it by assimilatory sulfate reduction; few microorganisms require a reduced form of sulfur such as cysteine.

**b - Micro or Minor Mineral Nutrients or Trace Elements**
The microorganisms, in general do not use only macro (major) elements but also others like **cobalt, copper, manganese, molybdenum, nickel, selenium, tungsten, vanadium and zinc** which are required in residual fraction by nearly all microorganisms. These elements are often referred to as minor (micro) nutrients or trace elements table 3. The micronutrients required in small amounts and usually not a growth limiting factor due to their ubiquitous

nature. They are metals playing the role of cells catalysts and many of them are play a structural role in various enzymes. The major micronutrients of living systems and gives examples of enzymes in which each plays a role. Some microorganisms.

**Table 3: Micronutrients (trace elements) and their cellular functions**

| **Micronutrient** | **Cellular function** |
|---|---|
| Cobalt (Co) | Vitamin B12; transcarboxylase (propionic acid bacteria) |
| Copper (Cu) | Respiration (cytochrome c oxidase); photosynthesis |
| Manganese (Mn) | Acts as activator or various enzymes; and in the photolytic (water-splitting) enzyme in oxygenic phototrophs (photosystem-II) |
| Molybdenum (Mo) | Present in some Flavin-containing enzymes, nitrogenase, nitrate reductase, sulphide oxidase, some format dehydrogenases |
| Nickel (Ni) | Present in most hydrogenase enzymes; coenzyme F430 of methanogenes; carbon monoxide Dehydrogenase; urease |
| Selenium (se) | Occurs in format dehydrogenase; oxotransferases of hyperthermophiles |
| Tungsten (W) | In some format dehydrogenases; oxotransferased of hyperthermophiles |
| Vanadium (V) | Vanadium nitrogenase; bromoperoxidase |
| Zinc (Zn) | In carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; many DNA-binding proteins |

Finally, Microorganisms required a balance mixture of nutrients for normal growth. If an essential nutrient is in short supply, microbial growth will be limited regardless of the concentrations of other nutrients.

## 2. Growth Factors

Besides the mineral nutrients, the microorganisms need some organic compounds. Most of the microorganisms are capable of synthesizing these organic compounds from simpler carbon resources; others cannot and need their supply from outside for their proper growth and development. Organic nutrients of this type are known collectively as growth factors (essential metabolites). **Growth factors** are organic compounds that are essential cellular components or precursors of such components but cannot be synthesized by the organism itself. And can be categorized into three groups.

1. Amino acids are needed for protein synthesis.
2. Purines and pyrimidines are needed for nucleic acid synthesis.
3. Vitamins are small organic molecules that usually make up all or part of enzyme cofactors and are needed in only very small amounts to sustain growth. Some important vitamins and their functions are summarized in the table 4.

**Table 4 : Vitamins and their Functions**

| Vitamins | Function |
|---|---|
| Riboflavin (B2) | Precursor of Flavin mononucleotide (FMN) and Flavin-adenine dinucleotide (FAD) which are involved in electron transport chain |
| Cobalamin (B12) | Reduction of and transfer of single carbon fragments; synthesis of deoxyribose |
| Biotin | In fatty acid biosynthesis; in b-decarboxylation; in some CO2-fixation reaction |
| p-Aminobenzoic acid | Precursor of folic acid |
| Folic acid | One-carbon metabolism; transfer of methyl group |
| Thiamine (B1) | Transketolase; a-decarboxylations |
| Nicotinic acid (niacin) | Precursor of Nicotinamide adenine dinucleotide (NAD+); electron transfer in oxidation-reduction reactions |
| Lipoic acid | Acyl group transfer in decarboxylations of Pyruvic acid and a-ketoglutaric acid |
| Pantothenic acid | Precursor of coenzyme A; activation acetyl and other acyl derivastions |
| Vitamin B6 | Amino acid and keto acid transformations |
| Vitamin K | Electron transport; in synthesis of sphingolipids |
| Hydroxamates | Solubilization of iron and transport into cell |

## B. Nutritional types of Microorganisms

Microorganisms can be grouped into nutritional classes based upon their sources of Carbon, energy and electron:

**Carbon Sources**:

a- Autotrophs M.O.: Organisms that use $CO_2$ as their principal source of carbon.

b- Heterotrophs M.O.: Organisms that reduced performed organic molecules as their carbon source.

**Energy Sources:**

a- phototrophs M.O: Use light as their energy source.

b- Chemotrophs M.O: obtain energy from oxidation of chemical compounds (either organic or inorganic).

**Electron sources**:

a- Lithotrophus M.O.: use reduced inorganic substances as their electron source.

b- Organotrophs M.O: extract electrons from reduced organic compounds.

Despite the great metabolic diversity seen in the microorganisms, most may be placed in one of five nutritional classes based on the primary sources of carbon, energy, and electrons as in table 5.

**Table 5: Major nutrition types of Microorganisms**.

| Nutritional Type | Carbon Source | Energy Source | Electron Source | Representative M.O. |
|---|---|---|---|---|
| Photolithoautotrophy | $CO_2$ | Light | Inorganic e - donor | Purple and green sulfur bacteria, cyanobacteria |
| Photoorganoheterotrophy | Organic carbon, but $CO_2$ may also be used | Light | Organic e - donor | Purple nonsulfur bacteria, green nonsulfur bacteria |
| Chemolithoautotrophy | $CO_2$ | Inorganic chemicals | Inorganic e - donor | Sulfur –oxidization bacteria Hydrogen-oxidizing bacteria |
| Chemolithoheterotrophy | Organic Carbon but $CO_2$ may also be used | Inorganic chemicals | Inorganic e - donor | Some sulfur_oxidizing bacteria |
| Chemoorganoheterotrophy | Organic carbon | Organic chemicals often same as C source | Organic e donor Often same as C source | Most nonphotosynthetic microbes , including most pathogens , fungi |

Although a particular species usually belongs to only one of the nutritional classes, some show great metabolic flexibility and alter their metabolic patterns in response to environmental changes (Mixotrophs). e.g., purple non sulfur bacteria

### C. Uptake of Nutrients by the cell

The first step in nutrient use is uptake of the required nutrients by the microbial cell. Uptake mechanisms must be specific that is, the necessary substances, and not others, must be acquired. Because microorganisms often live in nutrient poor habitats, they must be able to transport nutrients from dilute solutions into the cell against a concentration gradient. Finally, nutrient molecules must pass through a selectively permeable plasma membrane that prevents the free passage of most substances. Materials move into and out of cells through either passive transport or active transport:

• **Passive transport** - movement of molecules from a more crowded to a less crowded area WITHOUT the use of energy. Movement occurs when there are unequal concentrations of a substance inside and outside of the cell.

• **Active transport** - movement of molecules from a less crowded to a more crowded area WITH the use of energy. Molecules are "carried" into or out of the cell using some of the cell's energy. Active transport is also characterized by the carrier saturation effect at high solute concentration

Active and passive transport differ because: 1. Active transport makes use of energy in the form of ATP whereas passive transport does not utilize any. 2. Active transport involves the transfer of molecules or ions against a

concentration gradient whereas passive transport is the transfer along a concentration gradient.

Microorganism's uses of several different transport mechanisms include:
- **Passive transport**:
 Passive diffusion
 Facilitated diffusion

- **Active transport:**
 Group translocation (primarily prokaryotes)
 Membrane Bound Transport Systems
 Binding Protein Transport System

- **siderophores for iron uptake**
- **Endocytosis (Eukaryotes only)**

**Passive (Simple) diffusion**: is the process in which molecules move from an area of higher concentration to an area of lower concentration due to random thermal energy. The rate of Passive diffusion is dependent on the size of the concentration gradient between a cell exterior and its interior. Very Small Molecules such as glycerol, H2O, O2, and CO2 often move across membranes by passive diffusion. Large molecules must use another mechanism to be transported across a membrane.

**Facilitated diffusion:** differs in that the rate of diffusion is increased by using carrier molecules called **permeases** embedded in the plasma membrane. Diffusion involving carrier protein is called Facilitated diffusion. Each permease is specific for the particular molecule being transported.
Rate of diffusion increases as the concentration gradient increases, but the process can become "saturated", i.e., at a certain concentration all of the permeases are bound with transport molecules. No metabolic energy input required. After the solute molecule binds to the outside, the carrier may change its conformation and release the molecule on the cell interior (figure 11). The carrier subsequently changes back to its original shape and is ready to pick up another molecule, the process is reversible, i.e., if concentration is higher in the cell, molecules will move out into the environment. Facilitated diffusion is much more prominent in eukaryotic cells where it is used to transport a variety of sugars and amino acids.

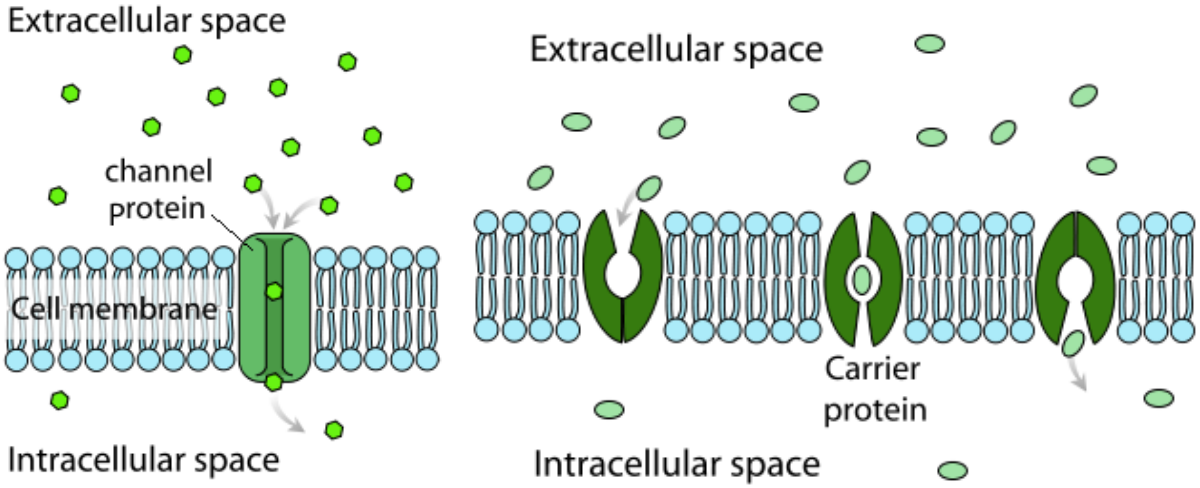



Figure 11: Facilitated diffusion involves the use of a protein to transport molecules across the cell membrane.

**Active transport:** is carried out in at least three different ways in bacteria. These include group translocation; membrane bound transport systems and bidding protein transport systems. **- Group translocation:** is a type of active transport because metabolic energy is used during uptake of the molecule whereby molecules are chemically altered while being transported into the cell (figure 12). Used by prokaryotes, not eukaryotes**.** Best example – phosphoenolpyruvate : sugar transferase system (PTS).
PEP + sugar (outside) → pyruvate + sugar- phosphate (inside)

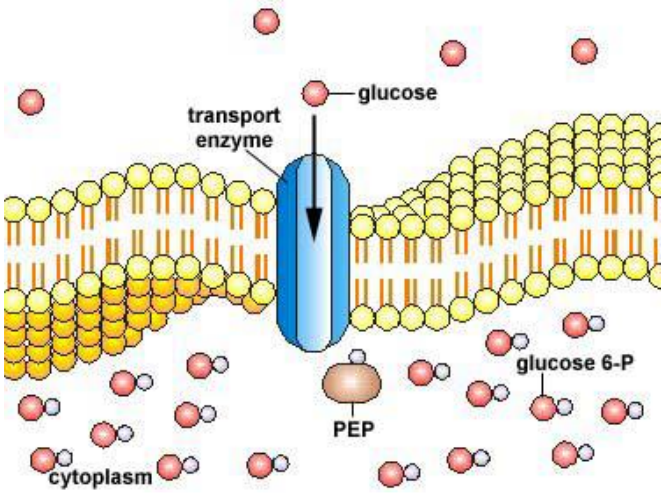



Figure 12: group translocation.

**- Membrane Bound Transport Systems**: In this type of transport system all the proteins required for transport are firmly bound to the cell membranes. In E. coli about 40% of the transport systems belong to this class, which includes transport systems for sugars, ions and several amino acids.

**- Binding Protein Transport System**: The bacterial has special transport problems arising from the presence of the cell wall and of the fluid filled periplasmic space between the inner and outer membranes of the cell. Binding proteins are present in the periplasmic space, and perhaps function in carrying the solute across the space. The solute is then donated to another transport receptor site at the surface of the inner membrane (plasma membrane).Binding protein transport systems utilize ATP or a related compound.

**Iron uptake:** Iron uptake is made difficult by extreme insolubility of ferric iron ($Fe^{3+}$) and its derivatives, which leaves little free iron available for transport many bacteria and fungi have overcome this difficulty by secreting siderophores. Siderophores are low molecular weight organic molecules that are able to complex with ferric iron and supply it to the cell. Microorganisms have developed specialized transport system for siderophores, once transported in molecule releases the iron ion which is reduced to its ferrous form ($Fe^{2+}$). Siderophore is recycled.

**Endocytosis:** is used to bring materials into the cell from the outside, the cell takes up solutes or particles by enclosing them in vesicles pinched off from the plasma membrane. In most cases the materials are delivered to a lysosome where they are digested Figure 13.

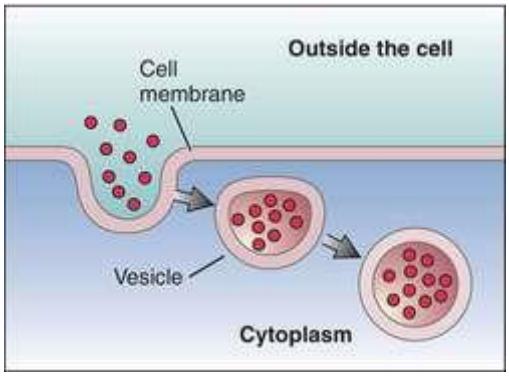



Figure 13: Endocytosis

## Microbial Cultivation:

Cultivation is the process of propagating organisms by providing the proper environmental conditions. The artificial culture of any organism requires a supply of the necessary nutrients, together with appropriate conditions such as temperature, pH and oxygen concentration. The nutrients and conditions provided in the laboratory are usually a reflection of those found in the organism's natural habitat.

**Cultivation Methods**:

Two problems will be considered: 1) the choice of a suitable medium and 2) the isolation of a bacterial organism in pure culture. The technique used and the type of medium selected depend upon the nature of the investigation. In general, three situations may be encountered: (1) One may need to raise a growth of cells of a particular species that is on hand; (2) one may need to determine the numbers and types of organisms present in a given material; or

(3) one may wish to isolate a particular type of microorganism from a natural source.

Culture media: is a solid or liquid preparation used to grow, transport, and store microorganisms. Must be containing the essential components necessary for microbial growth as well as be useful in the identification and characterization of microorganisms. Culture media can be classified on the basis of several parameters: the chemical constitution from which they are made, their physical nature, and their function (table 6)

Table 6: Types of Media

| Physical nature | Function type |
|---|---|
| Liquid Defined (synthetic) | Supportive (General purpose) |
| Semisolid | Complex Enriched |
| Solid | Selective Differential |

**Two types of general purpose culture media:**

- Synthetic (defined): all the components of the medium are known and well defined, e.g., glucose –salts broth.
- Complex: one or more of the components are comprised of a chemically unknown substance, e.g., tryptic soy broth.

**Two types of enriched culture media:**

- Selective: contain substances that favor the growth of one particular microbe over another.
- Differential: contain substances that distinguish between different groups of microbes.

Some enriched media can be both selective and differential, e.g., MacConkey's agar.

**Isolation of Microorganisms in Pure Culture**

In order to study the properties of a given organism, it is necessary to handle it in pure culture free of all other types of organisms. To do this, a single cell must be isolated from all other cells and cultivated in such a manner that its collective progeny also remain isolated. Several methods are available.

1) Plating: Unlike cells in a liquid medium, cell in or on a gelled medium are immobilized. Therefore, if few enough cells are placed in or on a gelled medium, each cell will grow into an isolated colony.

2) Dilution: A much less reliable method. The suspension is serially diluted, and samples of each dilution are plated. If only a few samples of a particular dilution exhibit growth, it is presumed that some of the colonies started from single cells.

## Preservation of microbial cultures

Microbial cultures are preserved by storage at low temperatures, in order to suspend growth processes. For short periods, most organisms can be kept at refrigerator temperature (around 4 ◦C), but for longer-term storage, more specialized treatment is necessary. Using deep freezing or freeze-drying, cultures can be kept for many years, and then bring to life again and recultured. Deep freezing requires rapid freezing to −70 ◦C to −95 ◦C, while freeze-drying (lyophilization) involves freezing at slightly less extreme temperatures and removing the water content under vacuum. Long-term storage may be desirable to avoid the development of mutations or loss of cell viability.

### Microbial growth

Growth is the orderly increase in the sum of all the components of an organism. Thus the increase in size that results when a cell takes up water or lipid or polysaccharide is not true growth. Cell multiplication is a consequence of growth; in unicellular organisms, growth leads to an increase in the number of individuals making up a population or culture.

Growth is defined as an increase in cellular constituents and may result in an increase in a microorganism's size, population number, or both.

Growth of a cell is the culmination of an ordered interplay among all of the physiological activities of the cell. It is a complex process involving

1. Entrance of basic nutrients into the cell
2. Conversion of these compounds into energy and vital cell constituents
3. Replication of the chromosome
4. Increase in size and mass of the cell
5. Division of the cell into two daughter cells, each containing a copy of the genome and other vital components.

Bacterial growth is the division of one bacterium into two daughter cells in a process called binary fission. Providing no mutational event occurs the

resulting daughter cells are genetically identical to the original cell. Hence, "local doubling" of the bacterial population occurs.

**The prokaryotic cell cycle (binary fission):**

The cell cycle is complete sequence of events extending from the formation of a new cell through the next division. Most prokaryotes reproduce by binary fission (figure 14), although some prokaryotes reproduce by budding, fragmentation, and other means. Binary fission is a relatively simple type of cell division: the cell elongates, replicates its chromosome, and separates the newly formed DNA molecules so there is one chromosome in each half of the cell. Finally, a septum (or cross wall) is formed at midcell, dividing the parent cell into two progeny cell, each having its own chromosome and a complement of other cellular constituents. Two pathways function during the cell cycle: one pathway replicates and partitions the DNA into the progeny cells, the other carries out cytokinesis (septum formation and formation of progeny cells).

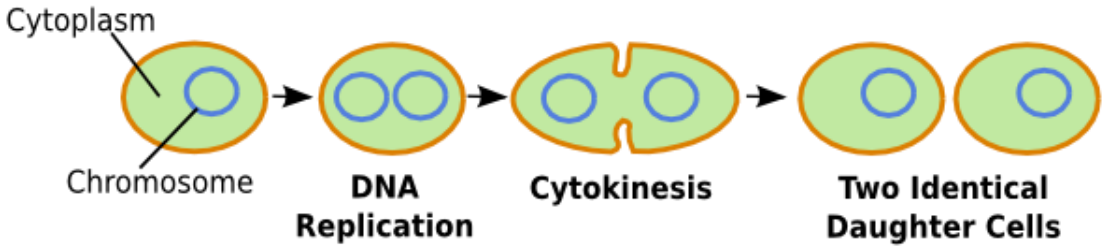
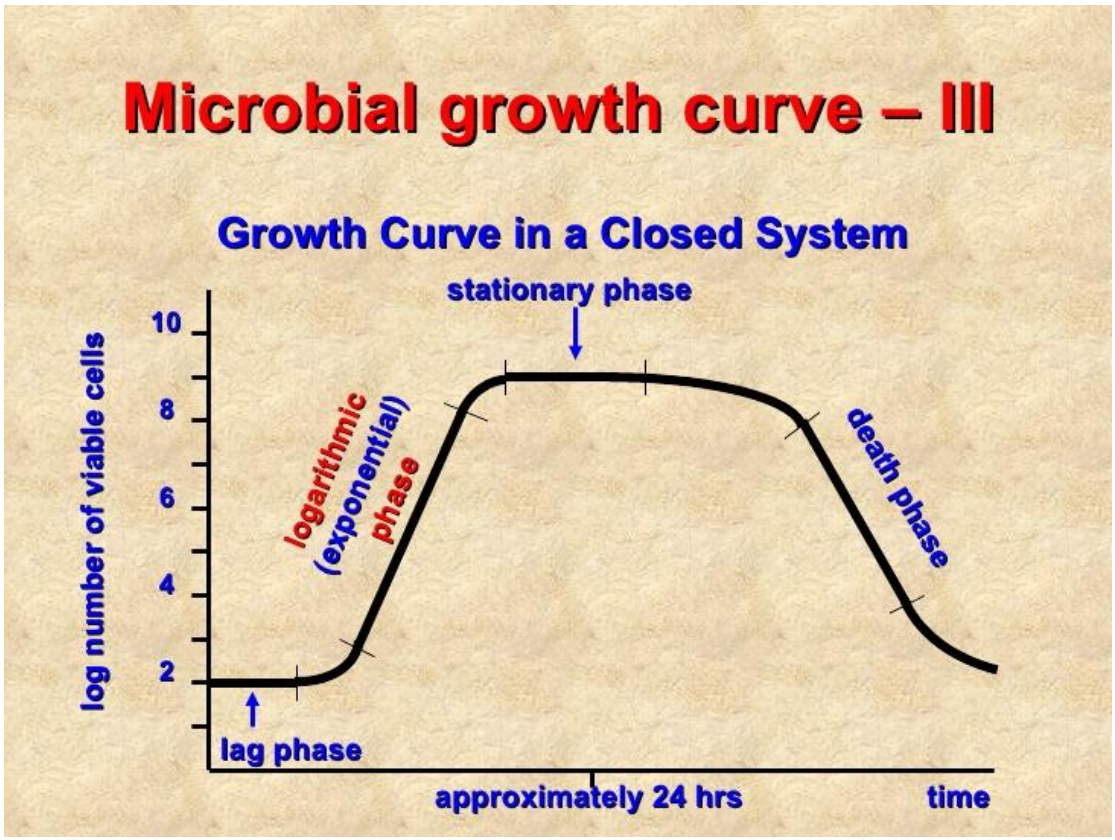



Figure 14: binary fission.

**The Growth curve**:

Binary fission and other cell division processes bring about an increase in the number of cells in a population. Population growth is studied by analyzing the growth curve of a microbial culture. When microorganisms are cultivated in liquid medium, they usually are grown in a batch culture or closed system- that is, they are incubated in a closed culture vessel with a single batch of medium. Because no fresh medium is provided during incubation, nutrient concentrations decline and concentrations of wastes increase. The growth of microorganisms reproducing by binary fission can be plotted as the logarithm of the number of viable cells versus the incubation time. The resulting curve has four distinct phases (Figure 15).



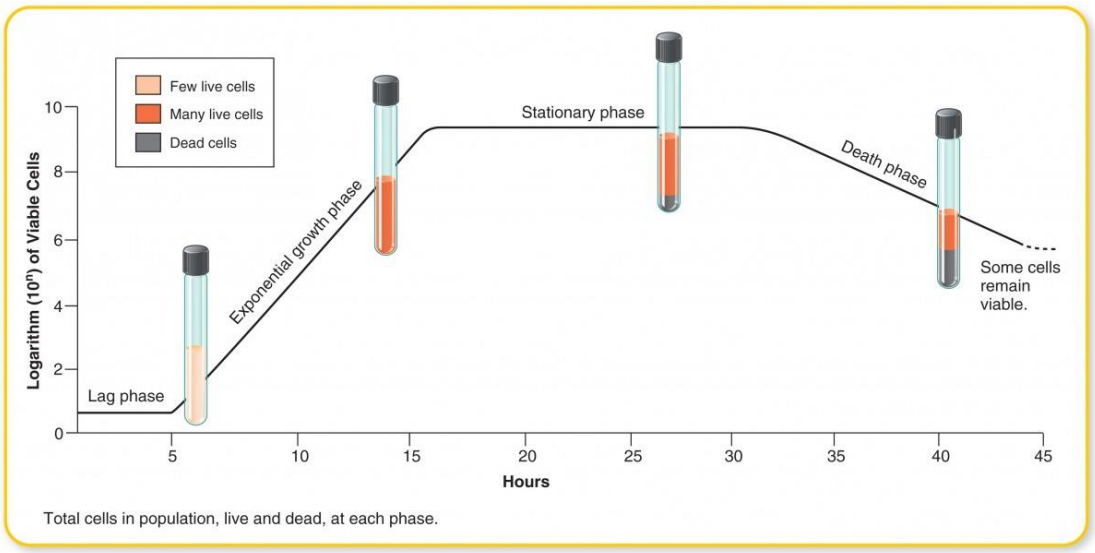




Figure 15: Microbial Growth Curve in closed system.

**Lag phase**: When microorganisms are introduced into fresh culture medium, usually no immediate increase in cell number occurs, so this period is called the lag phase. Although cell division does not take place right away and there is no net increase in mass, the cell is synthesizing new components. A lag phase prior to the start of cell division can be necessary for a variety of reasons. The cell May be old and depleted of ATP, essential cofactors and ribosomes; these must be synthesized before growth can begin. The medium may be different from the one the microorganism was growing in previously.

Here new enzymes would be needed to use different nutrients. Possibly the microorganisms have been injured and require time to recover. Whatever the causes, eventually the cells retool, replicate their DNA, begin to increase in mass, and finally divide.

The lag phase varies considerably in length with the condition of the microorganisms and the nature of the medium. This phase may be quite long if the inoculum is from an old culture or one that has been refrigerated. Inoculation of a culture into a chemically different medium also results in longer lag phase. On the other hand, when a young, vigorously growing exponential phase culture is transferred to fresh medium of the same composition, the lag phase will be short or absent.

**Exponential phase:** During the exponential or log phase, microorganisms are growing and dividing at the maximal rate possible given their genetic potential, the nature of the medium, and the conditions under which they are growing. Their rate of growth is constant during the exponential phase; that is, the microorganisms are dividing and doubling in number at regular intervals. Because each individual divides at a slightly different moment, the growth curve rises smoothly rather than in discrete jumps. The population is most uniform in terms of chemical and physiological properties during this phase; therefore exponential phase cultures are usually used in biochemical and physiological.

Exponential growth is balanced growth. That is, all cellular constituents are manufactured at constant rates relative to each other. If nutrient levels or other environmental conditions change, unbalanced growth results. This is growth during which the rates of synthesis of cell components vary relative to one another until a new balanced state is reached. Unbalanced growth is readily observed in two types of experiments: shift –up, where a culture is transferred from a nutritionally poor medium to a richer one; and shift-down, where a culture is transferred from a rich medium to a poor one. In a shift –up experiment, there is a lag while the cells first construct new ribosomes to enhance their capacity for protein synthesis. This is followed by increases in protein and DNA synthesis. Finally, the expected rise in reproductive rate takes place. In a shift-down experiment, there is a lag in growth because cells need time to make the enzyme required for the biosynthesis of unavailable nutrients. Consequently cell division and DNA replication continue after the shift –down, but net protein and RNA synthesis slow. The cells become smaller and reorganize themselves metabolically until they are able to grow again. Then balanced growth is resumed and the culture enters the exponential phase. These shifts-up and shift-down experiments demonstrate that microbial growth is under precise, coordinated control and responds quickly to changes in environmental conditions.

The microbial growth is limited by the low concentration of a required nutrient. The rate of growth also increases with nutrient concentration. At sufficiently high nutrient levels the transport systems are saturated, and the growth rate does not rise further with increasing nutrient concentration.

**Stationary phase**: Because this is a closed system, eventually population growth stopped and growth curve becomes horizontal this stationary phase. Usually is attained by bacteria at a population level of around $10^9$ cells per ml. Other microorganisms normally do not reach such high population densities; protozoan and algal cultures often have maximum concentrations of about $10^6$ cells per ml. Of course final population size depends on nutrient availability and other factors, as well as the type of microorganism being cultured. In the stationary phase the total number of viable microorganisms remains constant. This may result from a balance between cell division and cell death, or the population may simply stop to divide but remain metabolically active.

Microbial populations enter the stationary phase for several reasons. One factor is nutrient limitation; if an essential nutrient is depleted, population growth will slow. Aerobic organisms often are limited by $O_2$ availability. Oxygen is not very soluble and may be depleted so quickly that only the surface of a culture will have an $O_2$ concentration adequate for growth. The cells beneath the surface will not be able to grow unless the culture is shaken or aerated in another way. Population growth also may stop due to the accumulation of toxic waste products. This factor seems to limit the growth of many anaerobic cultures (cultures growing in the absence of $O_2$). For example, streptococci can produce so much lactic acid and other organic acids from sugar fermentation that their medium becomes acidic and inhibited. Streptococcal cultures also can enter the stationary phase due to depletion of their sugar supply. Finally, there is some evidence that growth may stop when a critical population level is reached.

**Senescence and death:** For many years, the decline in viable cells following stationary cells was described simply as the death phase. It was assumed that detrimental environmental changes like nutrient lack and the buildup of toxic wastes caused permanent harm resulting in loss of viability. That is even when bacterial cells were transferred to fresh medium, no cellular growth was observed. Because loss of viability was often not accompanied by a loss in total cell number, it was assumed that cells died but not lyse. This view is currently under discussed. There are two alternative hypotheses. Some microbiologist believes starving cells that show an exponential decline in density have not irreversibly lost their ability to reproduce. Rather, they suggest that microbes are temporarily unable to grow, at least under the laboratory conditions used. This phenomenon, which the cells are called viable but non culturable (VBNC), is thought to be the result of a genetic

response triggered in starving, stationary phase cells. Just as some bacteria form spores as a survival mechanism, but others are able to become dormant without changes in morphology. Once the appropriate conditions are available, VBNC microbes resume growth. VBNC microorganisms could cause a public health threat, as many assays that test for food and drinking water safety are culture-based.

The second alternative to a simple death phase programmed cell death. In contrast to the VBNC hypothesis whereby cells are genetically programmed to survive, programmed cell death predicts that a fraction of the microbial population is genetically programmed to commit suicide. In this case, non culturable cells are dead and the nutrients that they leak enable the eventual growth of those cells in the population that did not initiate suicide. The dying cells are sacrifice themselves for the benefit of the larger population.

**Phase of prolonged decline:**

Long-term growth experiments reveal that an exponential decline in viability is sometimes replaced by a gradual decline in the number of culturable cells. This decline can last months to years. During this time the bacterial population continually evolves so that actively reproducing cells are those best able to use the nutrients released by their dying brethren and best able to tolerate the accumulated toxins. This dynamic process is marked by successive waves of genetically distinct variants. Thus natural selection can be observed within a single culture vessel (figure 16).

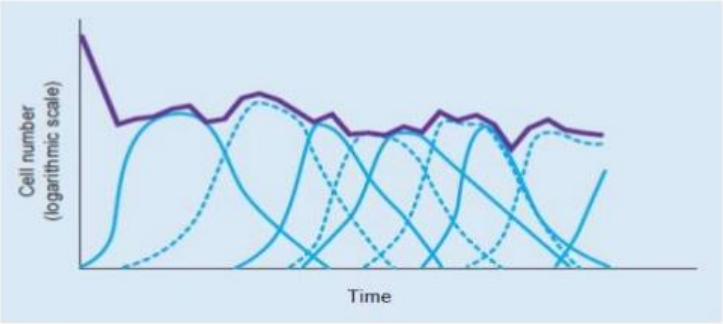



Figure 16: prolonged Decline in Growth.

**The continuous Culture of microorganisms**:

In an open system, it is possible to grow microorganisms, a system with constant environmental conditions maintained through continual provision of nutrients and removal of wastes. The conditions are met in the laboratory by a continuous culture system. A microbial population can be maintained in the exponential growth phase and at a constant biomass concentration for extended periods in a continuous culture system. Two major types of continuous culture systems commonly are used:

1) chemostats

2) turbidostats

**The chemostat**: is constructed so that sterile medium is fed into the culture vessel at the same rate as media containing microorganisms is removed (figure 17.) The culture medium for a chemostat possesses an essential nutrient (e.g., amino acid) in limiting quantities. Because one nutrient is limiting, the growth rate is determined by the rate at which new medium is fed into the growth chamber, and the final cell density depends on the concentration of the limiting nutrients.

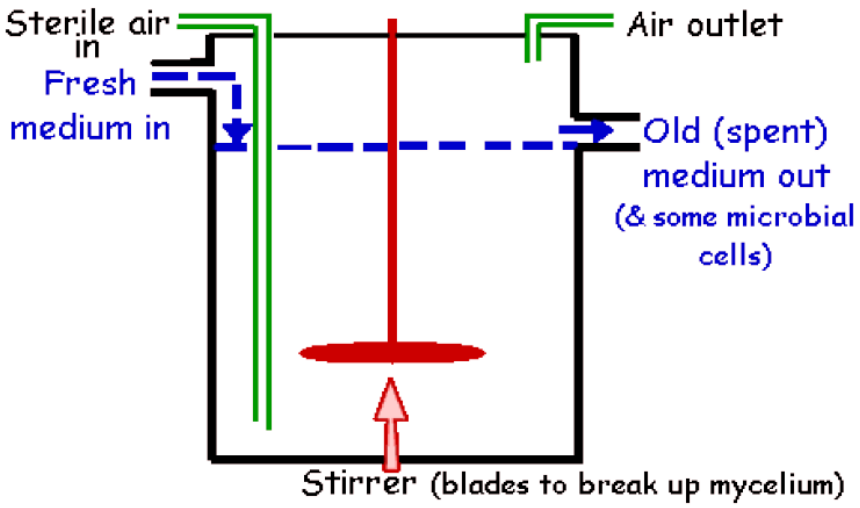



Figure 17: chemostat continuous culture.

**The turbidostats**: has a photocell that measures the absorbance or turbidity of the culture in the growth vessel. The flow rate of media through the vessel is automatically regulated to maintain a predetermined turbidity or cell density. The turbidostat differs from the chemostat in several ways. The dilution rate in a turbidostat varies rather than remaining constant, and its culture medium contains all nutrients in excess. That is, none of the nutrients is limiting. The turbidostats operates best at high dilution rated; the chemostat is most stable and effective at lower dilution rated.

Continuous culture systems are very useful because they provide a constant supply of cells in exponential phase and growing at a known rate. They make possible the study of microbial growth at very low nutrient levels, concentrations close to those present in natural environments. These systems are essential for research in many areas- for example, in studies on interactions between microbial species under environmental conditions resembling those in freshwater lake or pond. Continuous systems also are used in food and industrial microbiology.

**Environmental Factors Affecting Growth**:

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as pH, temperature, and aeration must be carefully controlled. The microbial growth also is greatly affected by the chemical and physical nature of their surroundings. An understanding of environmental effects aids in the control of microbial growth and the study of the ecological distribution of microorganisms.

**1) Nutrients:** All microorganisms require the following nutrients to grow, repair themselves, and to replicate:

(a) Carbon, Nitrogen, Sulfur, Phosphorus, and other Macro mineral nutrients.

(b) Various trace elements

(c) In addition, some microorganisms require various vitamins as well as additional growth factors (e.g., specific amino acids)

(d) "Although we are concerned with ways microorganisms satisfy their own nutritional needs, we can note that in satisfying such needs, they also help recycle elements in the environment."

(e) Fastidious Microorganisms: microorganisms whose nutritional needs are unusually complex are termed fastidious.

**2) Hydrogen Ion Concentration (pH):** Most organisms have a fairly narrow optimal pH range. The optimal pH must be experimentally determined for each species. Most organisms (Neutrophiles) grow best at a pH of 6.0–8.0, although some kinds (Acidophiles ) have optima as low as pH 3.0 and others (Alkaliphiles) have optima as high as pH 10.5.

Microorganisms regulate their internal pH over a wide range of external pH values by pumping protons in or out of their cells. Acidophiles maintain an internal pH of about 6.5 over an external range of 1.0–5.0; neutralophiles maintain an internal pH of about 7.5 over an external range of 5.5–8.5; and alkaliphiles maintain an internal pH of about 9.5 over an external range of 9.0–11.0. Internal pH is regulated by a set of proton transport systems in the cytoplasmic membrane, including a primary, ATP-driven proton pump and a Na+/H+ exchanger. A K+/H+ exchange system has also been proposed to contribute to internal pH regulation in neutralophiles.

**3) Temperature:** Different microbial species vary widely in their optimal temperature ranges for growth: Psychrophilic forms grow best at low temperatures (15–20 °C); mesophilic forms grow best at 30–37 °C; and most thermophilic forms grow best at 50–60 °C. Some organisms are hyperthermophilic and can grow at well above the temperature of boiling

water, which exists under high pressure in the depths of the ocean. Most organisms are mesophilic; 30 °C is optimal for many free-living forms, and the body temperature of the host is optimal for warm-blooded animals (Figure 18).

Microorganisms share with plants and animals the heat-shock response, a synthesis of a set of "heat-shock proteins," when exposed to a sudden rise in temperature above the growth optimum. These proteins appear to be unusually heat-resistant and to stabilize the heat-sensitive proteins of the cell.

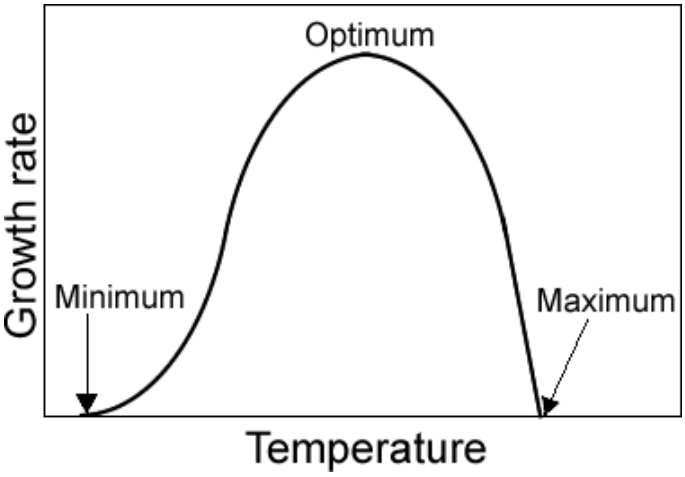



Figure 18: Temperature and growth

**4) Aeration:** Many organisms are obligate aerobes, specifically requiring oxygen as hydrogen acceptor; some are facultative, able to live aerobically or anaerobically; and others are obligate anaerobes, requiring a substance other than oxygen as hydrogen acceptor and being sensitive to oxygen inhibition.

The supply of air to cultures of aerobes is a major technical problem. Vessels are usually shaken mechanically to introduce oxygen into the medium, or air is forced through the medium by pressure. The diffusion of oxygen often becomes the limiting factor in growing aerobic bacteria; when a cell concentration of 4–5 x $10^9$/mL is reached, the rate of diffusion of oxygen to the cells sharply limits the rate of further growth.

Obligate anaerobes, on the other hand, present the problem of oxygen exclusion. Many methods are available for this: Reducing agents such as sodium thioglycolate can be added to liquid cultures; the culture vessel can be placed in a container from which the oxygen is removed by evacuation or by chemical means; or the organism can be handled within an anaerobic glove-box.

**5) Ionic Strength & Osmotic Pressure:** To a lesser extent, such factors as osmotic pressure and salt concentration may have to be controlled. For most organisms, the properties of ordinary media are satisfactory; however, for marine forms and organisms adapted to growth in strong sugar solutions, for example, these factors must be considered. Organisms requiring high salt concentrations are called halophilic; those requiring high osmotic pressures are called osmophilic.

Most bacteria are able to tolerate a wide range of external osmotic pressures and ionic strengths because of their ability to regulate internal osmolality and ion concentration. Osmolality is regulated by the active transport of K+ ions into the cell; internal ionic strength is kept constant.

**6) Light:** Phototrophic organisms require light in order to carry out photosynthesis. In the laboratory, care must be taken that light of the correct wavelength is used, and that the source used does not also act as a heat source. Fluorescent light produces little heat, but does not provide the wavelengths in excess of 750 nm needed by purple and green photosynthetic bacteria.

## Microbial Adaptation

Adaptation in biology; any feature in the structure or function of an organism that allows it to survive and reproduce more effectively in its environment. Much adaptation is inherited and is the result of many thousands of years of evolution. It is thought to occur as a result of random variation in the genetic make-up of organisms coupled with natural selection. Species become extinct when they are no longer adapted to their environment.

Adaptation is the process whereby a population becomes better suited to its habitat or better able to live in its habitats. This process takes place over many generations, and is one of the basic phenomena of biology. Also, the term **adaptation** may refer to a feature which is especially important for an organism's survival. For example, the adaptation of horses' teeth to the grinding of grass, or their ability to run fast and escape predators. Such adaptations are produced in a variable population by the better suited forms reproducing more successfully, that is, by natural selection.

Adaptedness is the state of being adapted: the degree to which an organism is able to live and reproduce in a given set of habitats. An adaptive trait is an aspect of the developmental pattern of the organism which enables or enhances the probability of that organism surviving and reproducing.

### Results of adaptation

Adaptation produces individuals whose genetically determined characteristics allow them to survive and reproduce more effectively. Thus, the *Flavobacterium* species that have evolved to eat nylon. Before nylon existed in their environment there would have been no advantage to the ability to digest it and turn it into useful chemical energy. Once it appeared in large amounts a single bacterium with a mutation for nylon metabolism living in a pool of nylon reproduced like mad, passing the gene on.

## General principles of adaptation

The significance of an adaptation can only be understood in relation to the total biology of the species.

Adaptation is, first of all, a process, rather than a physical part of a body. The distinction may be seen in an internal parasite (such as a fluke), where the bodily structure is greatly simplified, but nevertheless the organism is highly adapted to its unusual environment. From this we see that adaptation is not just a matter of visible traits: in such parasites critical adaptations take place in the life-cycle, which is often quite complex. Many aspects of an animal or plant can be correctly called adaptations, though there are always some features whose function is in doubt.

Another great principle is that **an organism must be viable at all stages of its development and at all stages of its evolution**. This is obviously true, and it follows that there are constraints on the evolution of development, behavior and structure of organisms. The main constraint is the requirement that changes in the system during evolution should be relatively small changes, because the body systems are so complex and interlinked.

All adaptations help organisms survive in their ecological. These **adaptative traits** may be structural, behavioral or physiological.

- **Structural adaptations** are physical features of an organism (shape, body covering, defensive or offensive armament; and also the internal organization).

- **Behavioral adaptations** are composed of inherited behavior chains and/or the ability to learn: behaviors may be inherited in detail, or a tendency for learning may be inherited. Examples: searching for food, mating.

- **Physiological adaptations** permit the organism to perform special functions (making venom, phototropism); but also more general functions such as growth and development, temperature regulation, ionic balance and other aspects of homeostasis. Adaptation, then, affects all aspects of the life of an organism.

Physiological adaption is a metabolic or physiologic adjustment within the cell, or tissues, of an organism in response to an environmental stimulus resulting in the improved ability of that organism to cope with its changing environment. Unlike evolutionary adaptation which involves transgenerational adjustment, physiological adaptation is generally narrow in scope and involves response of an individual to a range of stimuli. e.g. the ability of certain organisms to absorb nutrients under low oxygen tensions.

## Types of adaptation

### 1) Changes in habitat

Before Darwin, adaptation was seen as a fixed relationship between an organism and its habitat. It was not appreciated that as the climate changed, and as the habitat changed, so did the organisms. Also, habitats are subject to changes in their organisms: for example, invasions of species from other areas. The relative numbers of species in a given habitat are always changing. Change is the rule, though much depends on the speed and degree of the change.

When the habitat changes, three main things may happen to a resident population: habitat tracking, genetic change or extinction. In fact, all three things may occur in sequence. Of these three effects, only genetic change brings about adaptation.

- Habitat tracking

When a habitat changes, the most common thing to happen is that the resident population moves to another locale which suits it; this is the typical response of flying insects or oceanic organisms, who have wide (though not unlimited) opportunity for movement. This common response is called habitat tracking. It is one explanation put forward for the periods of apparent stasis in the fossil record.

- Genetic change

Genetic change is what occurs in a population when natural selection acts on the genetic variability of the population. By this means, the population adapts genetically to its circumstances. Genetic changes may result in visible structures, or may adjust physiological activity in a way that suits the changed habitat.

It is now clear that habitats and biota do frequently change. Therefore, it follows that the process of adaptation is never finally complete. Over time, it may happen that the environment changes little, and the species comes to fit its surroundings better and better. On the other hand, it may happen that changes in the environment occur relatively rapidly, and then the species becomes less and less well adapted. Seen like this, adaptation is a genetic tracking process, which goes on all the time to some extent, but especially when the population cannot or does not move to another, less hostile area. Also this process affects every species in a particular ecosystem.

### 2) Intimate relationships: co-adaptations

In co-evolution, where the existence of one species is tightly bound up with the life of another species, new or 'improved' adaptations which occur in one species are often followed by the appearance and spread of corresponding features in the other species. There are many examples of this; the idea emphasizes that the life and death of living things is intimately connected, not just with the physical environment, but with the life of other species. These relationships may continue on this way for millions of years, as has the relationship between flowering plants and insects (pollination).

### 3) Internal adaptations

There are some important adaptations to do with the overall coordination of the systems in the body. Such adaptations may have significant consequences. Examples, in vertebrates, would be temperature regulation, or improvements in brain function, or an effective immune system. An example in plants would be the development of the reproductive system in flowering plants.

## Bacterial adaptation

Bacteria have been designed to be adaptable. Their **surrounding layers** and the **genetic information** for these and other structures associated with a bacterium are capable of alteration. Some alterations are reversible, disappearing when the particular pressure is lifted. Other alterations are maintained and can even be passed on to succeeding generations of bacteria.

The adaptation of bacteria to an antibacterial agent such as an antibiotic can occur in two ways. The first method is known as **inherent (or natural) resistance**. Gram-negative bacteria are often naturally resistant to penicillin, for example. This is because these bacteria have another outer membrane, which makes the penetration of penicillin to its target more difficult. Sometimes when bacteria acquire resistance to an antibacterial agent, the cause is a membrane alteration that has made the passage of the molecule into the cell more difficult.

The second category of adaptive resistance is called **acquired resistance**. This resistance is almost always due to a change in the genetic make-up of the bacterial genome. Acquired resistance can occur because of mutation or as a response by the bacteria to the selective pressure imposed by the antibacterial agent. Once the genetic alteration that confers resistance is present, it can be passed on to subsequent generations. Acquired adaptation and resistance of bacteria to some clinically important antibiotics has become a great problem in the last decade of the twentieth century.

*Bacteria adapt to other environmental conditions as well. These include adaptations to changes in temperature, pH, concentrations of ions such as sodium, and the nature of the surrounding. An example of the latter is the response shown by *Vibrio parahaemolyticus* to growth in a watery environment versus a more viscous environment. In the more viscous setting, the bacteria adapt by forming what are called swarmer cells. These cells adopt a different means of movement, which is more efficient for moving over a more solid surface. This adaptation is under tight genetic control, involving the expression of multiple genes.

*Bacteria react to a sudden change in their environment by expressing or repressing the expression of a whole loss of genes. This response changes the properties of both the interior of the organism and its surface chemistry. A well-known example of this adaptation is the so-**called heat shock response** of *Escherichia coli*. The name derives from the fact that the response was first observed in bacteria suddenly shifted to a higher growth temperature.

*Another adaptation exhibited by *Vibrio parahaemolyticus*, and a great many other bacteria as well, is the formation of adherent populations on solid surfaces. This mode of growth is called a biofilm. Adoption of a biofilm mode of growth induces a many of changes, many involving the expression of previously unexpressed genes. In addition, de-activation of actively expressing genes can occur. Furthermore, the pattern of gene expression may not be uniform throughout the biofilm. Bacteria within a biofilm and bacteria found in other places, such as in a wound where oxygen is limited, grow and divide at a far slower speed than the bacteria found in the test tube in the laboratory. Such bacteria are able to adapt to the slower growth rate, once again by changing their chemistry and gene expression pattern.

## Control of Microorganisms Growth

• **Important Terms:**

1. **Viability** – ability to reproduce
2. **Sterilization** – absolute elimination of all microbes, any process that destroys or removes all infectious organisms including endospores and viruses.
3. **Disinfection** – destroys most microbes, not spores or some viruses. Any physical process or application of any chemical that will kill the growing microbial cells. These processes need not kill or inactivate endospores.

A **disinfectant** is a chemical capable of killing microbial cells. It should be understood that if a chemical is referred to as a disinfectant, it is to be used on inanimate objects and not to be used on body surfaces.

4. **Antisepsis (antiseptic**) – disinfection of living tissue. Those practices that keep microorganism from entering the sterile tissues. The application of these practices is referred to as aseptic technique. **Antiseptics** are those chemicals that can be applied to tissue surfaces to kill or inhibit the growth of microorganisms.

5. **Germicide** – **Microbicidal agents** are chemicals that will kill or destroy microorganisms. Among the microbicidal agents are those that target specific microorganisms including:

**a. fungicidal** agents which are designed to kill fungi.
**b**. **bactericidal** agents which are designed to kill bacteria.
**c. sporicidal** agents which are designed to destroy endospores.
**d. viricidal** agents which are designed to destroy viruses.

6. **Antibiotic** – substance that kills or reduces viability of microbes inside or on the Surface of the body.

7. **Microbiostasis** refers to the inhibition of growth of microorganisms. This does not mean that the organisms are killed simply but they are unable to grow. Refrigeration and many antimicrobial drugs exert a microbistatic effect.
a. **Bacteriostatic** agents are chemicals that inhibit the growth of bacteria.
b. **Fungistatic** agents are chemicals that inhibit growth of fungi.

8. **Aseptic** – exclude entry of microbe.

9. **Degerming** – removal of transient microbes from an area, mechanical removal.

10. **Sanitization** – reduce the number of microbes, not total elimination, any mechanical process (scrubbing, rinsing, etc.) that reduces the microbial load on a surface.

**Sanitizers** are chemical agents that assist in this task. These are usually soaps or detergents.

12. **Chemotherapy**- use of chemical agents to kill or inhibit the growth of microorganisms within the host tissue.

## Control of Microorganisms by physical and chemical agents

Although most microorganisms are beneficial and necessary for human well-being, microbial activities may have undesirable consequences, such as food spoilage and disease. Therefore it is essential to be able to kill a wide variety of microorganisms or inhibit their growth to minimize their destructive effects (figure 19). The goal is twofold:

1- To destroy pathogens and prevent their transmission.

2- To reduce or eliminate microorganisms responsible for the contamination of water, food, and other substances**.**

Microorganisms are not uniformly affected by physical and chemical decontamination. Susceptibility to the effects of physical and chemical agents depends upon **the type of microorganism** and at **what stage in the microorganism's lifecycle they are exposed to the agent**. When choosing and applying a method of decontaminating materials, it is important that you understand what type of organism is being targeted and the relative resistance of that organism.

1. The target with the **highest resistance** is the bacterial endospores. Endospores are ubiquitous in the environment. Many bacteria found in the soil are capable of forming these structures. Introduced into deep wounds or during surgical procedures, these spores can cause severe problems. Thus surgical equipment and other materials used in invasive procedures need to be decontaminated in such a way as to destroy these agents.

2. Targets with the **moderate resistance** include protist cysts, sexual fungal spores, non-enveloped viruses (many enteric viruses including those responsible for polio, Hepatitis A and Hepatitis E), Mycobacterium tuberculosis, Staphylococcus aureus and members of the genus Pseudomonas.

3. Targets with the **least resistance** include vegetative cells of most microbes, enveloped viruses (including those viruses responsible for AIDS and Hepatitis B), and asexual fungal spores.

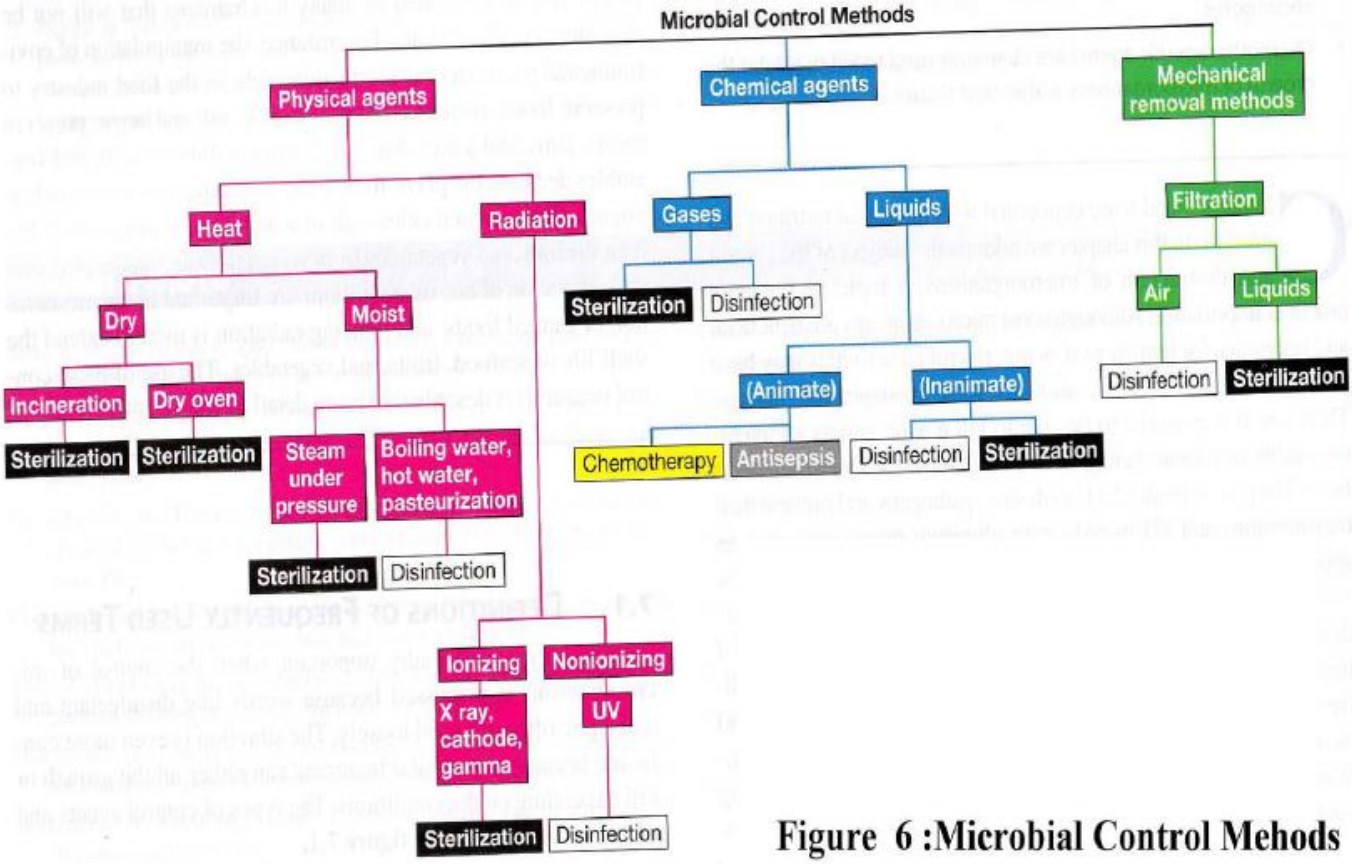



**Figure 6 :Microbial Control Mehods**

## • The pattern of microbial death

A microbial population is not killed instantly when exposed to a lethal agent. Population death, like population growth, is generally exponential or logarithmic that is, the population will be reduced by the same fraction at constant intervals (figure 20). When the population has been greatly reduced, the rate of killing may slow due to the survival of a more resistant strain of the microorganism.

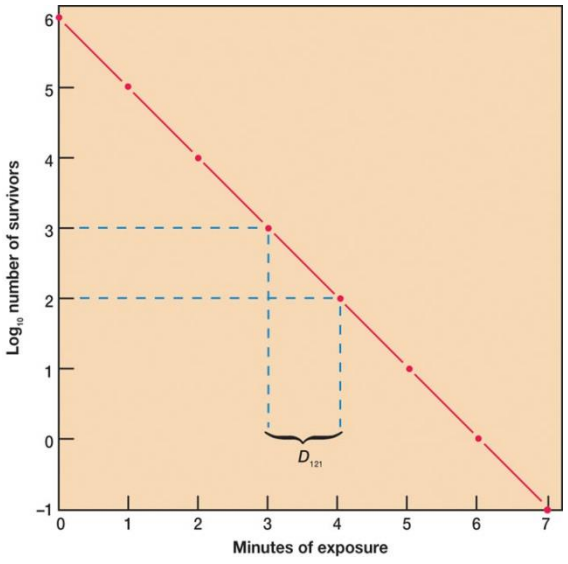



Figure 20: The Pattern of Microbial Death. An exponential plot of the survivors versus the minutes of exposure to heating at 121°C. In this example the $D_{121}$ value is 1 minute.

## • Conditions influencing the effectiveness of antimicrobial agents

Distraction of microorganisms and inhibition of microbial growth are not simple matters because the efficiency of an antimicrobial agent (an agent that kills microbes or inhibits their growth) is affected by at least six factors.

**A. Population size**:

Because an equal fraction of microbial population is killed during each interval, a larger population required a longer time to die than a smaller one. This can be seen in the theoretical heat-killing experiment. Death is logarithmic (Fig. 20)

**B. Population composition:**

The effectiveness of an agent varies greatly with the nature of the organisms being treated because microorganisms differ markedly in susceptibility. Bacterial endospores are much more resistant to most antimicrobial agents than are vegetative forms, and younger cells are usually more readily destroyed than mature organisms. Some species are able to withstand adverse condition better than others. For instance, *Mycobacterium tuberculosis*, which causes tuberculosis, is much more resistant to antimicrobial agents than most other bacteria.

**C. Concentration or intensity of an antimicrobial agent**

Generally the more concentrated chemical agent or intense physical agent, the more rapidly microorganisms are destroyed. However, agent effectiveness usually is not directly related to concentration or intensity. Over a short range a small increase in concentration leads to an exponential rise in effectiveness; beyond a certain point, increase may not raise the killing rate much at all. Sometimes an agent is more effective at lower concentrations. For example, 70% ethanol is more effective than 95% ethanol because its activity is enhanced by the presence of water.

**D. Duration of exposure (contact time):**

The longer a population is exposed to a microbial agent, the more organisms are killed (fig. 20). To achieve sterilization, exposure duration sufficient to reduce the probability of survival to $10^{-6}$ or less should be used.

**E. Temperature:**

An increase in the temperature at which a chemical acts often enhances its activity. Frequently a lower concentration of disinfectant or sterilization agent can be used at higher temperature.

**F. Local environment:**

The population to be controlled is not isolated but surrounded by environmental factors that may either offer protections or aid in its destruction. e.g., because heat kills more readily at an acidic PH, acidic foods and beverages such as fruits and tomatoes are easier to pasteurize than foods

with higher PHs like milk. A second important environmental factor is organic matter, which can protect microorganisms against heating and chemical disinfectants.

**• Use of Physical method in control**

Heat and other physical agents are normally used to control microbial growth and sterilize objects. The most frequently employed physical agents are Heat, low temperature and radiation.

**1-Heat**

Fire and boiling water have been used for sterilization and disinfection. Either moist or dry heat may be applied.

**a. Moist heat** readily kills viruses, bacteria, and fungi (table 7). Moist heat is thought to kill by degrading nucleic acids and by denaturing enzymes and other essential proteins. It may also disrupt cell membranes.

**a1- Boiling water:** Exposure to boiling water for 10 minutes is sufficient to destroy vegetative cells and eukaryotic spores. Unfortunately the temperature of boiling water (100°C or 212°F at sea level) is not high enough to destroy bacteria endospores, which may survive hours of boiling. Therefore boiling can be used for disinfection of drinking water and objects not harmed by water, but boiling does not sterilize.

Table 7: Approximate Conditions for Moist Heat killing.

| **Organism** | **Vegetative Cells** | **Spores** |
|---|---|---|
| **Yeasts** | 5 minutes at 50-60°C | 5 minutes at 70 -80 °C |
| **Molds** | 30 minutes at 62°C | 30 minutes at 80 °C |
| **Bacteria*** | 10 minutes at 60 -70 °C | 2 to over 800 minutes at 100°C |
| **Viruses** | 30 minutes at 60°C | |

Condition for mesophilic bacteria

**a2- Steam sterilization**: In order to destroy bacteria endospores, moist heat sterilization must be carried out at temperatures above 100° C, and this requires the use of saturated steam under pressure is carried out with an autoclave. Water is boiled to produce steam, which is released through the jacket and into the autoclaves chamber. The air initially present in the chamber is forced out until the chamber is filled with saturated steam and the outlets are closed. Hot, saturated steam continues to enter until the chamber reaches the desired temperature and pressure, usually 121°C and 15 pounds of pressure. At this temperature saturated steam destroys all vegetative cells and endospores in a small volume of liquid within 10 to 12 minute. Treatment is

continued for at least 15 minute to provide a margin of safety. Of course, larger containers of liquid such as flasks require much longer treatment times.

Autoclave must be carried out properly or the processed material will not be sterile. If all air has not been flushed out of the chamber, it will not reach 121° C even though it may reach a pressure of 15 pounds. The chamber should not be packed too lightly because the steam needs to circulate freely and contact everything in the autoclave.

**b. Pasteurization**: Many substances, such as milk, are treated with controlled heating at temperature well below boiling, a process known as **pasteurization** in honor of its developer **Louis Pasteur**. In the 1860s the French wine industry was plagues by the problem of wine spoilage, which made wine storage and shipping difficult. Pasteur examined spoiled wine under the microscope and detected microorganisms that looked like the bacteria responsible for lactic acid and acetic acid fermentations. He then discovered that a brief heating at 55 to 60° C would destroy these microorganisms and preserve wine for long periods. In 1886 the German chemists adapted the technique for preserving milk and reducing milk transmissible diseases. Milk pasteurization was introduced into the United States in 1889. Milk, beer, and many other beverages are now pasteurized. Pasteurization does not sterilize a beverage, but it does kill any pathogens present and slows spoilage by reducing the level of nonpathogenic spoilage microorganisms.

**c. Dry heat sterilization:** Many objects are best sterilized in the absence of water by dry heat sterilization. Some items are sterilized by incineration. For instance, inoculating loops, which are used routinely in the laboratory, can be sterilized in a small, bench-top incinerator. Other items are sterilized in an oven at 160 to 170° C for 2 to 3 hours. Microbial death apparently results from the oxidation of cell constituents and denaturation of portions. Dry air heat is less effective than moist heat. The spores of *Clostridium botulinum*, the cause of botulism, are killed in 5 minutes at 121° C by moist heat but only after 2 hours at 160° C with dry heat. However, dry heat has some definite advantages. It does not corrode glassware and metal instruments as moist heat does, and it can be used to sterilize powders, oils, and similar items. Most laboratories sterilize glassware and pipettes with dry heat. Despite these advantages, dry heat sterilization is slow and not suitable for heat sensitive materials like many plastic and under rubber items.

**2- Low Temperature**

Although our emphasis is on the destruction of microorganisms, often the most convenient control technique is to inhibit their growth and reproduction by the use of either freezing or refrigeration. This approach is particularly important in food microbiology.

**a. Freezing** items at -20° C or lower stops microbial growth because of the low temperature and the absence of liquid water. Some microorganisms will be killed by ice crystal disruption of cell membranes, but freezing does not destroy all contaminating microbes. In fact, freezing is a very good method for long –term storage of microbial samples when carried out properly, and many laboratories have a low –temperature freezer for culture storage at -30 or – 70° C. Because frozen food can contain many microorganisms, it should be thawed in a refrigerator and consumed promptly in order to avoid spoilage and pathogen growth.

**b. Refrigeration** greatly slows microbial growth and reproduction, but does not stop it completely, fortunately most pathogens are mesophilic and do not grow well at temperatures around 4 °C. Thus refrigeration is a good technique only for shorter term storage age of food and other items.

## 3- Radiation

Microbiologists take advantage of the effects of ultraviolet and ionizing radiation to sterilize or disinfect.

**a. Ultraviolet (UV)** radiation around 260 nm is quite lethal but does not penetrate glass, dirt films, water, and other substances very effectively. Because of this disadvantage, U**V** radiation is used as a sterilizing agent only in a few specific situations. UV lamps are sometimes placed on the ceilings of rooms or in biological safety cabinets to sterilize the air and any exposed surfaces. Because UV radiation burns the skin and damages eyes, people working in such areas must be certain the UV lamps are off when the areas are in use.

**b. Ionizing radiation** is an excellent sterilizing agent and penetrates deep into objects. It will destroy bacterial endospores and vegetative cells, both prokaryotic and eukaryotic; however, ionizing radiation is not always effective against viruses. Gamma radiation from a cobalt 60 source is used in the cold sterilization of antibiotics, hormones, sutures, and plastic disposable supplies such as syringes. Gamma radiation has also been used to sterilize and pasteurize meat other food. Irradiation can eliminate the threat of such pathogens as *E.coli* O157:h7, *Staphylococcus aureus*, and *Campylobacter jejuni*. Based on the results of numerous studies, both the food and drug administration and the WHO have approved food irradiation and declared it safe. Currently irradiation is being used to treat poultry, beef, pork, veal, lamb, fruits, vegetables, and spices.

• **The use of mechanical methods:**

**1) Filtration:**

Filtration is an excellent way to reduce the microbial population in solutions of heat – sensitive material, and sometimes it can be used to sterilize solutions. Rather than directly destroying contaminating microorganisms, the filter simply removes them.

There are two types of filters:

**a. Depth filters** consist of fibrous or granular materials that have been bonded into a thick layer filled with twisting channels of small diameter. The solution containing microorganisms is sucked through this layer under vacuum, and microbial cells are removed by physical screening or entrapment and also by adsorption to the surface of the filter material.

**b. Membrane filters** have replaced depth filters for many purposes. These circular filters are porous membranes, a little over 0.1 mm thick, made of cellulose acetate, cellulose nitrate, polycarbonate, polyvinylidene fluoride, or other synthetic materials. Although a wide variety of pore sizes are available, membranes with pores about 0.2 μm in diameter are used to remove most vegetative cells, but not viruses, from solutions ranging in volume from 1 ml to many liters. The membranes are held in special holders (figure 21) and are often preceded by depth filters made of glass fibers to remove larger particles that might clog the membrane filter. The solution is pulled or forced through the filter with a vacuum or with pressure from a syringe, collected in previously sterilized containers. Membrane filters remove microorganisms by screening them out much as a sieve separates large sand particles from small ones. These filters are used to sterilize pharmaceuticals, ophthalmic solutions, culture media, oils, antibiotics, and other heat – sensitive solutions.

Air also can be sterilized by filtration. Two common examples are surgical masks and cotton plugs on culture vessels that let air in but keep microorganisms out. Other important examples are laminar flow biological safety cabinets, which employ high – efficiency particulate air (HEPA) filters (a type of depth filter) to remove 99.97% of 0.3 μm particles. Laminar flow biological safety cabinets or hoods force air through HEPA filters, and then project a vertical curtain of sterile air across the cabinet opening. This protects a worker from microorganisms being handled within the cabinet and prevents contamination of the room (figure 22). A person uses these cabinets when working with dangerous agents such as *Mycobacterium tuberculosis* and tumor viruses. They are also employed in research labs and industries, such as the pharmaceutical industry. When a sterile working surface is needed for conducting assays, preparing media, examining tissue cultures, and the like.

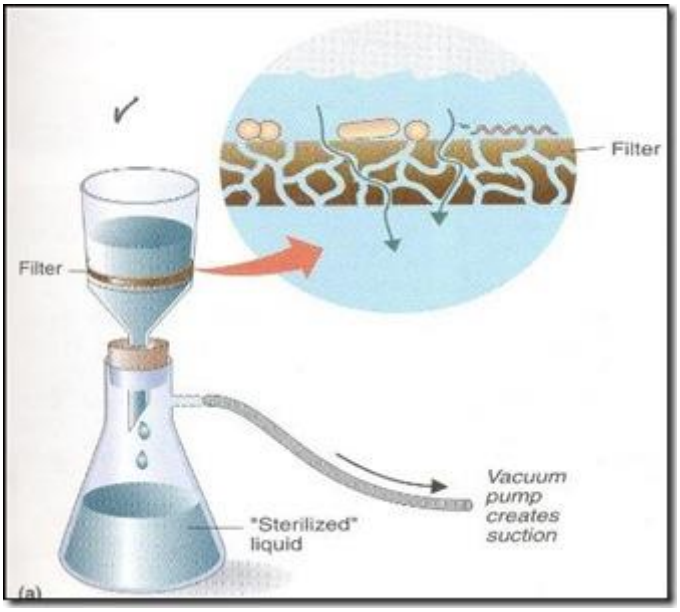



Figure 21: Membrane filter Sterilization. The liquid to be sterilized in pumped through a membrane filter and into sterile container. The inset shows a cross section of the filter and its pores, which are too small for microbes to pass through.

Figure 22: A laminar Flow Biological Safety Cabinet. a) a Technician using potentially hazardous material in a safety cabinet. b) A schematic diagram showing the airflow pattern.

## • The Use of Chemical Agents in Control

Physical agents are generally used to sterilize objects. Chemicals, on the other hand, are more often employed in disinfection and antisepsis. The proper use of chemical agents is essential to laboratory and hospital safety. Chemicals also are employed to prevent microbial growth in food, and certain chemicals are used to treat infectious disease.

Many different chemicals are available for use as disinfectants, and each has its own advantages and disadvantages. In selecting an agent, it is important to keep in mind the characteristics of a desirable disinfectant. **a)** Ideally the disinfectant must be effective against a wide variety of infectious agents (gram-positive and gram – negative bacteria, acid –fast bacteria, bacterial endospores, fungi, and viruses) at low concentrations and in the presence of organic matter. **b)** Although the chemical must be toxic for infectious agents, it should not be toxic to people or corrosive for common materials, in practice, this balance between effectiveness and low toxicity for animals is hard to achieve. Some chemicals are used despite their low effectiveness because they are relatively nontoxic. The ideal **c)** disinfectant should be stable upon storage, **d)** odorless or with a pleasant odor, **e)** soluble in water and lipids for penetration into microorganisms, **f)** have a low surface tension so that it can enter cracks in surfaces, and **g)** be relatively inexpensive.
One potentially serious problem is the overuse of antiseptics. For instance, the antibacterial agent triclosan is found in products such as deodorants, mouthwashes, soaps, cutting boards, and baby toys. Unfortunately, the emergence of triclosan bacteria has become a problem. For example, Pseudomonas actively pumps the antiseptic out of the cell. There is now evidence that extensive use of triclosan also increases the frequency of bacterial resistance to antibiotics. Thus overuse of antiseptics can have unintended harmful consequences.

**Some chemicals used as disinfectants and antiseptics;**
**a. Phenolics**

Phenol was the first widely used antiseptic and disinfectant. In 1867 Joseph Lister employed it to reduce the risk of infection during surgery. Today phenol and phenolics (phenol derivatives) such as cresols, xylenols, and orthophenylphenol are used as disinfectants in laboratories and hospitals. The commercial disinfectant Lysol is made of a mxture of phenolics. Phenolics act by **denaturing proteins and disrupting cell membranes**. They have some real advantages as disinfectants: phenolics are effective in the presence of organic material, and remain active on surfaces long after application. However, they have a disagreeable odor and can cause skin irritation.

## b. Alcohols

Alcohols are among the most widely used disinfectants and antiseptics. They are bacterial and fungicidal but not sporicidal; some liquid- containing viruses are also destroyed. The two most popular alcohol germicides are ethanol and isopropanol, usually used in about 70 to 80 % concentration. They act by **denaturing proteins and possibly by dissolving membrane lipids**. A 10 to 15 minute soaking is sufficient to disinfect thermometers and small instruments.

## c. Halogens

A halogen is any of the five elements (fluorine, chlorine, bromine, iodine, and astatine) in group VIIA (Group 17) of the periodic table. They exist as diatomic molecules in the Free State and form salt like compounds with sodium and most other metals. The halogens iodine and chlorine are important antimicrobial agents. Iodine is used as a skin antiseptic and kills by oxidizing cell constituents and iodinating cell proteins. At higher concentrations, it may even kill some spores. Although it is an effective antiseptic, the skin may be damaged, a stain is left, and iodine allergies can result. More recently iodine has been complexed with an organic carrier to form an iodophor. Iodophors are water soluble, stable, and nonstaining, and release iodine slowly to minimize skin burns and irritation.

Chlorine is the usual disinfectant for municipal water supplies and swimming pools and is also employed in the dairy and food industries. It may be applied as chlorine gas, sodium hypochlorite, or calcium hypochlorite, all of which yield hypochlorous acid (HCIO) and then atomic oxygen. The result is oxidation of cellular materials and destruction of vegetative bacteria and fungi, although not spores.

$$Cl_2 + H_2O \longrightarrow HCl + HClO$$

$$Ca(OCl)_2 + 2H_2O \longrightarrow Ca(OH)_2 + 2HClO$$

$$HClO \longrightarrow HCl + O$$

Death of almost all microorganisms usually occurs within 30 minute. Since organic material interferes with chlorine action by reacting with chlorine and its products, an excess of chlorine is added to ensure microbial destruction. Chlorine is also an excellent disinfectant for individual use because it is effective, inexpensive, and easy to employ. Small quantities of drinking water can be disinfected with halazone tablets. Halazone slowly releases chloride when added to water and disinfects it in about a half hour. It is frequently used by campers lacking access to uncontaminated drinking water.

Chlorine solutions make very effective laboratory and household disinfectants. An excellent disinfectant –detergent combination can be prepared if a 1/40 dilution of household bleach is combined with a nonionic detergent, such as a dishwashing detergent, to give a 0.8% detergent concentration. This mixture will remove both dirt and bacteria.

**d. Heavy Metals**

For many years the ions of heavy metals such as mercury, silver, arsenic, zinc, and copper were used as germicides. These have now been superseded by other less toxic and more effective germicides (many heavy metals are more bacteriostatic than bactericidal). There is little exception. In some hospitals, a 1%solution of silver nitrate is added to the eyes of infants to prevent ophthalmic gonorrhea. Sliver sulfadiazine is used on burns. Copper sulfate is an effective algicide in lakes and swimming pools.
Heavy metals combine with proteins, often with their sulfhydryl groups, and inactivate them. They may also precipitate cell proteins.

**e. Quaternary Ammonium compounds**

They are detergents that have antimicrobial activity and are effective disinfectants. Detergents are organic cleansing agents that are amphipathic, having both polar hydrophilic and nonpolar hydrophobic components. The hydrophilic portion of a quaternary ammonium compound is positively charged quaternary nitrogen; thus quaternary ammonium compounds are cationic detergents. Their antimicrobial activity is the result of their ability to disrupt microbial membranes; they may also denature proteins.
Cationic detergents like benzalkonium chloride and cetypyridinium chloride kill most bacteria but not *M. tuberculosis* or endospores. They have the advantage of being stable and nontoxic but they are inactivated by hard water and soap. Cationic detergents are often used disinfectants for food utensils and small instruments and as skin antiseptics.

**f. Aldehydes**

Both of the commonly used aldehydes, formaldehyde and glutaraldehyde are highly reactive molecules that combine with nucleic acid and proteins and inactivate them, probably by cross- linking and alkylating molecules. They are sporicidal and can be used as chemical sterilants. Formaldehyde is usually dissolved in water or alcohol before use. A 2 % buffered solution of glutaraldehyde is an effective disinfectant. It is less irritating than formaldehyde and is used to disinfectant hospital and laboratory equipment.

Glutaraldehyde usually disinfects objects within about 10 minute but may require as long as 12 hours to destroy all spores.

**g. Sterilizing (by gases)**

Many heat – sensitive items such as disposable plastic petri dishes and syringes, heart – lung machine components, sutures, and catheters are sterilized with ethylene oxide gas. Ethylene oxide (EtO) is both microbicidal and sporicidal and kills by combining with cell proteins. It is a particularly effective sterilizing agent because it rapidly penetrates packing materials, even plastic warps.

Sterilization is carried out in a special ethylene oxide sterilizer, very much resembling an autoclave in appearance, that controls the EtO concentration, temperature, and humidity. Because pure EtO is explosive, it is usually supplied in a 10 to 20 % concentration mixed with either $CO_2$ or dichlorodifluoromethane. The ethylene oxide concentration, humidity, and temperature influence the rate of sterilization. A clean object can be sterilized if treated for 5 to 8 hours at 38 ° C or 3 to 4 hours at 54 ° C when the relative humidity is maintained at 40 to 50 % and the EtO concentration at 700 mg/liter. Extensive aeration of the sterilized materials is necessary to remove residual EtO because it is so toxic.

Betapropiolactone (BPL) is occasionally employed as a sterilizing gas. In the liquid form it has been used to sterilize vaccines and sera. BPL decomposes to an inactive form after several hours. It also destroys microorganisms more readily than ethylene oxide but does not penetrate materials well and may be carcinogenic. For these reasons, BPL has not been used as extensively as EtO.

Vaporized hydrogen peroxide can be used to decontaminate biological safety cabinets, operating rooms, and other large places. These systems introduce vaporized hydrogen peroxide into the enclosure for some time, depending on the size of the enclosure and the materials within. Hydrogen peroxide is toxic and kills a wide variety of microorganisms. However, during the course of decontamination process, it breaks down to water and oxygen, both of which are harmless. Other advantages of these systems are that they can be used at a wide range of temperatures (4 to 80 ° C) and they do not damage most materials.

**• Chemotherapeutic Agents**

The chemical discussed thus far are appropriate for use either on inanimate objects or external host tissue. Chemotherapeutic agents are chemicals that can be used internally to kill or inhibit the growth of microbes within host tissues. They can be used internally because they have selective toxicity; that

is, they target the microbe and do relatively little if any harm to host. Most chemotherapeutic agents are antibiotic – chemicals synthesized by microbes that are effective in controlling the growth of bacteria. Since the discovery of the first antibiotics, pharmaceutical companies have developed numerous derivatives and many synthetic antibiotics. Chemotherapeutic agents for treating diseases caused by fungi, protists, and viruses have also been developed.

## Microbial Bioenergetics

Metabolism is the total of all chemical reaction that occur in cell. Metabolisms are thousands of different reactions; most of them fall into of two general categories:

**Anabolism**, sometimes also termed as biosynthesis, the reactions that consume energy in order to build large molecules and cellular structures from smaller and simpler one, they are **endergonic reactions**. It involve a series of stapes **(1)** conversion of the organism's carbon source into a set of small molecules called **precursor metabolites**; **(2)** synthesis of monomers and other building blocks (i.e., amino acids, nucleotides, simple carbohydrates, and simple lipids) from precursor metabolites; **(3)** synthesis of macromolecules (i.e., proteins, nucleic acids, complex carbohydrates, and complex lipids); and **(4)** assembly of macromolecules into cellular structures.

**Catabolism**, is the opposite or complement of anabolism that involve the breakdown of relatively large complex organic molecules into smaller and simpler molecules and often release energy, they are **exergonic reactions**

Considerable metabolic diversity exists in the microbial world. However, there are several biochemical principles common to all types of metabolism. These are;**(1)** the use of ATP to store energy captured during exergonic reactions so it is can be used to derive endergonic reaction; **(2)** the organization of metabolic reactions into pathways and cycles;**(3)** the catalysis of metabolic reactions by enzymes; and **(4)** the importance of oxidation – reduction reactions in energy conservation.

### Adenosine Triphosphate (Metabolic money)

In what way do cells extract energy from electrons of fuel compounds? To answer this question, we must look more closely to the chief energy carrier and powerhouse molecule **Adenosine triphosphate(ATP)**, which also been described as metabolic money because it can be earned, saved, spend, and exchanged. This molecule provides connection between energy yielding reactions (catabolism) and other cellular activities that require energy. Some

clues to its energy storage behavior lie in its unique molecular structure. The molecular structure of **ATP** first deduced by Lohman in 1930 and confirmed by Alexander Todd *et, al*., in 1948, it is three - part molecule, consist of; nitrogen base (adenine) linked to a 5 carbon sugar (ribose), with a chain of three phosphate groups (**figure 23**). The two terminal phosphoryl groups are highly energized, when **ATP** was utilize in anabolic processes the phosphoanhydride bonds that link these groups together are break down or hydrolyzes almost completely to produce **adenosine diphosphate (ADP), orthophosphate (Pi),** and large amount of energy is released.

**Adenosine PPP + $H_2O$ ⟶ Adenosine PP (ADP)+ Pi + energy (- 7.3 Kcal)**

**Adenosine PP+ $H_2O$ ⟶ Adenosine P (AMP) + Pi + energy (- 7.3Kcal)**

**Adenosine P + $H_2O$ ⟶ Adenosine+ P + energy (-7.3 Kcal)**

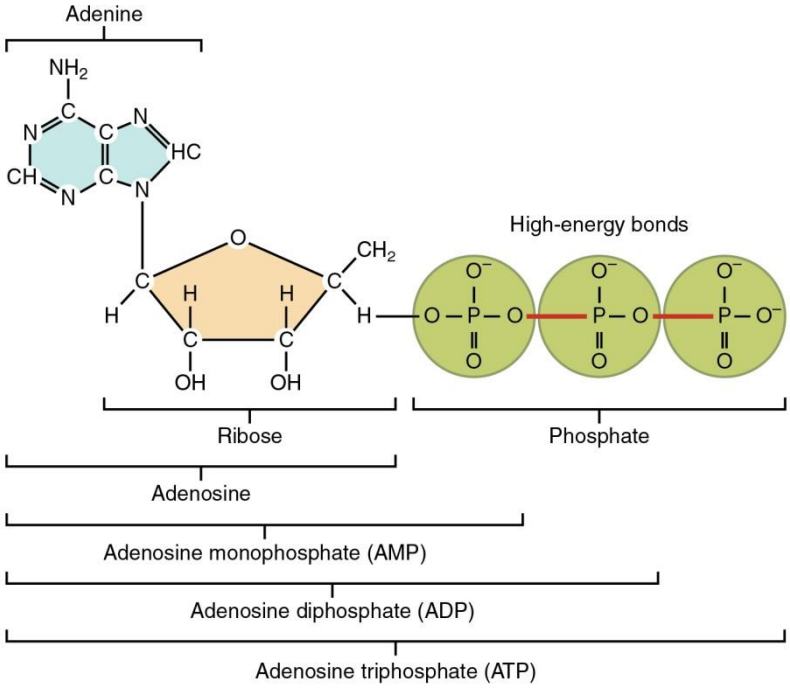



Figure 23: Structure of ATP, ADP, and AMP

The trapping of energy in form of ATP is called Phosphorylation; there are three types of phosphorylation processes followed by living cells:
Photophosphorylation: It occurs in the presence of light in photosynthetic cells with assistant of photo pigments (chlorophylls and carotenoids),which trap photo energy from solar radiation that exited and release electrons from photo pigments, these electrons pass through a series of electron carriers and generates phosphate bonds in form of ATP.

Oxidative phosphorylation: Is the processes in which ATP is formed as a result of the transfer of electrons ($e^-$) that collected by certain electron carriers like NAD, NADP, and FAD, and passed into electron transport chain (ETC), located in the inner membrane of mitochondria in eukaryotic cells and in plasma membrane in prokaryotic cells, finally the $e^-$ combined with oxygen, which act as final electron acceptor, the subsequent passage of electrons though electron carriers release energy used to generate ATP from ADP. One molecule of NAD generates three molecules of ATP when it enters the ETC, however one molecule of FAD generates only two molecules of ATP, this is due to the fact that FAD enters ETC later than NAD.

Substrate level phosphorylation: Is a type of metabolic reaction that results in generation of ATP or guanosine triphosphate (GTP) by direct transfer and donation of a phosphoryl group to ADP or GDP from phosphorylated reactive intermediate, for example:

**GTP + ADT** $\longrightarrow$ **ATP + GDP**

**Oxidation – Reduction Reactions**

The release of energy from an energy source in bio-system normally involves biological oxidation, fundamentally is the removal of electrons from various substrates (**electron donor**), which thereby oxidized, and does not necessarily involves oxygen. In living cells the removed **$e^-$** cannot be remain in a free state there must be immediately transferred to other compounds or molecules (**electron acceptor),** which thereby reduced. So oxidation is the loss of $e^-$, and reduction is gain of $e^-$ , an acceptor is oxidizing agent and the donor is reducing agent.

Oxidation – reduction are always coupled so termed **redox reaction**. The equilibrium constant for such reaction. In most cells oxidation of organic substrates is accomplished by removal of hydrogen (dehydrogenation), hydrogen atom provides one proton ($H^+$) and one electron ($e^-$). In living cells enzymes involved in dehydrogenation processes are dehydrogenase enzymes, these enzymes like other enzymes are highly specific for their respective substrates, and all of these include coenzymes, which are less restricted; one coenzyme may act in several different other Apoenzymes and reactions. Dehydrogenises coenzymes (electron carriers), resemble shutter that are alternately loaded and unloaded, repeatedly accepting and releasing e- and H to facilitate the transfer of redox energy. Most of coenzymes transfer both e- and H. The most common electron carriers are:

**NAD; nicotinamide adenine dinucleotide**

**NADP; nicotinamide adenine dinucleotide phosphate**

**FAD; Flavin adenine dinucleotide**

The essential portion of NAD and NADP is nicotinamide (Niacin, Vitamin B3), the pyridine ring of this group accepts substrates H or $e^-$, and being reduced to NADH and NADPH, the reduced form in turn transfers the accepted hydrogen to second acceptor that is at low energy level and being re-oxidized to NAD and NADP.

FAD, contain riboflavin (Vitamin B2), so far all vitamins are analogous part of coenzymes, thus is common to several dehydrogenases, when reduced by substrates hydrogen represented as FADH, the coenzyme very commonly re-oxidized to FAD by transfer of hydrogen to an iron – bearing respiratory pigments of cytochrome system.

**The Thermodynamics and Bioenergetics**

**Energy** may be most simply defined as the capacity to do work, the energy in living cells which is termed as bioenergy, mostly trapped as **ATP,** and is used to do cellular works like biosynthesis, transport, growth, and motility. The flow of energy through organisms at cellular and molecular level termed **Bioenergetics**; that concern with how organism extract energy from surrounding environment and how this energy used to fuel the myriad of life. To understand the basic concepts of bioenergetics some principles of **thermodynamics lows** (is the study of energy transformation that describe the relationships between thermal energy, or heat and other forms of energy, and how energy affects matter). These principles facilitate a perception of how an energy – producing and energy – utilizing reaction occurs within the same cell and how the organism is able to accomplish various work functions.

**The first law of thermodynamics:** state the energy neither created nor destroyed, but converted from one form to another, that the total amount of energy in system remains constant, which confirm the principle of energy conservation, such as, the plants do not produce energy but transform light energy to chemical energy. The first low alone cannot explain why energy released by a reaction and absorbed by other. Scientific explanation for this phenomenon requires some knowledge of the **second thermodynamics low,** which is about the quality of energy, it state that as energy is transferred or transformed. In fact any discussion of energy must begin with definition of **Entropy (S);** simply is the measure of disorder combined to system thermal change, may be also considered as a measure of a system thermal energy that is available.

Chemical reactions classified as **exergonic** and **endergonic** based on free energy, an exergonic reaction proceeds with a net release of energy, for the overall reaction of cellular respiration,

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

An endergonic reaction is one that absorbs free energy from its surroundings and never proceeds spontaneously that requires input of energy for conversion of $CO_2$ and water to sugar.

$$6\ CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6$$

## Photosynthesis

Microorganisms derive energy not only from the oxidation of inorganic and organic compounds, but also from light energy, which they capture and use to synthesize ATP and reducing power (e.g., NADPH). They harvest the energy of sunlight and use it to power the synthesis of ATP. The conversion of solar energy to chemical energy is called Photosynthesis. The general reaction of photosynthesis can be summarized as follow:

$$6\ CO_2 + 12\ H_2X \longrightarrow C_6H_{12}O_6 + 12\ X + H_2O$$

(X depends on the source of electron for reducing power and can be a number of different compounds).

Usually a phototrophic organism reduces and incorporates $CO_2$. Photosynthesis is one of the most significant metabolic processes on Earth because almost all our energy is ultimately derived from solar energy. It provides photosynthetic organisms with the ATP and reducing power necessary to synthesize the organic material required for growth. In turn these organisms serve as the base of most food chains in the biosphere. One type of photosynthesis is also responsible for replenishing our supply of $O_2$, a remarkable process carried out by a variety of organisms, both eukaryotic and prokaryotic. Although photosynthesis is performed differently by different organisms, the process always involves two reactions:

- **Light - dependent reactions;** are those reactions used to derive energy from sunlight.
- **Dark - dependent reactions;** the reactions which use that energy to fix $CO_2$.

### The Role of Photosynthetic Pigments

Photosynthetic organisms are highly visible in their natural habitat because they possess photo pigments, Chlorophylls and Carotenoids. Colored pigments, used to capture light energy, these pigments vary in color because they absorb different wavelengths of light.

The primary light – absorbing pigments are chlorophylls. Most photosynthetic organisms, including an important group of bacteria called cyanobacteria, use

chlorophyll a. A different group of photosynthetic bacteria, the purple and green bacteria, use bacteriochlorophyll. This absorbs wavelengths not absorbed by chlorophyll a, enabling the green and purple bacteria to grow in habitat where other photosynthetic organisms cannot.

The carotenoids are accessory pigments that increase the efficiency of light utilization; they do this by absorbing wavelengths of lights not absorbed by chlorophylls and transferring that energy to chlorophyll.

Chlorophyll and other light absorbing pigments are organized in protein complex called Photosystem located in special photosynthetic membrane. In cyanobacteria, these are found in stacked membrane called Thylakoids. Plants and algae also have thylakoids, in the stroma of their chloroplasts; the photosynthetic pigments in purple and green bacteria are either contained within cytoplasmic membrane or within structure called Chlorosome attached to the membrane.

**Photophosphorylation**

Within the photosystem there are two components that work together to harvest energy, the antenna complex, is composed of hundreds of light – gathering pigments (chlorophyll and accessory pigments), this complex act as a funnel, capturing the energy of light and then transferring it to reaction-center chlorophyll. When this chlorophyll absorbs that energy, one of its electrons become excited, or raised to higher energy level, in a manner similar to oxidative phosphorylation. The high energy electron is sequentially pass along series of electron carriers in membrane embedded electron transport chain, the flow of $e^-$ dawn an ETC that leads to the ultimate reduction of NADH to NADPH. In addition this results in translocation of protons ($H^+$) across membrane, generating Proton motive force (PMF). ATP synthase permits the flow of protons back across the membrane and uses the energy to generate ATP. The high energy electron from reaction – center chlorophyll that passes along the ETC may or may not be returned to their original source.

In cyclic photophosphorylation (a cyclical pathway), the chlorophyll molecule regains the lost $e^-$ from water , In non – cyclical photophosphorylation, PMF is produced, but in addition, high energy electrons are drawn off to generate reducing power, electrons must still be returned to chlorophyll, but they must come from a different source, such as hydrogen sulfide**.** (Figure 24).

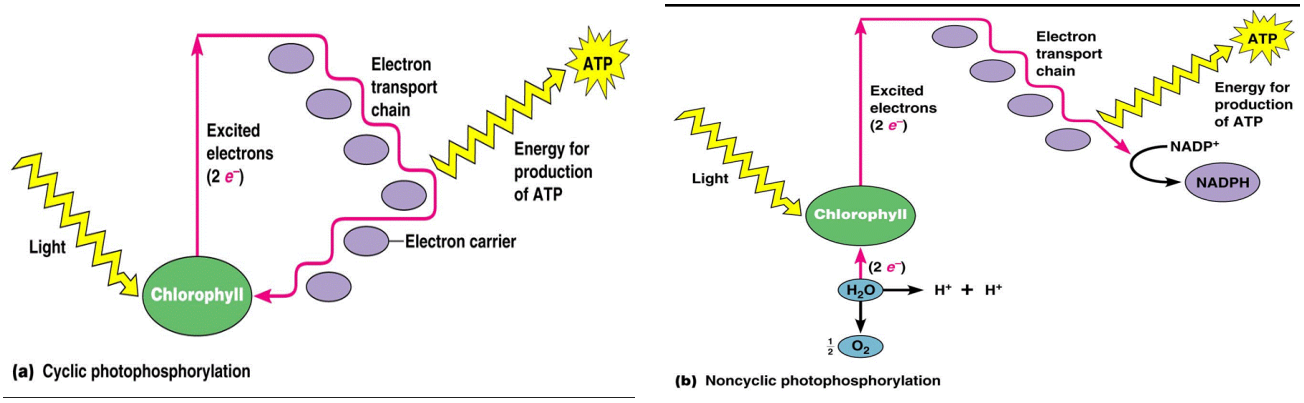



Figure 24: Cyclical and non - cyclical Photosynthesis in Bacteria

**Electron Source**

Photosynthetic organisms require electron source to generate reducing power in form of NADPH, the compound used to obtain electrons dictates whether or not oxygen ($O_2$) is evolved during photosynthesis.

**Oxygenic Photosynthesis**

When plants, algae, and cyanobacteria use non – cyclical photophosphorylation to make reducing power, they use water as electrons source that are returned to the chlorophyll. When electron extracted from water proton and ($O_2$) are released. It is because of oxygenic photophosphorylation that obligate aerobes, including humans and other animals are able to inhabit earth.

**Un-oxygenic Photosynthesis**

Un-oxygenic photosynthesis such as green and purple bacteria, lack the sophisticated photosystem that the oxygenic photosynthetic organisms us to generate NADPH. Instead they must use alternative mechanism such as hydrogen sulfide, hydrogen gas, and reducing organic molecules. Because water not used as electron source, un-oxygenic phototrophs do not evolve ($O_2$).

ATP and NADPH which produced from photophosphorylation processes have short life spam so they must recycled by producer cells quickly because they cannot store in cells as storage compounds, the cells overcome this situation by auto fix of $CO_2$ to high energy storage compounds.

**Fixation of $CO_2$ by Autotrophs**

Autotrophs use $CO_2$ as their only or principle carbon source and the reduction and incorporation of $CO_2$ Requires much energy. Many autotrophs obtain energy by trapping light during photosynthesis, but some derive energy from the oxidation of reduced electrons donors. Autotrophic $CO_2$ fixation is crucial to life on Earth because it provides the organic matter on which heterotrophs depend.

Four different $CO_2$ – fixation pathways have been identified in microorganisms. Most autotrophs use the Calvin cycle, which is also called the Calvin – Benson cycle or the Reductive Pentose Phosphate Cycle. The Calvin cycle is found in photosynthetic eukaryotes and most photosynthetic bacteria. It is absent in some obligatory anaerobes and microaerophilic bacteria.

**Anabolic Pathways – Synthesizing Subunit from Precursor Molecules**

While prokaryotes as a group are highly diverse with respect to the compounds they use for energy, they are remarkably similar when comparing their biosynthetic processes. Using precursor metabolites, reducing power in form of NADPH, and ATP, cells synthesize the necessary subunits using specific anabolic pathways. Organisms that lack one or more enzymes in a given pathway must have the end product of that pathway provided from an external source. This is why fastidious bacteria such as the lactic acid bacteria require many different growth factors. Once the subunits are either synthesized or supplied, they can be assembled to make macromolecules. Various different macromolecules can then be joined to form the structures that make up the cell.

- **Lipid Synthesis**

Synthesis of lipids in microorganisms can be viewed as having two essential components – Fatty acid synthesis and Glycerol synthesis.
Synthesis starts with the transfer of the acetyl group of acetyl-CoA to a carrier protein called acyl carrier protein (ACP). This carrier serves to hold the fatty acid chain as it elongated by progressively adding 2- carbon units. When the newly synthesized fatty acid reaches its required length, usually 14, 16, or 18 carbons long, it is released from ACP. The glycerol components are synthesized from glyceraldehyde 3 – phosphate.

- **Amino Acids Synthesis**

Proteins are composed of various combinations of 20 different amino acids. Amino acids can be grouped into structurally related families that share common pathway of biosynthesis. Some are synthesized from precursor metabolites formed during glycolysis, while other are derived from compounds of the TCA cycle.

***Glutamate**

Although all amino acids are necessary for protein synthesis, glutamate is especially important because it is used to form many other amino acids. In

addition, its synthesis provides a mechanism for bacteria to incorporate nitrogen into organic material.

Bacteria that synthesize glutamate use single – step reaction that incorporates ammonia into precursor metabolites α - ketoglutarate, produced in the TCA cycle (Figure 25a). Once glutamate has been produced, its amino group can be transferred to other carbon compounds to produce amino acids such as aspartate (Figure 25 b).

This transfer of amino group, a transamination, regenerates α - ketoglutarate from glutamate. The α- ketoglutarate can then be used again to incorporate more ammonia.

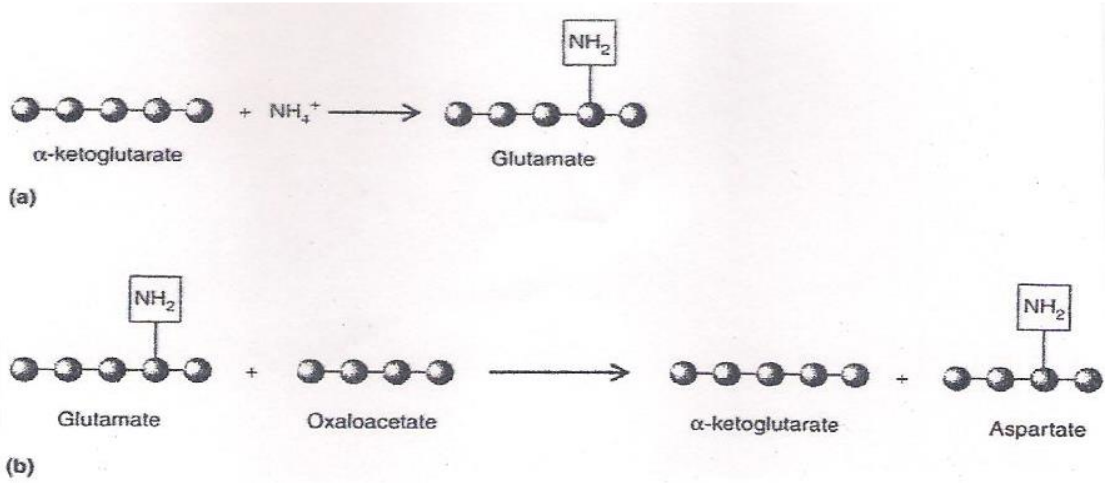



Figure 25: Glutamate synthesis in Microbial cell

## Microbial Enzymes

Chemical reactions of life are organized and complex, but even when they are highly organized cannot proceed without the tool of life, **Enzymes,** are biological catalysts , designed to do the work of life , each enzyme has one specific job to do. They are proteins act as catalysts, speed up a chemical reaction without being altered during the reaction, this means enzymes can be recycled and do not need to be made in large amount. This due to enzymes lower the **activation energy** (the amount of energy necessary to push the reactants over an energy barrier so that the reaction can proceed) needed to derive biochemical reactions in normal cellular temperature, in this manner cellular proteins are not damaged by excess heat for reaction.

### Enzymes structure

Enzymes are globular proteins ranging in size from 1300 to millions Dalton, with a thousands of amino acids are bounded together with covalent bonds called peptide bonds, linked as polypeptide. Like all proteins an enzyme exhibits levels of molecular complexity includes primary, secondary, and tertiary structures, the larger enzymes exhibits quaternary organization (figure

26). The first three structural levels arise from polypeptide chains folding process and. this folding cause the surface of apoenzyme acquire distinct tree dimensional forms (3D) known as a " native conformation". The enzymes native conformation which is essential for functional activity maintained and stabilized by hydrogen and bisulfide bonds, disruption (breakage) of these bonds lead to the loss of enzyme function known as denaturation. 3D conformation relates to enzymes specificity cause the surface feature of the tertiary structure provides a unique and specific site usually a crevices or groove called the active site or catalytic site, which is small protein (include a few amino acids) compared to the overall size of the protein molecule, used for attachment and accepting of substrate that certain enzyme acted on.

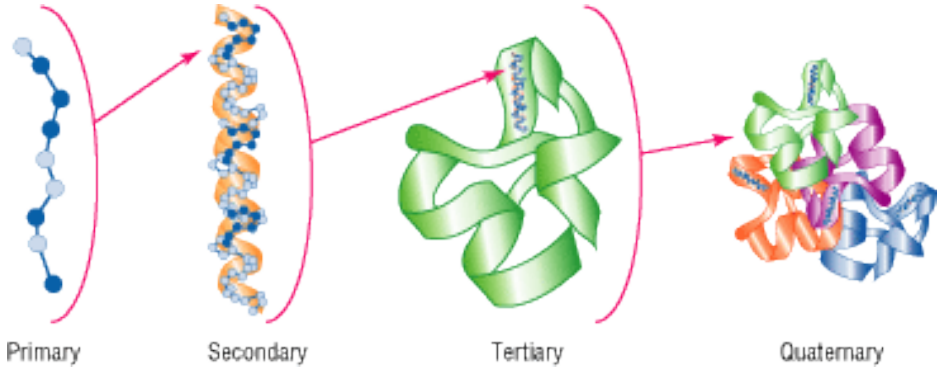



Figure-26: Levels of Enzymes Structure

## Cofactors and Coenzymes

Many enzymes are composed only of protein, sometimes referred as simple enzymes. However some enzymes consist of protein (**apoenzyme)** and nonprotein component (**prosthetic groups**). non protein portions are required for participation in the catalytic reaction. The complete enzyme consisting of apoenzyme and its prosthetic group called the **holoenzyme**. Prosthetic groups fall into two major categories: Cofactors and Coenzymes.

**Cofactors:** are commonly metal ions, bind permanently or reversibly to the enzyme, so most of trace elements used by living cells are primarily used as cofactors to serve as structural or functional role, such elements include magnesium, manganese, zinc, iron, copper, cobalt, and selenium. For example $Mg^{2+}$ needed for DNA polymerase to form a growing DNA molecule. Metal cofactors participate in precise enzyme functions, in general help to form a metal – bridge between enzyme and substrate that assists to bring active site and substrates closely together or as an electron acceptor/donor in redox reactions. In addition to these roles metals also bind directly to the enzyme to stabilize it in the active conformation or perhaps to induce the formation of active site.

**Coenzymes:** are small organic compounds works in conjugation with apoenzyme, are present in cells in reasonably constant concentration and play a dynamic role in metabolism, often derived of vitamins. Many important coenzymes like Coenzyme A, NAD, NADP, and FAD are derived from vitamin B complex, so vitamins are clearly important nutrients required as growth factors in majority of living cells, in that vitamins deficiencies prevent the complete holoenzymes from forming, which compromise both the chemical reactions and structure or function dependent upon that reaction. The general action of coenzymes is transmission of functional groups within substrates that act as carrier of these groups so they are loosely attached to the apoenzyme and can dissociate from the protein after products have been formed, and some of them used in generation of energy throughout catabolic pathways.

## Classification of Enzymes

Enzymes can be classified in different ways, which based on many criteria, the most common and followed classification categories are: (**1**) **Location of enzymes;** In the basement of enzymes location in and site of activity, enzymes generally divided into two main groups, **Exoenzymes,** these type of enzymes after their initial biosynthesis inside cells are transported extracellularly where they break down large food molecules and harmful chemicals like; cellulose, amylase, and penicillinase. **Endoenzymes**, these enzymes are retained intracellular and function there, most metabolic pathways enzymes are of this variety. (**2**) **Presence in the cell**, enzymes are not all produced in equal amounts or at equal rates, according to this criteria, two types of enzymes figured in microbial cells, **Constitutive enzymes,** are always present relatively in constant amounts regardless of the amount of their substrates, such as the enzymes involved in utilizing of glucose, and, **Inducible enzymes,** these enzymes not constantly present in cells ,they are selectively synthesis depending on metabolic requirements**,** and when their specific substrates they acted on are present. This property of selective synthesis prevents cells from wasting energy and it is an important metabolic control that ceasing none demands pathways**.** (**3**) **Systematic classification,** Despite the large number and diversity of enzymes present in cells, they are classified as belonging to one of **six classes** (includes other subclasses according to the type of reaction catalyzed), recommended in 1973 by international union of biochemistry and molecular biology (**IUBMB**), in this system the arbitrary name is end with the suffix ***ase,*** and usually enzymes named using the prefix that related to the type of chemical reaction they catalyze.

**The systematic major classes of enzymes**

| **Enzyme class Type of reaction** | **catalyzed** | **Example** |
|---|---|---|
| Oxidoreductase | Oxidation/reduction reactions | Lactate dehydrogenase<br>Pyruvate + NADH + H ===<br>Lactate + $NAD^+$ |
| Transferases | Transfer of functional groups | Hexokinase<br>Glucose + ATP === Glucose-6-phosphate + ADP |
| Hydrolases | Hydrolytic reactions **(**Cleave bonds in molecules with addition of water) | glucose -6- phosphate<br>Glucose- 6 –P + $H_2O$ === glucose + Pi |
| Lyases | Removal of a group from substrate (No hydrolysis) | Fumarate hydratase<br>L-malate ====== Fumarate + H2O |
| Isomerases | Isomerization Reactions (change substrates into other isomeric form) | Aldose-1-epimerase<br>α-D-glucose ===== β -D-glucose |
| Ligases | The joining of two molecules at the expense of bond energy in ATP and removal of water | Glutamamine synthetase<br>L-glutamate+ $NH_3$ +ATP === L-glutamine + ADP +orthophosphate (pi) |

**The Mechanism of Enzyme Reactions**

It is important to keep in mind that enzymes speed up chemical reactions without altering the energy barrier or equilibrium constant. If the reaction is endergonic, the presence of enzyme will not shift its equilibrium so that more products can be formed. Enzymes simply speed up the rate at which a reaction speeds toward its final equilibrium. How do enzymes catalyze reactions? First it must bind the substrate to form the **enzyme – substrate complex** (**ES)** "**transition state complex**"

E + S ========= ES ======== P + E

Which resemble both substrate and product and falls between the process of conversion of substrate to product, that many molecular changes occurs in this level like, breakage, formation or rearrangement of bonds, and developments of other changes. This state doesn't take place if not supplied with amount of energy equivalent to the **activation energy** which required to attain the transition state which falls at the top of energy barrier that has to be overcome and to complete the reaction. Presence of Enzyme accelerate the reaction by lowering the activation energy, therefore more substrate molecules will have sufficient energy to come together even though the equilibrium constant is unchanged **.** In fact enzymes bring substrates together at their **active site** to form the **ES.** An enzyme can interact with the substrate that acted on in two general ways; it may be rigid and shaped to precisely fit the substrate so the correct substrate binds specifically and is positioned properly in active site for reaction. This mechanism referred to as **Lock – and – key model** (figure 27). An enzyme also may be change shape when it binds the substrate so that the active site surround and precisely fit the substrate, this has been called **Induced fit model**. The formation of an **ES complex** can lower the activation energy in many ways, in most cases, substrates are held in the active site by weak interactions, such as **hydrogen bonds** and **ionic bonds**, as this interaction achieved the enzyme may put stress on bonds that must be broken, making it easier for the reaction to reach the transition state.

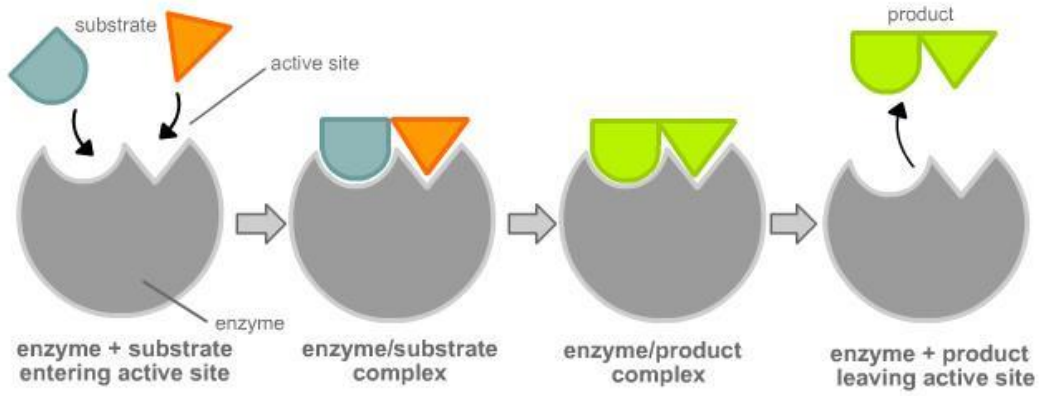



Figure-27: Lock – and – Key Model of Enzyme Function

## The effect of environment conditions on enzymes activity

Enzymes activity varies greatly with the change in environmental factors. One of the most important is the substrate concentration. Substrate concentration (**S**) usually low within cells, at very low concentration , an enzyme make product slowly because it seldom contract substrate molecules, if more substrate molecules are present, the enzyme binds substrate more often, and the reaction velocity (**V**), usually expressed in term of the rate of product formation, greater than at a lower substrate concentration, the initial reaction

velocity (**V°**), is proportional to substrate concentration so it's in 1st order reaction thus, if more substrate molecules present, an enzyme binds more often, under initial velocity that increase with substrate concentration. Eventually further increase in substrate concentration does not affect the reaction rate and the latter become constant "Zero order reaction" because the available enzyme molecules are binding substrate and converting it to the product as rapidly as possible. That is the enzyme is saturated with substrate and operating at maximal velocity ($\mathbf{V_{max}}$). The resulting substrate concentration curve. It is useful to know the substrate concentration an enzyme needs to function adequately.

Usually the **Michaelis – Menten** (**Km**), the substrate concentration required for the enzyme to achieve half maximal velocity, is used as a measure of the apparent affinity of an enzyme for its substrate. The lower **Km** value, the lower substrate concentration at which an enzyme catalyzes its reaction, enzyme with a low Km value said to have a high affinity for their substrate. **Km** value is not fixed value but varies according to substrate, temperature, and pH (figure 28).

When the reciprocal values of **S** and **V** are used in plotting of **V** versus **S,** a straight line curve is obtain as represented by **Line weaver – Burk**, used as accurate determination of required substrate concentration for a certain enzyme.

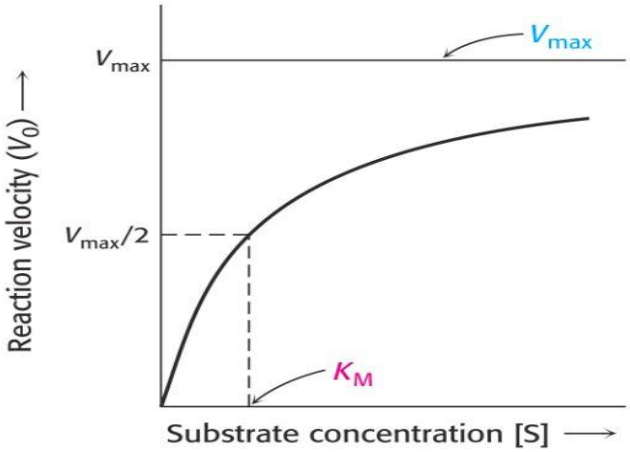



Figure 28: Michaelis – Menten Kinetics of Enzymes

Enzymes activity also affected by general environmental conditions, such as with alteration in Temperature and pH (figure 29). Each enzyme works best at certain optimal conditions which favor the most active conformation for the enzyme molecule. Temperature has a major impact on reaction rate; each enzyme has temperature optima for maximum activity. If temperature rises too much above optimum, thermal agitation begins to disrupt the weak bonds that stabilize the protein active conformation and the enzymes structure disrupted

and its activity lost. This phenomenon known as **denaturation.** Enzymes likewise function most rapidly at specific pH optimum, when the pH deviated to greatly form an enzyme optimum activity slow and the enzyme may be damaged. The temperature and pH optima of a microorganism's enzymes often reflect the temperature and pH of its habitat.

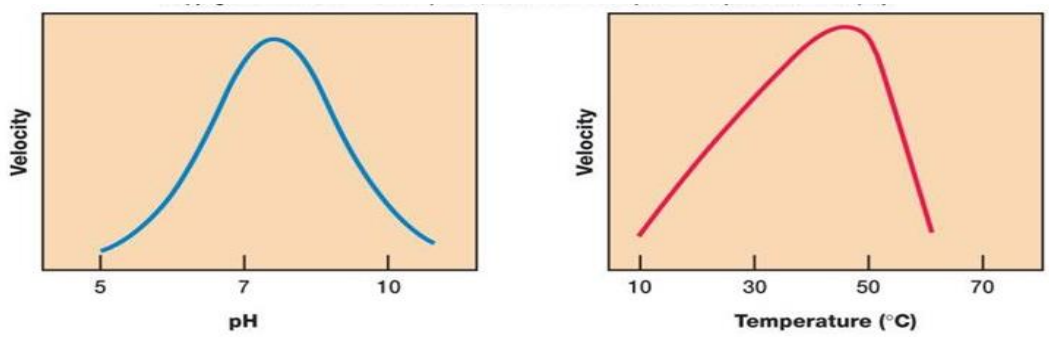



Figure 29: The Effect of pH and Temperature on Enzymes Activity

## Allosteric Enzymes

Allosteric is derived from the Greek root ***allo***, meaning "the other". These are regulatory enzymes; their catalytic activity depends upon non – covalent binding of specific small molecules as ligands at a unique region of the enzyme quite different and away from the active site known as **regulatory site, or allosteric site.** Ligands binding can be either activators or inhibitors (figure 30). Most allosterically regulated enzymes are constructed of two or more polypeptide chain each subunit has its own active site, the allosteric site are often located where subunits join. The ligands that bind at the allosteric site are called **allosteric effectors** or **modulators,** binding of modulator to an enzyme alter the **3D** conformational structure of the active site and thus affected the affinity for substrate, activator stabilizes the conformation that has functional active site, while the binding of an inhibitor stabilize the inactive form of the enzyme, so most of allosteric enzymes are key enzymes play a major role in balancing the flow of traffic between anabolic and catabolic pathways. For example, ATP binds to several catabolic enzymes allosterically, inhibiting their activity by lowering their affinity for substrate. The substrate saturation curve of these enzymes is often sigmoidal rather than hyperbolic.

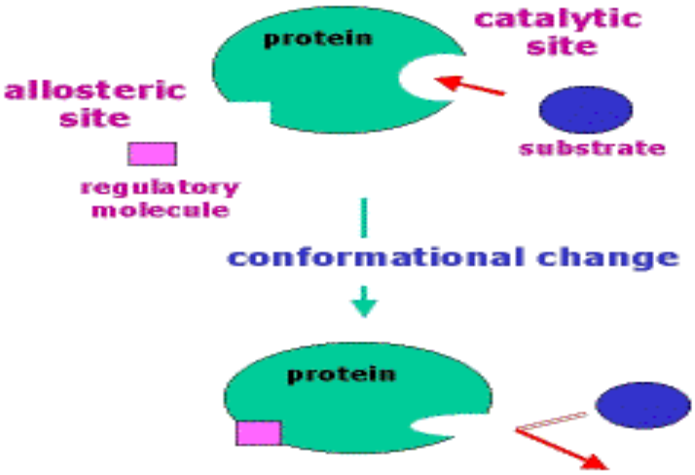



Figure 30: Allosteric Regulation

## Enzymes Inhibitors

An enzymes inhibitor is a molecule that binds to an enzyme and decreases its activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance. As many drugs and pharmaceutical compounds are enzyme inhibitors, and much of metabolic pathways was determined by using of inhibitors of specific enzymes. Inhibitors compounds can be bind to the free enzyme or to the ES complex and affect the velocity of reaction. Several classes of inhibitors are defined based on their reaction kinetics:

### Types of enzymes inhibitors:

#### 1. Reversible inhibitors

Reversible inhibitors attach to enzymes with non – covalent interactions such as hydrogen bonds, hydrophobic interactions, and ionic bonds. Multiple weak bonds between the inhibitors and the active site combine to produce strong and specific binding, reversible inhibitors generally do not undergo chemical reactions when bound to enzyme and can be easily removed by dilution and dialysis.

There are two main kinds of reversible enzyme inhibitors, they are classified according to the effect of the varying the concentration of the enzyme's substrate on the inhibitor.

**• Competitive Inhibitors:**

A competitive inhibitor is any compound which closely resembles the chemical structure and molecular geometry of the substrate that competes with the substrate for binding to free enzyme. The inhibitor may interact with the enzyme at the active site, but no reaction takes place, or inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the

enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site (figure 31).

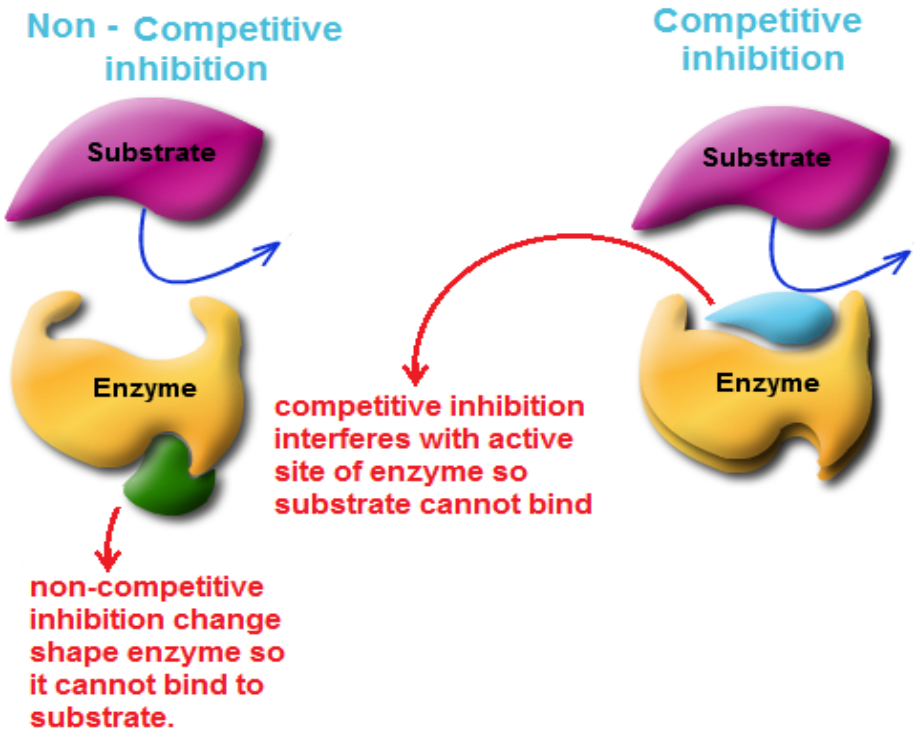



Figure 31: Enzymes reversible inhibitors

**• Non-competitive Inhibitors:**

A noncompetitive inhibitor is a substance that interacts with the enzyme, but usually not at the active site, reacts either remote from or very close to the active site. The net effect of a non-competitive inhibitor is reduction the activity and the enzyme reaction not proceed efficiently, but does not affected the binding of substrate. This inhibition model are not influenced by concentrations of the substrate as is the case for competitive inhibitor, and are depends only on the concentration of inhibitors (figure 31).

2. **Irreversible inhibitors**

These inhibitors form strong covalent bonds with an enzyme that may act at, near, or remote from the active site. Consequently, they may not be displaced by the addition of excess substrate. In any case, the basic structure of the enzyme is modified to the degree that it stops to work.

Since many enzymes contain sulfhydryl (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as $Ag^{+}$, $Hg^{2+}$, $Pb^{2+}$ have strong

affinities for -SH groups. Oxalic and citric acid inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

**Regulation of Metabolic Pathways**

The physiological integration of enzymes into metabolic pathways, which are proceed in highly organized manner, so it's necessary to regulate the activity of enzymes participates in pathways, most of regulatory behavior in cells related to the key enzyme **(Pacemaker),** usually are allosteric enzyme, which respond to various regulation signals, and control the flow of metabolic pathways as cellular demands. Cells regulate these enzymes in two procedures: **long – term regulation,** occurs by change the rate of de novo (synthesis of complex molecules from simple molecules such as sugars or amino acids) synthesis genetically, at either transcriptional or translational level. **Short – term regulation** of an enzyme occurs through modulation of the activity of key enzymes by activators and inhibitors. This is referred to as **feedback inhibition** (figure 32). The end product of pathway is feedback to reaction system that fits to allosteric site of key enzyme, by this fitness enzyme distorted and can no longer bind to its substrates, this attachment does not denature the enzyme and it's quite reversible.

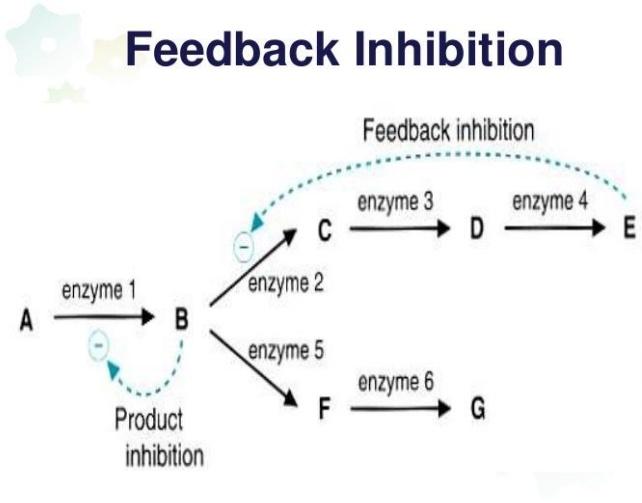



Figure 32: Short-Term Regulation of Enzymes (Feed Back Inhibition)

# Microbial Metabolism

## Catabolic Pathways

Catabolism is the set of metabolic pathways that breaks down complex compounds to simpler units, often supply material needed for biosynthesis, including precursor metabolites, and reducing power.

Precursor metabolites serve as starting molecules for biosynthetic pathways. Reducing power is used the carbon provided by the precursor metabolites, as they are transformed into amino acids, nucleotides, and other small molecules needed for synthesis of macromolecules .

Pathways that function both catabolically and anabolically are called **amphibolic** pathways (Greek *amphi*, on both side).

The central catabolic (**amphibolic)** pathways that take place in microbial cells are:

**Glycolysis** (glucose breaking down) and **Tricarboxylic acid cycle (TCA)**. Many of these reactions are freely reversible and can be used to synthesize or degrade molecules depending on the nutrient available and the need of microbe.

## Glycolysis

Microorganisms employ several metabolic pathways to catabolize glucose (fuel molecule) and other sugars. Because of this metabolic diversity their metabolism is often confusing. To avoid confusion as much as possible, the ways in which microorganism degrade sugars to **pyruvate** and similar intermediates are introduced by focusing on only three routes: **(1) Embden-meyerhof pathway**, **(2) Pentose phosphate pathway**, and **(3) Entner – Doudoroff pathway**.

These three pathways collectively termed **glycolytic pathways** or **glycolysis** [Greek *glyco*, sweet, and *lysis*, a loosening].

**Glycolysis** is the most important type of mechanism by which organism obtains energy from organic compound in the absence of oxygen. As it occurs in the absence of oxygen, there, it is also called anaerobic fermentation.

### *The Embden-Meyerhof pathway (EMP)

The sequence anaerobic reactions from glucose to pyruvate is called Embden-Meyerhof pathway, because the complete pathway were elucidated by Gustav Embden (who gave the manner of cleavage of fructose 1, 6- diphosphate and pattern of subsequent steps) and Otto Meyerhof (who confirmed Embden's work and studied the energetic of glycolysis). It is an important amphibolic pathway that provides several essential precursor metabolites, found in all major groups of organisms, and occurs in the cytoplasmic matrix of prokaryotes.

The pathway as a whole may be divided in to two phases (figure 33):

**The six - carbon phase 6C** (preliminary phase), in this phase energy is consumed**. The three – carbon phase 3C** (energy conserving phase).

The EMP degrade one glucose to two **pyruvates** by the sequence of reactions, and **ATP** , **NADH** are also produced. The yields of **ATP** and **NADH** may be calculated by considering the two phase separately.

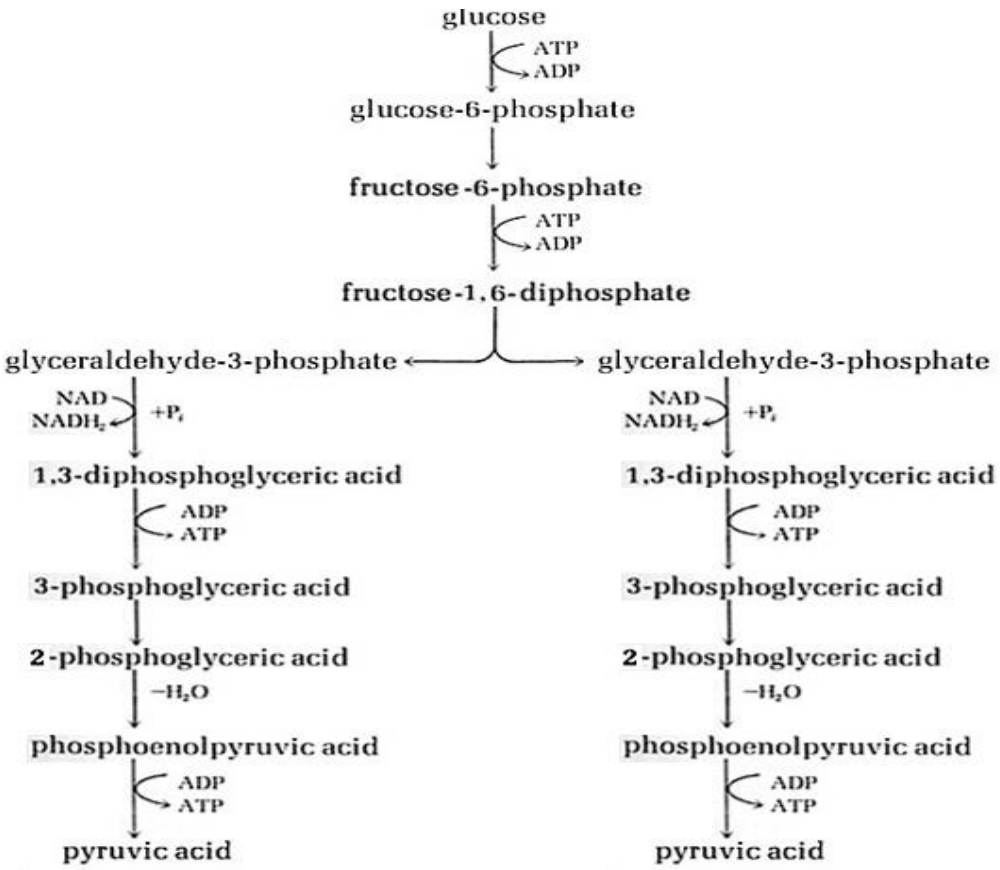



Figure 33: The Embden – Meyerhof Pathway (EMP)

*** The Pentose Phosphate Pathway (PPP)**

The **Pentose phosphate pathway,** also called **Hexose monophosphate shunt or Phosphogluconate pathway,** a second glycolysis pathway, may be used at the same time as either the EMP or the Entener- Doudoroff pathway. It can operate either aerobically or anaerobically, is important in both biosynthesis and catabolism and is the major source for NADPH require for anabolic processes. There are two distinct phases in PPP; oxidative phase, the linear portion of pathway carries out oxidation and decarboxylation of G-6-P, producing 5C sugar ribulose – 5 – phosphate, $CO_2$ and NADHs are generated during these oxidations (figure 34). The overall reaction for this phase is:

**3 G-6-P + 6 NADP + H2O ⟶ 2 ribulose 5- phosphate + $3CO_2$+ 6 NADPH + $6H^+$**

Ribulose 5- phosphate is then converted to a mixture of three, four, five, six, and seven – carbon sugar phosphate in a series of non – oxidative reactions.

Two enzymes play a central role in non – oxidative subsequent transformations:
**Transketolase** and **Transaldolase**, catalyze transfer of 2-C and 3-C molecular fragments respectively. These enzymes create a reversible link between PPP and EMP.

The overall result is that three G-6-P are converted to:
**3 G-6-P + 6NADP + $3H_2O$ ⟶ 2 F-6-P + G3P + $3CO_2$ + 6 NADPH + $H^+$**

The PPP is a good example of an **amphibolic** pathway as it has several catabolic and anabolic functions that are summarized as follow:
**1**. **NADPH** from the pentose phosphate pathway serves as: a source of electrons for the reduction of molecules during biosynthesis
**2**. The pathway produces two important precursor metabolites: Erythrose -4-phosphate, which is used to synthesis aromatic amino acids and vitamin B6 (pyridoxal), and ribose 5 –phosphate, which is a major component of nucleic acids. Note that when a microorganism growing on pentose carbon source, the pathway in turn can function biosynthetically to supply hexose sugars (*e*,*g*. glucose needed for biosynthesis of peptidoglycan).
**3**. Intermediates in the pathway may be used to produce ATP, G3P and F-6-P from the pathway can enter the EMP and can be converted to pyruvate, as ATP is produced by substrate – level phosphorylation. Pyruvate may be oxidized in TCA to provide more energy.

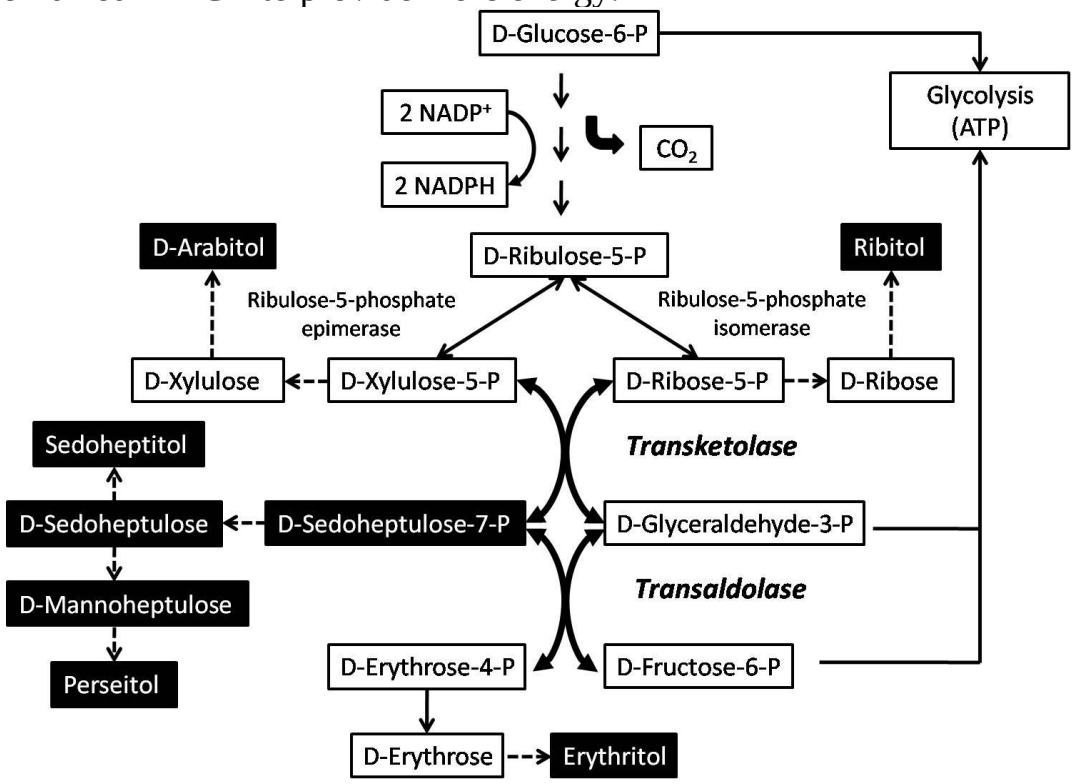


Figure 34: The Pentose Phosphate Pathway (PPP)

***The Entner – Doudoroff Pathway (ED pathway)**

Although the Embden – Meyerhof pathway is the most common rout for conversion of **hexoses** to **pyruvate**, the **Entner – Doudoroff pathway** for sugars breaking down is used by only prokaryotes, like some soil microbes, such as *Pseudomonas*, *Rhizobium*, *Azotobacter*, and a few other gram-negative bacteria. Very few gram-positive bacteria have this pathway, with the intestinal bacterium *Enterococcus faecalis* being a rare exception.

The **ED pathway** catabolizes glucose to pyruvic acid using enzymes distinct either from those used in EMP or PPP. The pathway begins with the same reaction as the PPP, the formation of g-6-p, which is then converted to 6-phosphogluconate (figure 35) Instead of being further oxidized, 6-phosphogluconate is dehydrated to form 2-keto-3-deoxy-6-phosphogluconate or **KDPG**, the key intermediate in this pathway. **KDPG** is then cleaved by **KDPG aldolase** to pyruvate and G3P. The G3P is converted to pyruvate in the EMP. If the Entner – Doudoroff pathway degrades glucose to pyruvate in this way, a net yield 1 **ATP** for every one glucose molecule processed, as well as 1 **NADH** and **NADPH.**

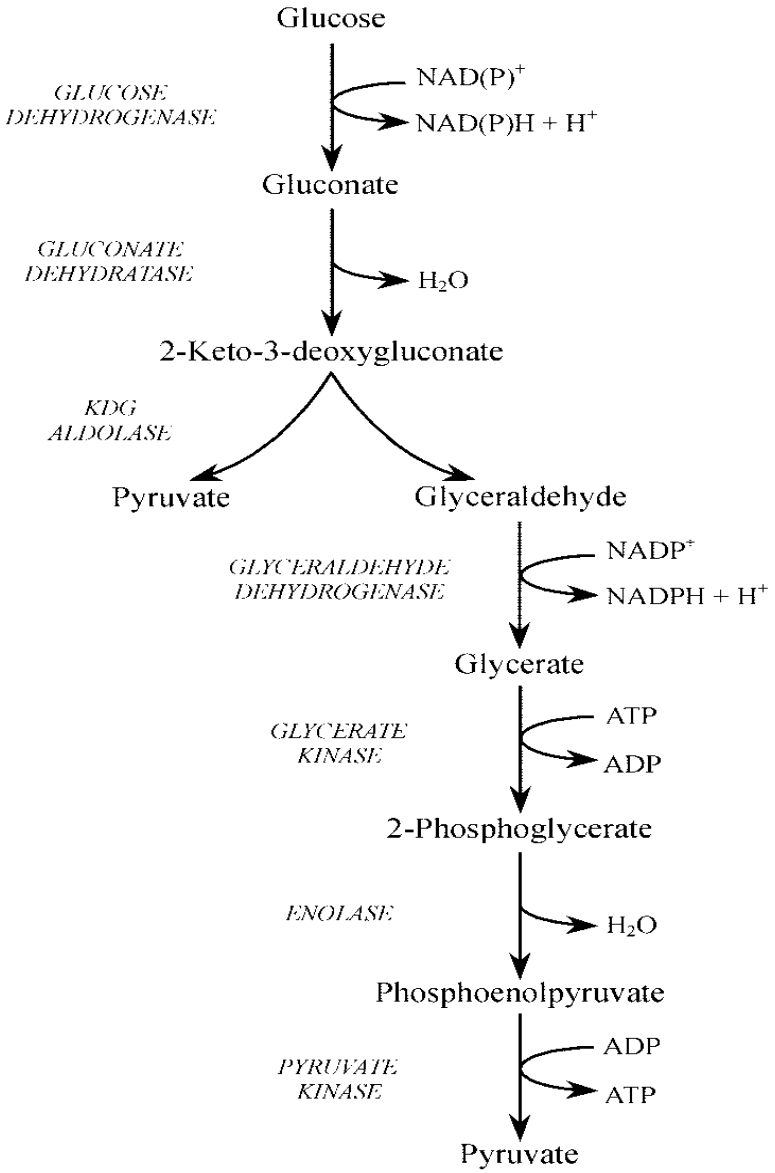



Figure 35: The Entner–Doudoroff Pathway (ED pathway)

**The Tricarboxylic Acid Cycle (TCA Cycle)**

The TCA also known as the **citric acid cycle** or **Krebs cycle,** is a series of chemical reactions used by all aerobic organisms to generate energy through the oxidation of acetate derived from carbohydrates, fats and proteins into $CO_2$ and chemical energy in the form of ATP. In the glycolytic pathways, the energy captured by the oxidation of glucose to pyruvate is limited to no more than two ATP generated by substrate- level phosphorylation, so during the aerobic respiration, the catabolic process continues by oxidizing pyruvate to three $CO_2$ through TCA cycle**.** The first step of this process employs a multi enzyme system called pyruvate dehydrogenase complex. It oxidize, cleaves and decarboxylate pyruvate to form two carbon- molecule, acetyl-coenzyme A (acetyl-CoA), is the starting point for the cycle (figure 36).

In the first reaction acetyl-coA is condensed with a 4C intermediate, oxaloacetate, to form citrate, a molecule with 6C. Citrate is rearranged to give isocitrate, a more readily oxidized. Isocitrate is subsequently oxidized and decarboxylated twice to yield -ketoglutarate (five carbons), and then succinyl-CoA (four carbons), a molecule with a high - energy bond.

At this point two NADH molecules have been formed and two carbons lost from the cycle as $CO_2$. The cycle continues when succinyl-CoA is converted to succinate. This involves breaking the high – energy bond in succinyl – CoA and using the energy released to form one GTP by substrate-level phosphorylation. GTP is also high – energy molecule and it is functionally equivalent to ATP. Two oxidation steps follow, yielding one FADH and one NADH. The last oxidation step regenerates oxaloacetate, and as long as there is a supply of acetyl-CoA the cycle can repeat itself. The TCA cycle generates two $CO_2$ molecules, three NADH molecules, one FADH, and one GTP for each acetyl-CoA molecule oxidized.

TCA cycle enzymes are widely distributed among microorganisms. In prokaryotes, they are located in the cytoplasmic matrix. In eukaryotes, they are found in the mitochondrial matrix. The complete cycle appears to be functional in many aerobic bacteria and fungi. This is not surprising because the cycle is such an important source of energy. Even those microorganisms that lack the complete TCA cycle usually have most of the cycle enzymes, because the TCA cycle is also a key source of carbon for use in biosynthesis.

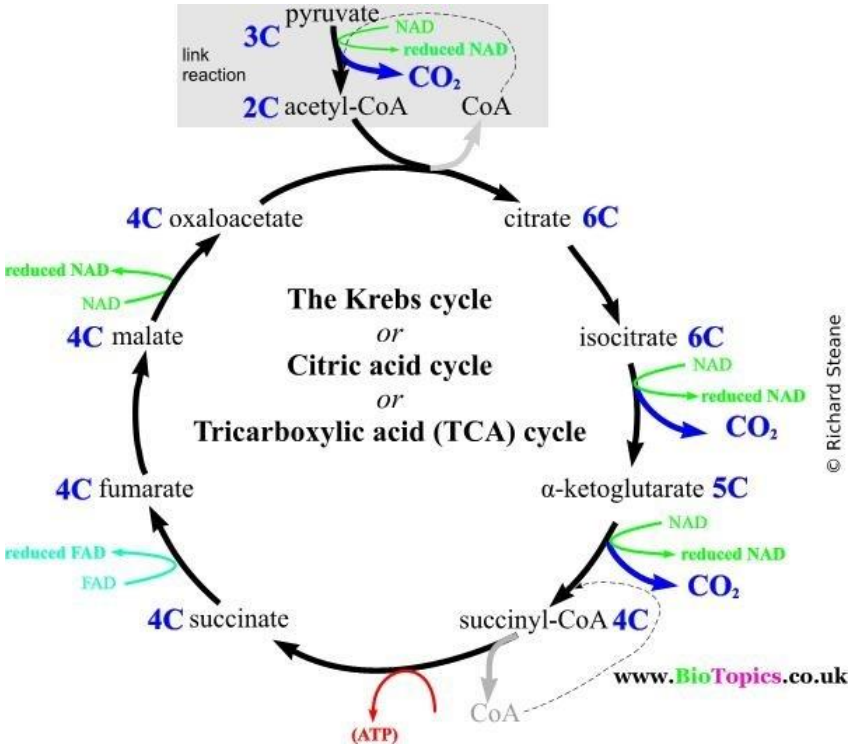



Figure 36: The Tricarboxylic Acid Cycle (TCA)

# Respiration

## Aerobic Respiration

Aerobic respiration is a series of enzymes – catalyzed reaction in which electrons are transferred from fuel molecule such as glucose to oxygen as a final electron acceptor. This pathway is the principal energy - yielding for aerobic microorganisms, and it provides both ATP and metabolic intermediates for many other pathways in the cell, including those of protein, lipid, and carbohydrate synthesis.

Aerobic respiration in microorganisms can be summarized by an equation:

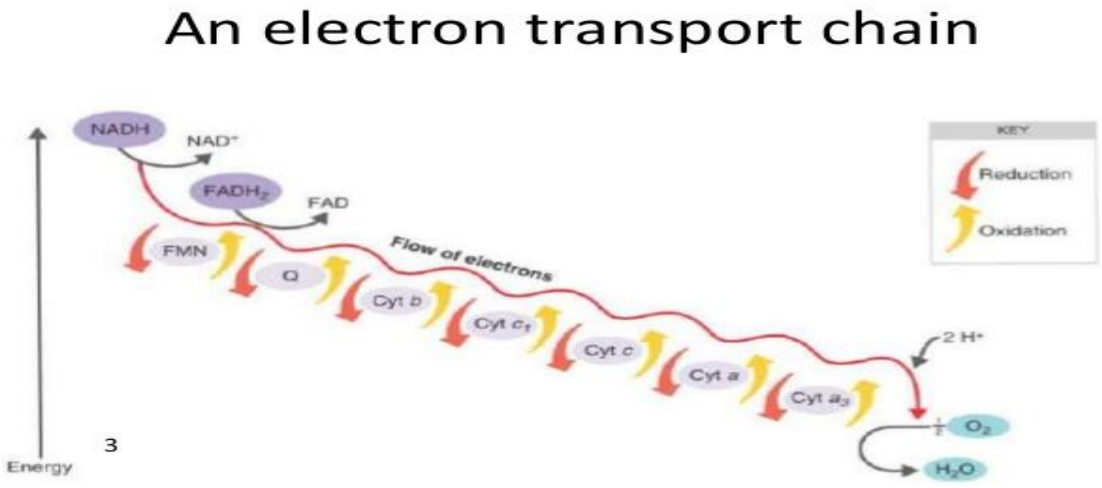
**Glucose ($C_6H_{12}O_6$) + $6O_2$ +38ADP + 38 Pi $\longrightarrow$ $6CO_2$ + $6H_2O$ + 38ATP**

## The Respiratory Chain: (Electron Transport System)

We now come to energy chain, which is the final "processing mill" for electron and hydrogen, and the major generator of ATP. Overall, the electron transport system (ETS) consists of a chain of special redox carriers that receive electrons from reduced carriers (NADH, $FADH_2$) generated by TCA cycle and glycolysis, and shuttle them in a sequential and orderly fashion. The flow of electrons down this chain is highly energetic and gives off ATP at various points. At its end, an enzyme catalyzes the final acceptances of electrons and hydrogen by oxygen, producing water. Some variety exists from one organism to another, but the principal compounds that carry out this complex reaction are:

**NADH dehydrogenase**, **flavoproteins**, **coenzyme Q** (**ubiquinone**), and **cytochromes**. These complex compounds contain a metal that readily facilitates receiving and donating electrons (being reduced and oxidized).
The highly compartmentalized structure of the respiratory chain is an important factor in its function. Not in that the electron transport carriers and enzymes are embedded in the inner mitochondrial membrane in eukaryotes. Bacteria carry them in the cell membrane (figure 37).

**Elements of electron Transport (the energy cascade):**
Although the biochemical details of this process are rather complicated, the basic reaction consists of a number of redox reactions. In general the seven carrier compounds and their enzymes are arranged in linear sequence and are reduced and oxidized in turn.
The sequence of electron carriers in the respiratory chain of most aerobic organisms is: (**1**) **NADH dehydrogenase**, (**2**) **flavin mononucleotide (FMN)**, (**3**) **coenzyme Q**, (**4**) **cytochrome *b*,** (**5**) **cytochrome *c1***, (**6**) **cytochrome *c*,** and (**7**) **cytochrome *aa3*** (figure 37).
Conveyance of the NADHs from TCA cycle and glycolysis to the first carrier sets in motion the remaining six steps. With each redox exchange, the energy level of the reactant is lessened. The released energy is captured and used by the ATP synthase complex station near the ETS.
Each NADH that enters electron transport gives rise to 3 ATPs. This coupling of ATP to electron transport is termed oxidative phosphorylation. Since FADH2 from the TCA cycle is metabolically equivalent to FMN, it releases only enough energy to synthesize 2 ATPs.



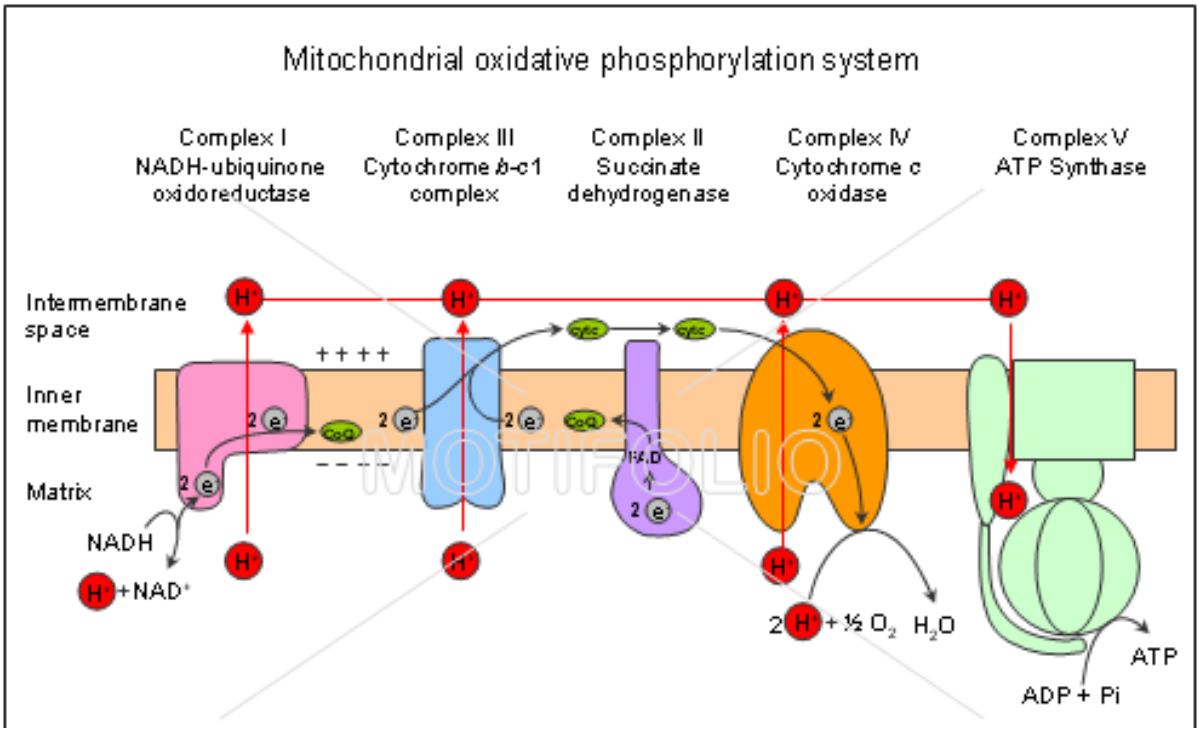



Figure 37: Electron Transport System in Prokaryotes

**The Theory of ATP formation by Oxidative Phosphorylation**

What biochemical processes are involved in the coupling electron transport to the production of ATP? As we know that in eukaryotes, the component of electron transport is embedded in a precise sequence on mitochondrial membrane. This stations essentially between two compartments: the inner mitochondrial matrix, and the outer mitochondrial membrane and cytoplasm. According to a widely accepted concept called the *chemiosmotic hypothesis*, as the electron transport carriers shuttle electrons, they actively pump hydrogen ions (protons) into the outer compartment of the mitochondrion. This process set up a concentration gradient of hydrogen ions called the proton motive force (PMF). The PMF also generates a difference in charge between the outer membrane compartment (+) and the inner membrane compartment (-). The potential energy inherent in such a separation of charge can be harnessed to produce ATP (figure 38). Although the exact mechanism is not completely understood, it appears that special pores penetrating the ATP synthase complex transport hydrogen ions (protons) from outside compartment back into the mitochondrial matrix. This process releases sufficient free energy for the synthesis of ATP from an ADP and pi. Bacterial ATP synthesis occurs by means of this same overall process. However, bacteria have the ETS stationed in the cell membrane and the direction of the proton movement is from the cytoplasm to the periplasmic space. In both cell types, the chemiosmotic theory has been supported by test showing that the

oxidative phosphorylation is blocked if the mitochondrial or bacterial cell membranes are disrupted.

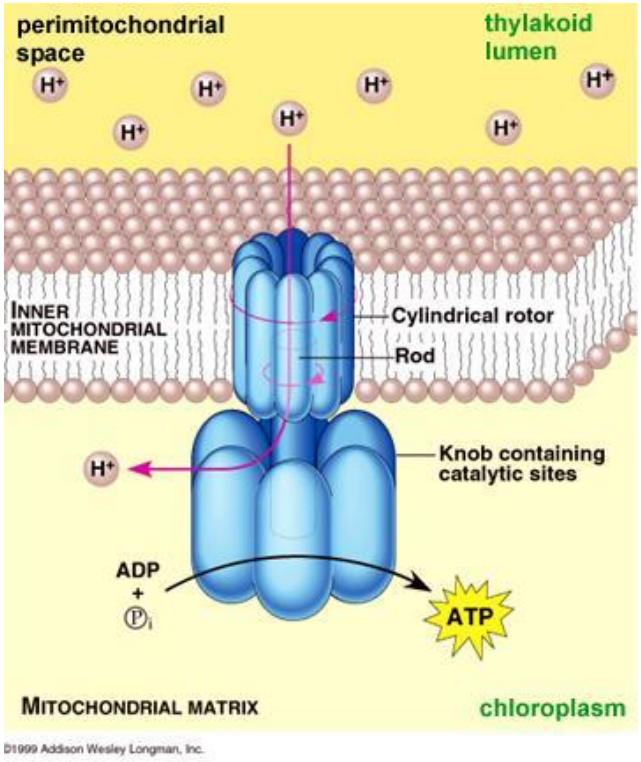



Figure 38: ATP formation by Oxidative Phosphorylation

**Yield of ATPs from oxidative phosphorylation**

The total of five NADHs (4 from TCA cycle and one from glycolysis) can be used to synthesize:

15 ATPs from ETS (5 ×3 pre electron pair)
And
15 × 2 = 30 ATPs per glucose

The single FADH2 producing during the TCA cycle results in:
2 × 2 = 4 ATPs per glucose

**Summary of aerobic respiration**

**(1)** The total yield of ATP is 40 molecules: 4 from glycolysis, 2 from TCA cycle, and 34 from electron transport.
However, since 2 ATPs were expended in early glycolysis, these have a maximum of 38 ATPs. The actual number may be lower in certain eukaryotic cell and bacteria.
**(2)** Six carbon dioxide molecules are generated during TCA cycle.
**(3)** Six oxygen molecules are consumed during electron transport.
**(4)** Six water molecules are reduced in electron transport and one in glycolysis, but one is send in the TCA cycle, this leaves a net of 6.

## Anaerobic respiration

Some bacteria have evolved an anaerobic respiratory system that function like aerobic cytochrome system except that it utilizes oxygen containing salts, rather than free oxygen, as the final electron acceptor. Of these, the nitrate ($NO_3$) and nitrite ($NO_2$) reduction system are best known. The reaction in species such as *Escherichia coli* is represented as:

Nitrite reductase

$NO_3 + H_2 \longrightarrow NO_2 + H_2O$

Reduction here is the removal of oxygen from nitrate. A test for this reaction is one of the best physiological tests in identifying bacteria.

Some species of *Pseudomonas* and *Bacillus* possess enzymes that can further reduce nitrate to nitric oxide (NO), nitrous oxide ($N_2O$), and even nitrogen gas ($N_2$). This process called denitrification is very important step in recycling nitrogen in the biosphere. Other oxygen containing nutrients reduced anaerobically by various bacteria are carbonates and sulfates. None of anaerobic pathways produce as much ATP as aerobic respiration

## *Fermentation*

Some chemoorganotrophic microbes do not respire because either they lack electron transport chain or they repress the synthesis of electron transport chain component under some conditions, making anaerobic respiration impossible. Yet NADH produced by glycolysis pathway must still be oxidized back to NAD. If NAD is not regenerated, oxidation of glyceraldehyde 3-phosphate will stop and glycolysis will stop. Many microorganisms solve this problem by slowing or stopping pyruvate dehydrogenase activity and using pyruvate or one of its derivatives as an electron acceptor for reoxidation of NADH in fermentation process (figure 39).

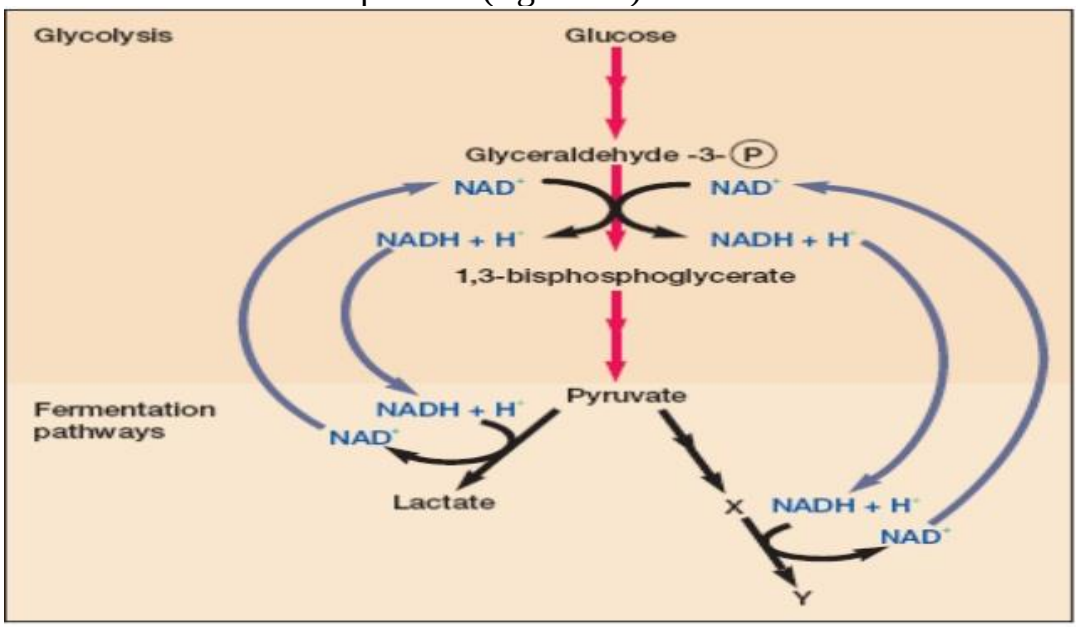



Figure 39: Reoxidation of NADH during Fermentation

There are many kinds of fermentation, and they often characteristic of particular microbial groups. Three unifying themes should be kept in mind when microbial fermentation is examined:

**(1)** NADH is oxidized to NAD

**(2)** The electron acceptor is often either pyruvate or pyruvate derivatives.

**(3)** Oxidative phosphorylation cannot operate.

**(4)** ATP is generated exclusively by Substrate level phosphorylation, and oxygen not participates.

**Major pathways for fermentation of sugars**

1- Alcoholic fermentation
2- Lactic acid fermentation: homolactic fermentative and heterolactic fermentative
3- Propionate fermentation
4- Mixed acid fermentation
5- Formic acid fermentation
6- Butarate and acetone – butanol fermentation

# Microbial physiology

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