STUDY MATERIAL FOR B.Sc MICROBIOLOGY

MICROBIAL PHYSIOLOGY AND METABOLISM

SEMESTER – II

ACADEMIC YEAR 2023-24

INDEX

MICROBIAL PHYSIOLOGY AND METABOLISM
SYLLABUS

UNIT – I

Physiology of microbial growth: Batch – continuous - synchronous cultures; Growth Curve and measurement method (turbidity, biomass, and cell count). Control of microbial growth.

UNIT – II

Nutrition requirements - Photoautotrophs, Photoorganotrophs, Chemolithotrophs (Ammonia, Nitrite, Sulfur, Hydrogen, Iron oxidizing Bacteria), Chemoorganotrophs. Nutrition transport mechanisms – Passive diffusion and Active transport. Factors affecting microbial growth.

UNIT - III

An overview of Metabolism - Embden Meyerhof Pathway, EntnerDoudoroff Pathway, Pentose Phosphate Pathway, Tricarboxylic Acid Cycle. Electron Transport Chain and Oxidative Phosphorylation. ATP synthesis. Fermentation-Homolactic Fermentation, Heterolactic Fermentation, Mixed Acid Fermentation, Butanediol Fermentation.

UNIT – IV

Photosynthesis - An Overview of chloroplast structure. Photosynthetic Pigments, Light Reaction-Cyclic and non-cyclic Photophosphorylation. Dark Reaction - Calvin Cycle.

UNIT- V

Bacterial reproduction - Binary fission, Budding, Reproduction through conidia, cyst formation, endospore formation. Fungi asexual and sexual reproduction, Microalgae reproduction. Asexual and sexual reproduction of protozoa.

UNIT – I

PHYSIOLOGY OF MICROBIAL GROWTH

Physiology of Microbial Growth:

- In batch fermentation, all the medium components are placed in the reactor at the start of cultivation except for atmospheric gases, acid or base for pH control, and antifoaming agents.
- There is a continuous change in the nutrient concentrations over time, and the system remains unsteady.
- The microbial metabolites may be produced at a primary or secondary stage of the microbial cultivation period.
- Fermentation is terminated when either all the nutrient is exhausted or the desired concentration of product is achieved.

Batch culture has the following mention advantages:

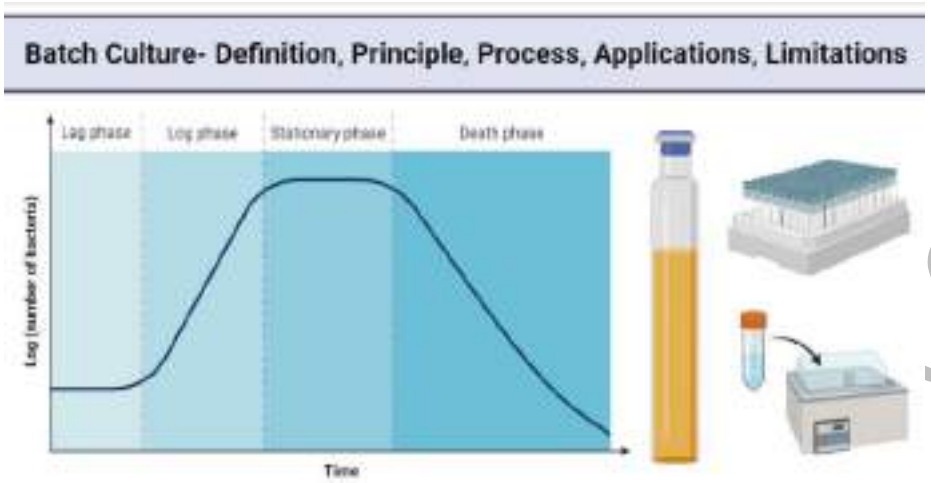
1. Reduced risk of contamination or cell mutation as the growth period is short.
2. Lower capital investment when compared to continuous processes for the same bioreactor volume.
3. More flexibility with varying product/biological systems.
4. Higher raw material conversion levels, resulting from a controlled growth period.

OTD in Space - November 12: Philae Spacecraft Gets Lost On Comet

Batch culture principle:

- A batch fermentation system is a closed system.
- At time t=0, the sterilized nutrient solution in the fermenter is inoculated with microorganisms, and incubation is allowed to proceed at a suitable temperature and gaseous environment for a suitable period.
- In the course of the entire fermentation, nothing is added, except oxygen (in the case of aerobic microorganisms), an antifoam agent, acid, or base to control pH.
- The composition of the medium, the biomass concentration, and the metabolite concentration generally change constantly as a result of the metabolism of the cells.
- After the inoculation of a sterile nutrient solution with microorganisms and cultivation under physiological conditions, four typical phases of growth are observed.

- Lag phase
- Log phase
- Stationary phase
- Death phase



BATCH CULTURE PROCESS:

- A batch culture begins with sterilization, and the sterile culture is then inoculated with microbes (about 2-5 % of the total volume).
- The percentages of nutrients, vitamins, and microbial cells in the reaction mixture and the temperature difference during the reaction cycle.
- The proper mixing keeps them at acceptable concentrations and temperatures.
- The process is carried out in anaerobic conditions by bubbled oxygen in or out, while acidic or alkaline solutions are added to control the pH.
- Antifoaming agents are added when indicated by a foam sensor.
- The microorganism growth is allowed to take place for days, weeks, or months.
- In the lag phase, little or no growth is observed at the beginning of fermentation, depending on a physiochemical equilibrium between the microorganism and the environment.
- Once the cells have adapted to the new conditions of growth, they enter the exponential phase.
- Primary metabolites are produced during the log or exponential phase with their formation decreasing when growth ceases. For example, Saccharomyces cerevisiae produces ethanol as a primary metabolite.

- When the cells enter the stationary phase, secondary metabolites are produced. Most antibiotics are metabolized as secondary metabolites. For example, Penicillium chrysogenum produces Penicillin as a secondary metabolite.
- The fermentation is terminated when one or more of the following has been reached:
- The microbial growth has stopped due to the depletion of the nutrients or the build of toxic compounds;
- After a fixed predetermined period;
- The concentration of desired product has been achieved.

Applications:

1. It is beneficial for the construction of biomass (Baker's yeasts) and primary metabolites (lactic acid, citric acid, acetic acid, or ethanol production).
2. In food industries, organic acids are used as preservatives or acidifiers(lactic acids, citric acids, and acetic acids), alcoholic beverages (wine, beer, and distilled spirits i.e. brandy, whisky, and rum), and sweeteners (e.g., aspartate) or amino acids used as flavouring agents (e.g., monosodium glutamate)are the various product manufactured by batch cultivation. Top of Form Bottom of Form

Batch culture Limitations:

1. Due to nutrient consumption and waste build-up, microbes are exposed to constantly changing environmental conditions in batch culture.
2. After reaching an endpoint, batch cultures must be restarted. In large bioreactors, it takes a long time to empty, clean, and refill the reactor.
3. In batch culture, the low productivity is a consequence of the high downtime (nonproduction time spent cleaning, sterilizing, and starting up another batch cultivation) during two consecutive batches.
4. A build up of toxic metabolites can sometimes inhibit cell growth and product synthesis.

Continuous culture

Continuous culture is a continuous process where nutrients are continually added to the bioreactor and the culture broth (containing cells and metabolites) is removed at the same time. The volume of the culture broth is constant due to a constant feed-in and feed-out rate (i.e consumed nutrients are replaced and toxic metabolites are removed from the culture).

Some advantages of continuous culture are:

1. In continuous culture, keeping the working volume constant simplifies culture scale-up based on a constant- power-to-volume strategy.

2. It is possible to set up the optimum conditions for maximum and long-term product synthesis.
3. Ability to obtain stable product quality (the steady-state consists of homogeneous cell culture with a constant biomass and metabolite concentration).
4. It also results in higher productivity per unit volume, as time-consuming tasks, such as cleaning and sterilization are unnecessary.
5. Cultures in a steady-state can last for days, weeks, or even months, thus greatly reducing the downtime and making the process more economically competitive.

Principles of continuous culture:

A continuous flow system consists of a reactor into which reactants are pumped at a steady rate and from which products are emitted.

The factors governing their operation are:

- how material passes through the reactor (which depends upon its design);
- the kinetics of the reaction taking place.
- In continuous culture, growth-limiting nutrients can be maintained at steady-state concentrations, which permits microorganisms to grow at submaximal rates.
- In a steady-state, the cellular growth rate and environmental conditions, like the concentrations of metabolites, stay constant
- Moreover, in continuous culture, parameters such as pH, oxygen tension, the concentration of excretion products, and population densities can easily be monitored and controlled.

Process of continuous culture:

- In continuous culture, an open system is set up in which one or more feed streams containing the necessary nutrients are fed continuously, while the effluent stream containing the cells, products, and residuals is continuously removed.
- A steady-state is established by maintaining an equal volumetric flow rate for the feed and effluent streams.
- The culture volume is kept constant, and all nutrient concentrations remain at constant steady-state values.
- During this process, the exponential growth phase is prolonged and the formation of byproducts is avoided.

- Continuous fermentation is monitored either by microbial growth activity or by-product formation, and these processes are referred to as-

A. Turbidostat method

- Cell growth is controlled and remains constant in this system, but the flow rate of fresh media varies.
- Cell density is controlled based on the set value for turbidity, which is created by the cell population while fresh media is continuously supplied.

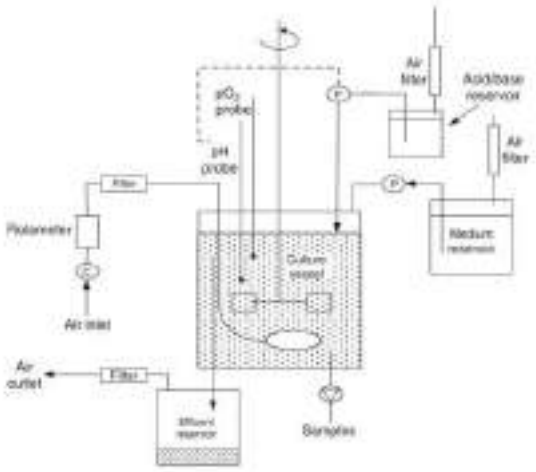


Fig: Turbidostat with light source used to detect cell turbidity and bacterial optical density.

B. Chemostat method

- In a chemostat, the nutrients are continuously supplied at a constant flow rate, and the density of the cells is adjusted according to the supplied essential nutrients for growth.
- In a chemostat, the growth rate is determined by adjusting the concentration of substrates like carbon, nitrogen, and phosphorus.

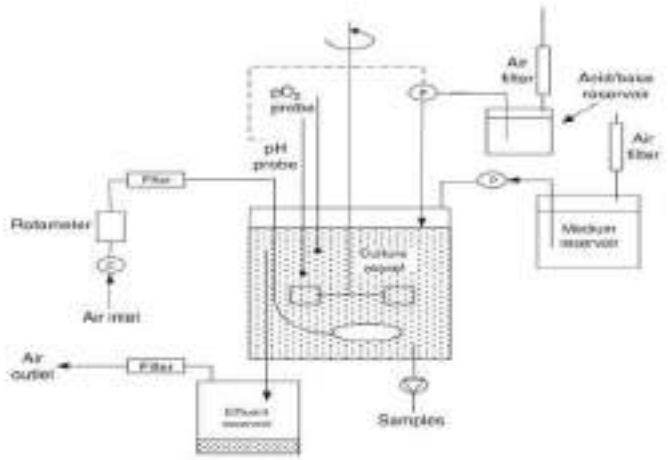
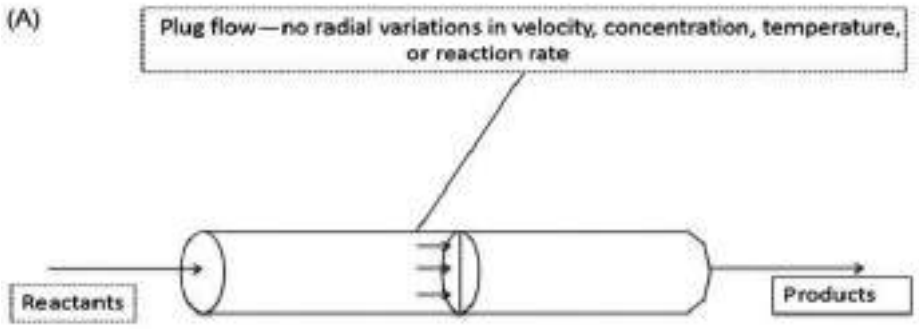


Fig: Chemostat continuous culture. The continuous cultures of chemostat and turbidostat systems have the following criteria:

- Medium and cells are continuously changing
- The cell density is constant
- Steady-state growth
- Open system

C. Plug-flow reactor

- In this type of continuous culture, the culture solution flows through a tubular reactor without back mixing.
- In a plug flow reactor, nutrients (reactants) enter the reactor in the form of "plugs," which flow in an axial direction through the reactor.
- The culture medium flows steadily through a tube and the cells are recycled from the outlet to the inlet.



Application of Continuous culture:

Continuous culture fermentation has been used for the production of single-cell protein, organic solvents, starter cultures, etc.

It has been used in the production of beer, fodder yeast, vinegar, baker's yeast, etc.

In the industrial production of secondary metabolites (such as antibiotics from a Penicillium or a Streptomyces sp.)

Continuous culture has been tested for L-lysine-producing C. glutamicum mutant B-6

It has been used in municipal waste treatment processes.

Limitations of Continuous culture

1. In the long-term cultivation process, sterility maintenance can be tricky, and downstream processing can prove challenging.
2. Controlling the production of some non-growth-related products is not easy Because of this, continuous culture often requires fed-batch culturing as well as continuous nutrient supply.
3. As a result of the viscosity and heterogeneous nature of the mixture, it may be challenging to maintain filamentous organisms.
4. If a faster-growing strain overtakes the original product strain, it may be lost over time.

Synchronous culture:

A synchronous or synchronized culture is a microbiological culture or a cell culture

- The Synchronous culture also known as the synchronous growth.
- The synchronous growth of a bacterial culture means all the cells of the culture remain at the same stage of growth and they grow all together from one phase to another.
- A population can be synchronized by manipulating their physical environments or physical composition of the medium.

If we keep an organism under unfavourable conditions there they will metabolize very slowly, but will not divide. If we keep the organisms under favourable conditions, then all cells undergo division and stay at the same phase.

- The cells of the synchronously growing culture divide at a time, their growth curve forms a Zig Zag pattern.
- The easiest way to synchronize bacterial growth is to add some cytostatic agents so that cells don't divide and they all maintain the same state of metabolism and cell cycle. When the cytostatic agent is removed, all cells start to divide at the same time.
- Synchronous culture/Synchronous growth of bacteria consists of two phases such as stationary phase and exponential phase.

Obtaining synchronous culture:

There are present different method for obtaining a Synchronous culture such as;

To arrest the growth of all cells in the culture, alter the External conditions, and then again alter the conditions to continue the growth. As a result of this, all the fresh growing cells are now beginning to grow at the same stage, and they are synchronized.

- Synchronous culture can be obtained by eliminating the essential nutrient from the growth medium and later re-introduce it.
- The chemical growth inhibitors can be used to stop cell growth. When the growth is completely stopped for all cells, then remove the inhibitor from the culture and the cells will begin to grow synchronously. For example, Nocodazole is used in biological research for synchronization.
- Cells in various growth stages contain distinct physical properties. Cells in culture can thus be physically divided depending on their density or size, for instance. This can be done by using centrifugation (for density) or filtration (for size).
- The Helmstetter-Cummings technique can be used to obtain a Synchronous culture. In this method, the bacterial culture is passed through a membrane. Some of these cells will remain bound to the membrane. After that a fresh medium is added to the membrane, as result, the membrane-bound bacteria will start to grow. Fresh bacteria that separated from the membrane are now all at the identical stage of growth; they are accumulated in a flask that now harbors as a synchronous culture.

Application of synchronous culture:

- Synchronous culture helps in the separation of the smallest cells from an exponentially growing culture. In the laboratory it is used to study the cell cycle.

GROWTH CURVE:

Bacterial Growth

Bacteria are unicellular organisms that tend to reproduce asexually by the means of binary fission. Bacterial growth is the increase in the number of bacterial cells rather than the increase in their cell size. The growth of these bacterial cells takes place in an exponential manner, i.e., one cell divides into 2, then 4, then 8, 16, 32 and so on.

The time taken for a bacterial cell to double is called generation time. The generation time varies among different species of bacteria based on the environmental conditions they grow in. Clostridium perfringens is the fastest growing bacteria that has a generation time of 10 minutes while Escherichia coli has a doubling time of 20 minutes. Mycobacterium tuberculosis is one of the slowest growing bacteria, taking about 12 to 16 hours to double.

Growth Curve

In a closed system with enough nutrients, a bacteria shows a predictable growth pattern that is the bacterial growth curve. It consists of four different phases. Read on to learn about the phases in detail.

Phases of the Bacterial Growth Curve

Upon inoculation into a new nutrient medium, the bacteria shows four distinct phases of growth. Let us dive into each of the phases in detail –

Lag Phase

The bacteria upon introduction into the nutrient medium take some time to adapt to the new environment. In this phase, the bacteria does not reproduce but prepares itself for reproduction. The cells are active metabolically and keep increasing in size. The cells synthesise RNA, growth factors and other molecules required for cell division.

Log Phase

Soon after the lag phase, i.e., the preparation phase, the bacterial cells enter the log phase. The log phase is also known as the exponential phase. This phase is marked by the doubling of the bacterial cells. The cell number increases in a logarithmic fashion such that the cell constituent is maintained. The log phase continues until there is depletion of nutrients in the setup. The stage also comes to a stop if toxic substances start to accumulate, resulting in a slower growth rate. The cells are the healthiest at this stage and researchers prefer to use bacteria from this stage for their experimental processes.

Plotting this phase on the bacterial growth curve gives a straight line. Upon calculation of the slope of this line, the specific growth rate of the organism is obtained. It is the measure of divisions per cell per unit of time.

Stationary Phase

In the stationary phase, the rate of growth of the cells becomes equal to its rate of death. The rate of growth of the bacterial cells is limited by the accumulation of toxic compounds and also depletion of nutrients in the media. The cell population remains constant at this stage. Plotting this phase on the graph gives a smooth horizontal linear line.

Death Phase

This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells. Lack of nutrients, physical conditions or other injuries to the cell leads to death of the cells.

MEASUREMENTS OF MICROBIAL GROWTH

INTRODUCTION

- When a few bacteria are inoculated into a liquid growth medium or any solid culture media and the population is counted at intervals.
- The growth of microbial populations can be measured in a number of ways. Some methods measure cell numbers; other methods measure the population's total mass, which is often directly proportional to cell numbers.
- It is orderly increase in a cellular constituent.
- When microorganisms reproduce by binary fission or budding then it also leads to increase in the number of cells.

MEASUREMENTS OF MICROBIAL GROWTH

- There are various ways to measures microbial growth for the determination of growth rates and generation times.
- For the measurement of growth either mass or population number is followed because growth leads to increase in both.
- Growth can be measured by one of the following types of measurements:

1. **Cell count** this method involves the measurement of growth either by microscopy or by using an electronic particle counter or indirectly by a colony count.
2. **Cell mass** in this growth can be measured directly by weighing or by a measurement of nitrogen concentration in cells or indirectly by the determination of turbidity using spectrophotometer.
3. **Cell activity** in this growth can be measured indirectly by analysis of the degree of biochemical activity to the size of population.

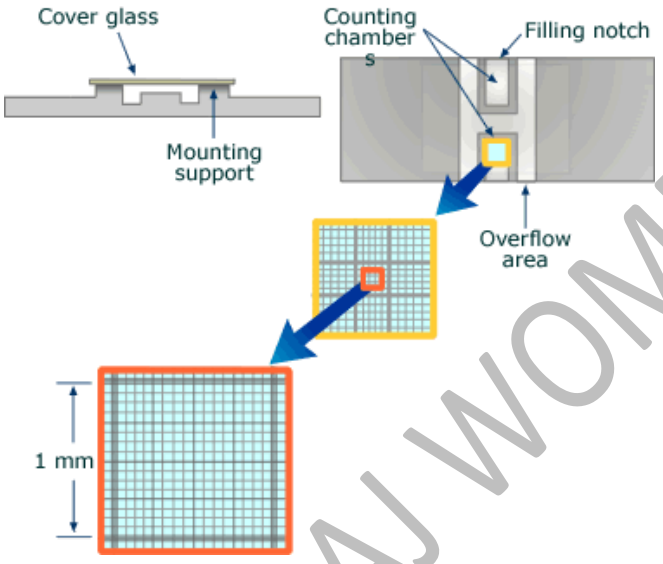
SOME SPECIFIC PROCEDURE WILL ILLUSTRATE THE APPLICATION OF EACH TYPE OF MEASUREMENT

1. Direct microscopic count
2. Electronic enumeration of cell numbers
3. The plate count method
4. Turbidity estimation of bacterial numbers
5. Determination of nitrogen content
6. Determination of dry weight of cells

7. Filtration method
8. Most Probable Number (MPN) Method

DIRECT MICROSCOPIC COUNT

- The most obvious way to count microbial numbers is through direct counting.
- Petroff-hausser counting is one of the easiest and accurate way to count bacteria.
- Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension.
- A top view of the chamber. The grid is located in the center of the slide.
- An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at X400 to X500 magnification.

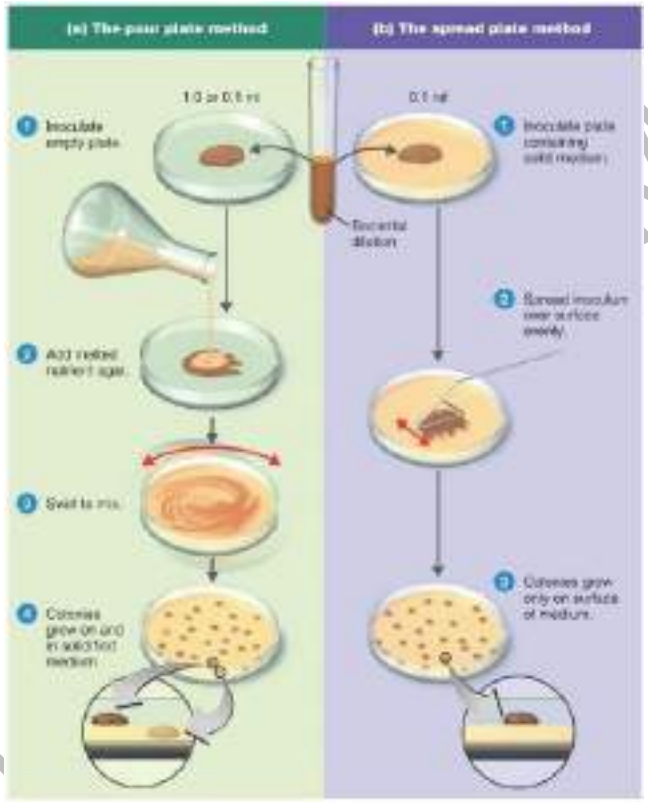


- Concentration of the cells can be calculated by using the average no. of bacteria the avg. number of bacteria in these squares.
- There are 25 squares covering a part of area of 1 mm2, then the entire number of bacteria in 1 mm2 of the chamber is (number/square) (25 squares). The chamber is 0.02 mm deep and thus, bacteria/mm3 = (bacteria/square) (25 squares) (50).
- The amount of bacteria per cm3 is 103 times this value. For example, imagine the average count per square is 28 bacteria: bacteria/cm3 = (28 bacteria) (25 squares) (50) (103) = 3.5X 107.

ELECTRONIC ENUMERATION OF CELL NUMBERS

- In this method of microbial growth measurement, bacterial suspension is kept inside an electronic particle counter, within which the bacteria are passed through tiny orifice 10 to 30 µm in diameter.
- This orifice is then connected to the two compartments of the counter which contains an electrically conductive solution.
- The electrical resistance between two compartments will increases momentarily, when bacterium passes through the orifice. This generates an electrical signal which is automatically counted.
- The main disadvantage of this method is that there is no way to determine whether the cell counted is viable or not.

THE PLATE COUNT METHOD

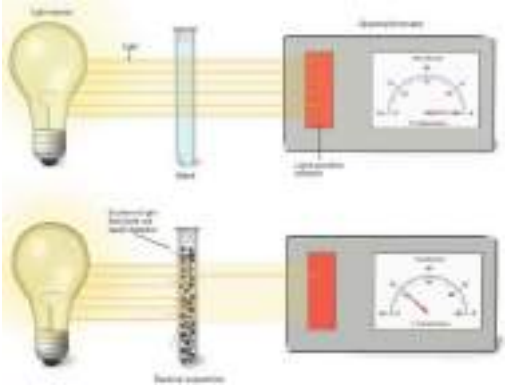


Methods of preparing plates for plate counts. (a) The pour plate method. (b) The spread plate method.

- This method allows the determination of the number of cells that will multiply under certain defined conditions.

- Plate count method can be done in two ways either by spread plate method or by pour plate method.
- This method of bacterial counting is most commonly used with satisfactory results for the estimation of bacterial populations in milk, water, foods and many other materials.
- This technique has some drawbacks because some relatively heat-sensitive microorganisms may be damaged by the melted agar and will therefore be unable to form colonies

TURBIDITY ESTIMATION OF BACTERIAL NUMBERS



- For some experimental work, turbidity is a only practical way of monitoring bacterial growth.
- actical way of monitoring bacterial growth. As bacteria multiply in a liquid medium, the medium becomes turbid, or cloudy with cells.
- Turbidity is the Cloudiness or haziness of a media or fluid caused by large no. of individual particles.
- The instrument used to measure turbidity is a spectrophotometer (or colorimeter).
- Microbial mass can be determined by determination of absorption of light.
- In the spectrophotometer, a beam of light is transmitted through a bacterial suspension to a light-sensitive detector, as the bacterial numbers increase, less light will reach the detector.
- As the population increases, absorbance of the light increases by the cells, so the turbidity also increases. Turbidity can be measured by using an instrument spectrophotometer.
- The absorbance is used to plot bacterial growth.

DETERMINATION OF NITROGEN CONTENT

- The major constituents of cell material are protein, and since nitrogen is characteristics part of proteins.
- Bacterial population or cell crop can measure in terms of bacterial nitrogen.
- In this growth can be measured by first harvesting the cells and wash them free of medium and then perform a quantitative chemical analysis of nitrogen.

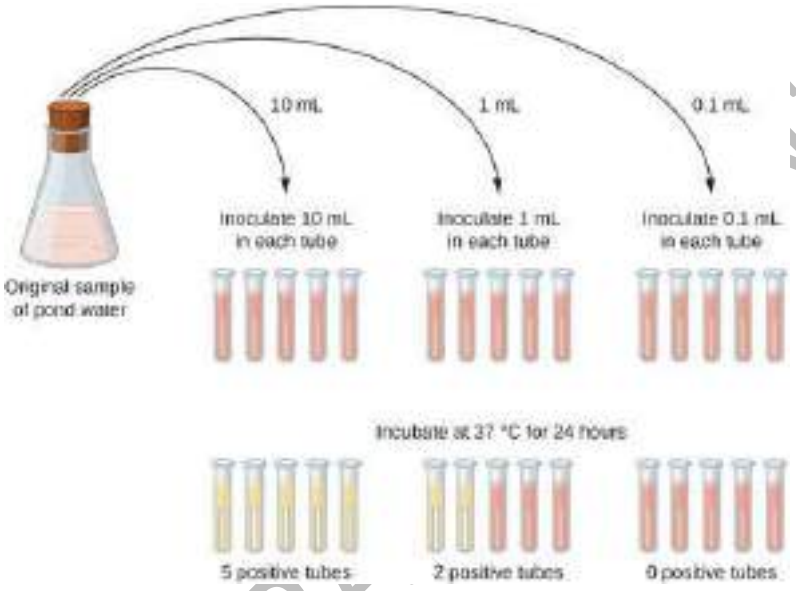
DETERMINATION OF DRY WEIGHT OF CELLS

- For filamentous bacteria and molds, the usual measuring methods are less satisfactory. A plate count would not measure this increase in filamentous mass.
- In plate counts of actinomycetes and molds, it is mostly the number of asexual spores that is counted instead.
- This is not a good measure of growth. One of the better ways to measure the growth of filamentous organisms is by dry weight.
- In this procedure, the fungus is removed from the growth medium, filtered to remove extraneous material, and dried in a desiccator, it is then weighed.
- Growth measurement by measuring cell mass is one of the easiest ways, a known volume of culture sample from the ferment or withdrawn and centrifuged.
- It is the most direct approach for quantitative measurement of a mass of cells.

COUNTING BACTERIA BY FILTRATION METHOD

- When the number of bacteria is extremely few, as in lakes or relatively pure streams, bacteria are often counted by filtration methods.
- During this technique, a minimum of 100 ml of water are passed through a thin membrane filter whose pores are too tiny to permit bacteria to pass.
- After filtration bacteria are filtered out and present on the surface of the filter. Then filter is transferred to a Petri plate containing a in liquid nutrient medium, where colonies grow from the bacteria on the filter's surface.
- This method is applied frequently to detection and enumeration of coliform bacteria, which are indicators of fecal contamination of food or water.

MOST PROBABLE NUMBER (MPN) METHOD



- Another method for determining the number of bacteria in a sample is the most probable number (MPN) method.
- This statistical estimating technique is based on the fact that the greater the number of bacteria in a sample, the more dilution is needed to reduce the density to the point at which no bacteria are left to grow in the tubes in a dilution series.
- The MPN method is most useful when the microbes being counted will not grow on solid media (such as the chemoautotrophic nitrifying bacteria).
- It is also useful when the growth of bacteria in a liquid differential medium is used to identify the microbes (such as coliform bacteria, which selectively ferment lactose to acid, in water testing).

- The MPN is only a statement that there is a 95% chance that the bacterial population falls within a certain range and that the MPN is statistically the most probable number.

CONTROL OF MICROBIAL GROWTH:

The control of microbial growth may involve sterilization, disinfection, antisepsis, sanitization, or degerming. Sterilization is the destruction of all forms of microbial life, with particular attention to bacterial spores. Disinfection and antisepsis both refer to destruction of microbial pathogens, although some organisms, such as bacterial spores, may remain alive. Disinfection refers to the destruction of pathogenic organisms on an inanimate (lifeless) object, such as a table-top, while antisepsis refers to that destruction on a living object, such as the skin surface.

Sanitization refers to the reduction in the number of pathogens to a level deemed safe by public health guidelines. Degerming is the physical removal of microorganisms by using such things as soaps or detergents.

Any chemical agent that kills microorganisms is known as a germicide. An agent that destroys bacteria is called a bactericide, one that kills fungi is a fungicide, and one that kills viruses is a viricide. A bacteriostatic agent prevents the further multiplication of bacteria without necessarily killing all that are present.

Among the conditions affecting the use of a germicide are temperature, the type of microorganism, and the environment. Germicides are more effective at high temperatures because the chemical breaks down at lower temperatures. Microorganisms vary in their susceptibility depending on such things as the composition of their cell wall, the presence or absence of a capsule, and the ability to form spores or cysts. The environment can affect the activity of a germicide, as, for example, when organic matter is present. This material shields microorganisms from germicides and often reacts with the germicide.

Physical Methods of Control

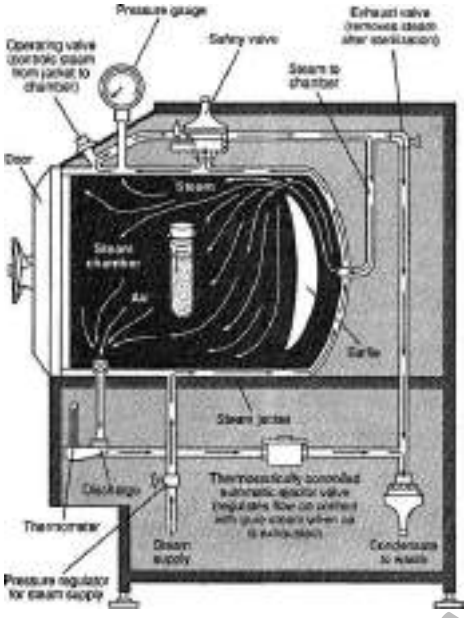
Physical methods for controlling the growth of microorganisms can be divided into heat methods and nonheat methods. The lowest temperature at which all microorganisms are killed in 10 minutes is the thermal death point, while the minimum amount of time required to kill microorganisms at a given temperature is known as the thermal death time. The time for destruction of 90 percent of the microbial population is the decimal reduction time.

Dry heat. Dry heat kills microorganisms by reacting with and oxidizing their proteins. Dry heat can be used in incineration devices, such as the Bunsen burner or the hot-air oven. In the hot-air oven, a temperature of about 170°C for two hours will bring about sterilization.

Moist heat. Moist heat is used to kill microorganisms in such things as boiling water. Most vegetating microorganisms are killed within two or three minutes, but over two or three hours may be required for destruction of bacterial spores. In moist heat, the microbial proteins

undergo denaturation, a process in which the three-dimensional form of the protein reverts to a two-dimensional form, and the protein breaks down.

Moist heat is used in the autoclave, a high-pressure device in which steam is superheated (Figure 1). Steam at 100°C is placed under a pressure of 15 pounds per square inch, increasing the temperature to 121°C. At this temperature, the time required to achieve sterilization is about 15 minutes. The autoclave is the standard instrument for preparing microbial media and for sterilizing instruments such as syringes, hospital garb, blankets, intravenous solutions, and myriad other items.



The autoclave, a pressurized steam generator used for sterilization processes.

Although pasteurization is used to lower the bacterial content of milk and dairy products, it does not achieve sterilization. The conditions of pasteurization are set up to eliminate the tuberculosis bacillus and the rickettsia that causes Q fever. Milk is pasteurized for 30 minutes at about 62°C or for 15 to 17 seconds at about 72°C. The first method is known as the holding method, the second method as the flash method. Dairy products can be pasteurized at 82°C for three seconds, a process known as ultra pasteurization.

An alternative heating method is tyndallization, also called intermittent sterilization. Liquids and other items are subjected to free-flowing steam for 30 minutes on each of three successive days. During the first day, all vegetating microorganisms, except spores, are killed. In the overnight period, the spores germinate, and they are killed by the steam on the second day. The last few remaining spores germinate on the second evening and are killed on the third day.

Non heat methods. A number of non heat methods are also available to control the growth and presence of microorganisms. Among these is filtration, a process in which a liquid or gas passes through a series of pores small enough to retain microorganisms. A vacuum can be created

to help pull the liquid or gas through the filter. A filter is often used when heat-sensitive materials such as vaccines are to be sterilized.

Filter materials can be of various types. For example, certain filters consist of diatomaceous earth, the skeletal remains of diatoms. Membrane filters composed of nitrocellulose can also be used. The effectiveness of the filter depends upon the pore size, which can be established to trap the microorganisms desired. For instance, if bacteria are to be removed, the pore size would be about 0.15 µm, while if viruses are to be removed, the pores size should be about 0.01 µm.

Drying can be used to control the growth of microorganisms because when water is removed from cells, they shrivel and die. To dry foods, they are mixed with salt or sugar. Either draws water out of microbial cells by osmosis, or they quickly die. One method for achieving drying is lyophilization, a process in which liquids are quick-frozen and then subjected to evacuation, which dries the material. Salted meat and sugared fruits are preserved this way.

Cold temperatures are used in the refrigerator to control microbial growth. At low temperatures, microbial metabolism slows considerably, and the reproductive rate is reduced. However, cold temperatures do not necessarily kill microorganisms. At freezing temperatures, ice crystals kill many microorganisms present.

Radiations are also used to control microorganisms when food or other materials are subjected to gamma rays or X rays. The radiations change the chemical composition of microorganisms by forming ions in the organic materials of the cytoplasm. Highly reactive toxic radicals also form.

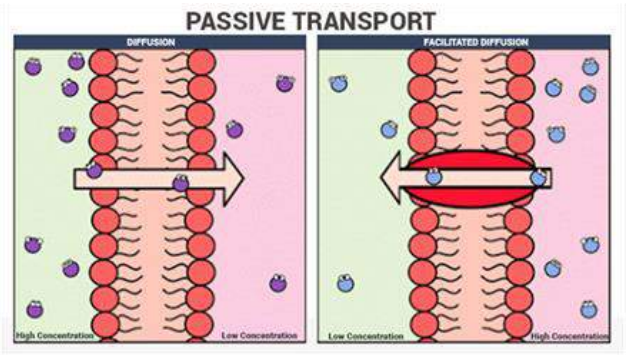
Nonionizing radiations are typified by **ultraviolet light.** Ultraviolet light affects the nucleic acids of microorganisms, inducing adjacent thymine residues in DNA molecules to bind to one another forming dimers. This binding changes the character of the DNA, making it unable to function in protein synthesis. Cell death soon follows. Although microwaves are a form of radiation, their direct effect on microorganisms is minimal. Microwaves induce water molecules to vibrate at high rates, creating heat. The heat is the killing agent rather than the microwaves.

UNIT – II

NUTRITION TRANSPORT MECHANISM

PASSIVE TRANSPORT:

Passive transport is the fundamental movement of ions and other molecular substances within the cells along the concentration gradient, without any external energy. It is also known as passive diffusion.



Passive Transport

Types of Passive Transport

There are four types of passive transport:

1. Simple Diffusion
2. Facilitated Diffusion
3. Filtration
4. Osmosis

Simple Diffusion

Types of Passive Transport – Simple Diffusion

Diffusion is the movement of substances from a region of higher concentration to a lower concentration. The difference in the concentration of the two areas is termed a concentration

gradient and the process of diffusion continues until this gradient neutralizes. Diffusion occurs in liquids and gases because their particles move randomly from one place to another. It is an important process in living things required for different life processes. The substances move in and out of the cells by simple diffusion.

Facilitated Diffusion

Facilitated diffusion is the passive transportation of ions or molecules across the cell membrane through specific transmembrane integral proteins. The molecules, which are large and insoluble require a carrier substance for their transportation through the plasma membrane. This process does not require any cellular or external energy.

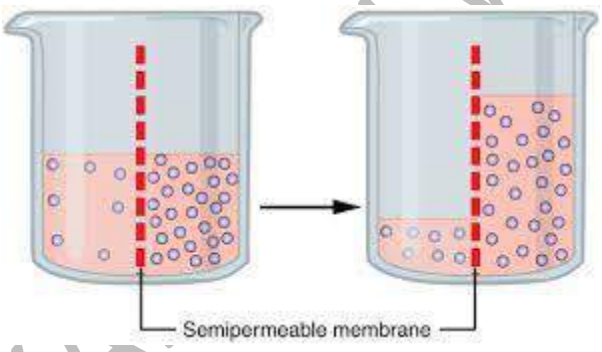
Glucose transporter, ion channels and aquaporins are some examples of facilitated diffusion. The cell membrane is permeable only to a few molecules that are smaller in size and non-polar. Therefore, facilitated diffusion with the help of transmembrane proteins is important.

Filtration

Filtration is the process of separating solids from liquids and gases. The selective absorption of nutrients in the body is an example of filtration. This process does not require any energy and takes place along the concentration gradient. The kidneys are an example of a biological filter. The blood is filtered by the glomerulus and the necessary molecules are reabsorbed.

In the process of filtration, the cell membrane permits only those substances which are soluble and could easily pass through its pores.

Osmosis



Passive transport represented by the process of osmosis

In the process of osmosis, water and other molecules pass through a selectively permeable membrane in order to balance the concentration of other substances.

Osmosis is affected by the concentration gradient and temperature. The greater the concentration gradient, the faster the rate of osmosis. Also, the rate of osmosis increases with the increase in temperature.

There is a theory of conflict about the process of osmosis. Few biologists suggest that osmosis is an active transport and not passive transport.

Examples of Passive Transport

Following are some examples of passive transport:

1. Ethanol enters our bodies and hits the bloodstream. This happens because the ethanol molecules undergo simple diffusion and pass through the cell membrane without any external energy.
2. Reabsorption of nutrients by the intestines by separating them from the solid waste and transporting the nutrients through the intestinal membrane into the bloodstream.
3. When a raisin is soaked in water the water moves inside the raisin by the process of osmosis and it swells.

Active Transport

Transportation is an essential, natural and the physiological process which occurs in all the higher organisms including plants, animals, and humans. In order to sustain life, this process is important as it functions by constantly transporting, different essential materials to and from all parts of the body including cells, tissues, and organs.

The essential materials mainly include water, hormones, gases, mineral nutrition, organic material, etc. The different means of transport in a living organism are:

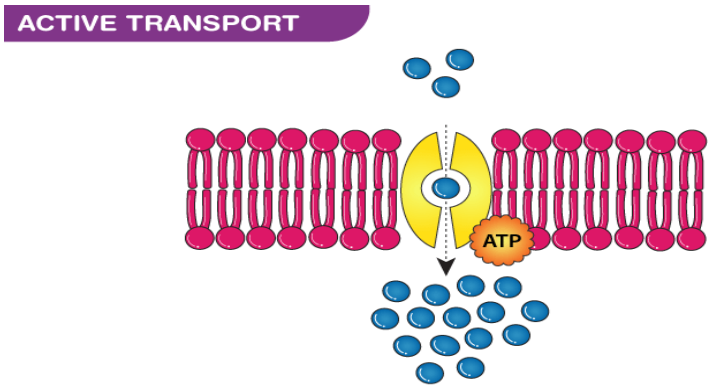
- Diffusion
- Facilitated diffusion
- Active transport
- Passive transport.

Active Transport

"Active Transport is defined as a process that involves the movement of molecules from a region of lower concentration to a region of higher concentration against a gradient or an obstacle with the use of external energy."

During the process of active transport, a protein pump makes use of stored energy in the form of ATP, to move molecules

The below diagram shows the process of active transport, which uses an external energy ATP for the movement of the molecules.



The uptake of glucose in the intestine of the human body and also the uptake of minerals or ions into the root hair cells of the plants are some of the examples of active transport.

Types of Active transport

There are two types of active transport namely – Primary active transport and secondary active transport.

Primary active transport

In this process of transportation, the energy is utilized by the breakdown of the ATP – Adenosine triphosphate to transport molecules across the membrane against a concentration gradient. Therefore, all the groups of ATP powered pumps contain one or more binding sites for the ATP molecules, which are present on the cytosolic face of the membrane. Basically, the primary active transport uses external chemical energy such as the ATP.

Sodium-potassium pump, the most important pump in the animal cell is considered as an example of primary active transport. In this process of transportation, the sodium ions are moved to the outside of the cell and potassium ions are moved to the inside of the cell.

Secondary active transport

Secondary active transport is a kind of active transport that uses electrochemical energy. It takes place across a biological membrane where a transporter protein couples the movement of an electrochemical ion (typically Na+ or H+) down its electrochemical gradient to the upward movement of another molecule or an ion against a concentration or electrochemical gradient.

Electrochemical Gradient

Electrochemical gradient exists whenever there is a net difference in charges. The positive and negative charges of a cell are separated by a membrane, where the inside of the cell has extra negative charges than outside. The membrane potential of a cell is -40 to -80 millivolts.

The cell has higher potassium concentration inside the cell but lower sodium concentration than the extracellular fluid. The sodium ions will move inside the cell based on the concentration gradient and voltage across the membrane. The voltage across the membrane facilitates the

movement of potassium into the cell, but its concentration gradient drives it out of the cell. The combination of voltage across the membrane and the concentration gradient that facilitates the movement of ions is called the electrochemical gradient.

Active Transport in Plants

Like humans and animals, plants also require transport systems which are mainly involved in the transport of materials, such as water, minerals, and necessary nutrients to all parts of the plant for its survival.

Active transport is a mode of transportation in plants, which uses stored energy to move the particles against the concentration gradient. In a plant cell, it takes place in the root cells by absorbing water and minerals. Active transport always leads to accumulation of molecules are ions towards one side of the membrane. This mode of transportation in plants is carried out by membrane proteins and transports the substance from the lower concentration to higher concentration.

Examples of Active Transport

Some of the best examples of active transport include:

- Phagocytosis of bacteria by Macrophages.
- Movement of Ca2+ ions out of cardiac muscle cells.
- Transportation of amino acids across the intestinal lining in the human gut.
- Secretion of proteins like enzymes, peptide hormones, and antibodies from different cells.
- Functioning of the White Blood Cells by protecting our body by attacking diseases causing microbes and other foreign invaders.

PHOTOAUTOTROPHS:

Definition

Photoautotrophs are organisms that can make their own energy using light and carbon dioxide via the process of photosynthesis. The word photoautotroph is a combination of autotroph, the word for an organism that makes its own food, and the prefix photo-, which means “light”. Green plants and photosynthetic bacteria are examples of photoautotrophs. They are not to be confused with photo heterotrophs, which also make energy from light but cannot use carbon dioxide as their sole source of carbon, and instead use organic materials.

Function of Photoautotrophs

Photoautotrophs essentially make their own food, which is how they can survive and reproduce. However, they are also important for the survival of heterotrophs, organisms that can’t make their food and must eat other organisms to survive. Heterotrophs eat autotrophs; for example, cattle

eat grass, and then humans eat those cattle. Photoautotrophs and other autotrophs are at the bottom of the food chain; they provide food for other organisms and are vital in all ecosystems. They are known as producers in the food chain, since they produce nutrients that all other animals need to survive. Without them, humans along with other animals would not survive because they would not have food.

Photoautotrophs are also important because they take in carbon dioxide, a by product of respiration in heterotrophs. In addition, phototrophs give off oxygen as a result of photosynthesis, and animals need this oxygen in order to survive.

Types of Photoautotrophs

Green Plants

Nearly all plants are photoautotrophs, which a few exceptions like Indian Pipe (Monotropa uniflora). This category of green plants includes all of the different forms of plant life, such as trees, mosses, and grasses. Plants are important sources of food in terrestrial ecosystems. They can make their own energy from light because they produce the molecule chlorophyll in organelles called chloroplasts within their cells. Chlorophyll absorbs light and transfers its energy to parts of the plant that can use that energy. It also gives plants their green color. Indian Pipe has lost the ability to produce chlorophyll, which is why it cannot produce its own energy from light. Instead, it parasitizes certain species of trees and fungi and “steals” their nutrients.

Bacteria

Some bacteria are photoautotrophs; most of these are called cyanobacteria or blue-green bacteria (formerly called blue-green algae). Like plants, cyanobacteria also produce chlorophyll. In fact, cyanobacteria are responsible for the origin of plants. Millions of years ago, cyanobacteria were taken up into cells, where they were able to make food for those cells in return for a place to live. This means that the chloroplasts in plant cells are actually cyanobacteria. Since cyanobacteria reproduce asexually, these chloroplasts are copies of the cyanobacteria that entered plant cells long ago. Green sulfur bacteria are another type of photoautotrophic bacteria that are ecologically similar to cyanobacteria, but they use sulfide ions instead of water during photosynthesis, and do not produce oxygen.

Algae

Algae come in many forms; they can be single-celled or multicellular (seaweed is a type of algae). They are important producers in aquatic ecosystems, but they can also found in terrestrial ones. Not all algae evolved from the same common ancestor, and as a result, only some species of algae are photoautotrophs. Like other photoautotrophs, algae are important producers of oxygen. Algae produce about half of the oxygen in the atmosphere.

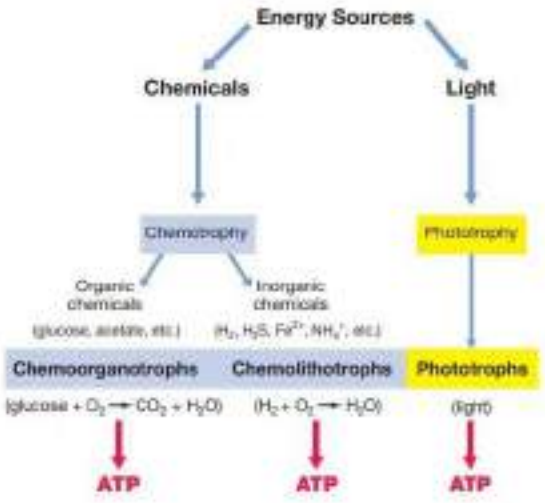
If too much algae flourishes in an algal bloom, this can disrupt the ecosystem by producing certain toxins and making nutrients less available. Algal blooms are often caused by human activities such as using nitrogen-containing fertilizers and improperly treating wastewater.

However, algae are efficient users of carbon dioxide in the atmosphere and may also be able to be used as a source of biofuel in the future to replace fossil fuels.

Chemoorganotrophs

Organisms that conserve energy from organic chemicals are called chemoorganotrophs. Thousands of different organic chemicals can be used by one or another microorganism. Indeed, all-natural and even most synthetic organic compounds can be metabolized. Energy is conserved from the oxidation of the compound and is stored in the cell in the energy-rich bonds of the compound adenosine triphosphate (ATP).

Aerobes obtain energy from an organic compound in the presence of oxygen, anaerobes obtain energy in the absence of oxygen and facultative anaerobes can break down organic compounds in both aerobic and anaerobic conditions.



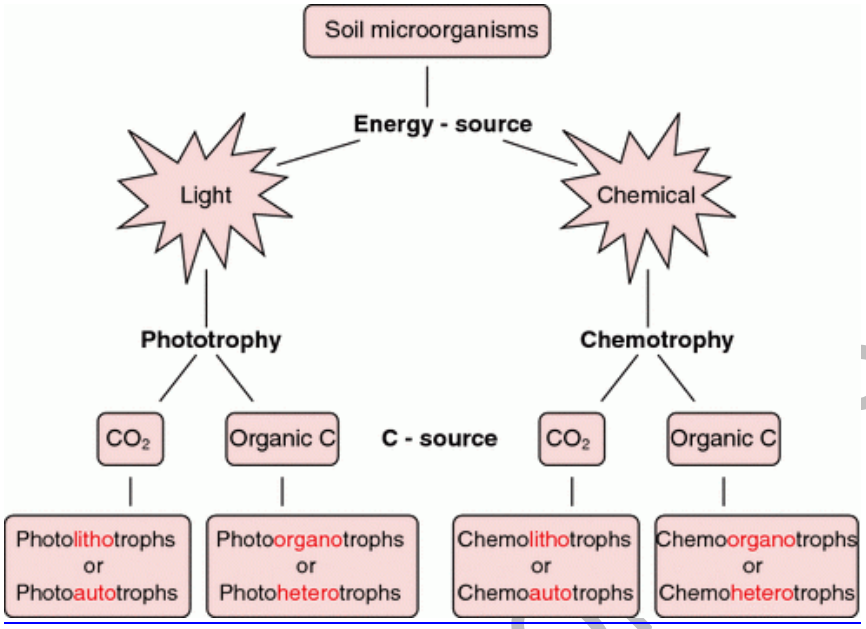
Metabolic process for conserving energy

Chemolithotrophs

The oxidation of inorganic compounds to yield energy is known as **chemolithotrophy.** Many prokaryotes can tap the energy available from the oxidation of inorganic compounds. This phenomenon was discovered by the Russian microbiologist Winogradsky. Organisms that carry out chemolithotrophic reactions are called **chemolithotrophs.** Like phototrophic organisms**, chemolithotrophic bacteria are also autotrophs.**

Chemolithotrophy occurs only in prokaryotes and is widely distributed among species of Bacteria and Archaea. Several inorganic compounds can be oxidized; for example, H2, H2S (hydrogen sulfide), NH3 (ammonia), and Fe21 (ferrous iron). Typically, a related group of chemolithotrophs specializes in the oxidation of a related group of inorganic compounds, and thus we have the “sulfur” bacteria, the “iron” bacteria, and so on.

The capacity to conserve energy from the oxidation of inorganic chemicals is a good metabolic strategy because competition from chemoorganotrophs, organisms that require organic energy sources, is not an issue. In addition, many of the inorganic compounds oxidized by chemolithotrophs, for example, H2 and H2S, are actually the waste products of chemoorganotrophs. Thus, chemolithotrophs have evolved strategies for exploiting resources that chemoorganotrophs are unable to use, so it is common for species of these two physiological groups to live in close association with one another.



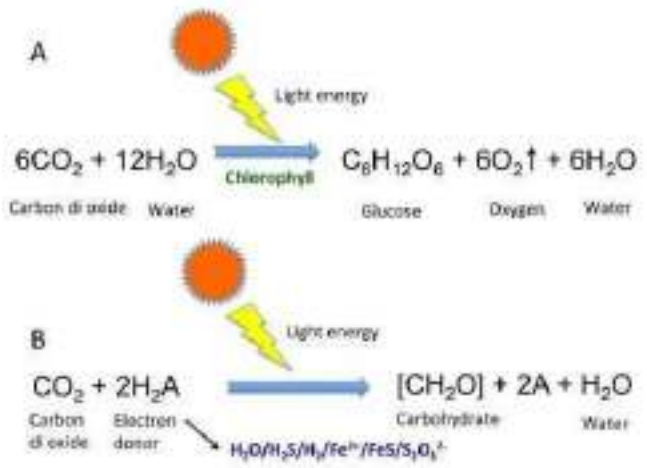
Phototrophs

Sunlight is available in many microbial habitats on Earth, phototrophic microorganisms living in those areas harvest energy from sunlight. They contain pigments that allow them to convert light energy into chemical energy, and thus their cells appear colored. Unlike chemotrophic organisms, **phototrophs do not require chemicals as a source of energy.**

Purple bacteria appeared on Earth long before oxygenic phototrophs evolved. Green sulfur bacteria were some of the first phototrophs to evolve on Earth.

Two major forms of phototrophy are known in prokaryotes.

1. **Oxygenic photosynthesis:** oxygen (O2) is produced. Among microorganisms, oxygenic photosynthesis is characteristic of cyanobacteria and algae (oxygenic phototrops).
2. **Anoxygenic photosynthesis**: does not yield O2. Purple sulfur bacteria, green bacteria, and heliobacteria are anoxygenic phototrophs.



A. Oxygenic photosynthesis B. Anoxygenic photosynthesis of autotrophs

Photolithotrophs

Among phototrophic bacteria are species that use inorganic compounds as their source of electrons and are called **photolithotrophs.** For example, Chromatium okenii.

Photoorganotrophs

Some phototrophic microorganisms use organic compounds such as fatty acids and alcohols as electron donors and are therefore **photoorganotrophs**. For example, Rhodospirillum rubrum.

UNIT – III

EMBDEN- MEYERHOF PATHWAY

Introduction

EMP pathway is the other name of glycolysis. It is named after the three scientists Gustav Embden, Otto Meyerhof, and J. Parnas, who gave the scheme of glycolysis. It is the pathway of glucose catabolism. It occurs in the cytoplasm of all living cells, aerobic as well as anaerobic.

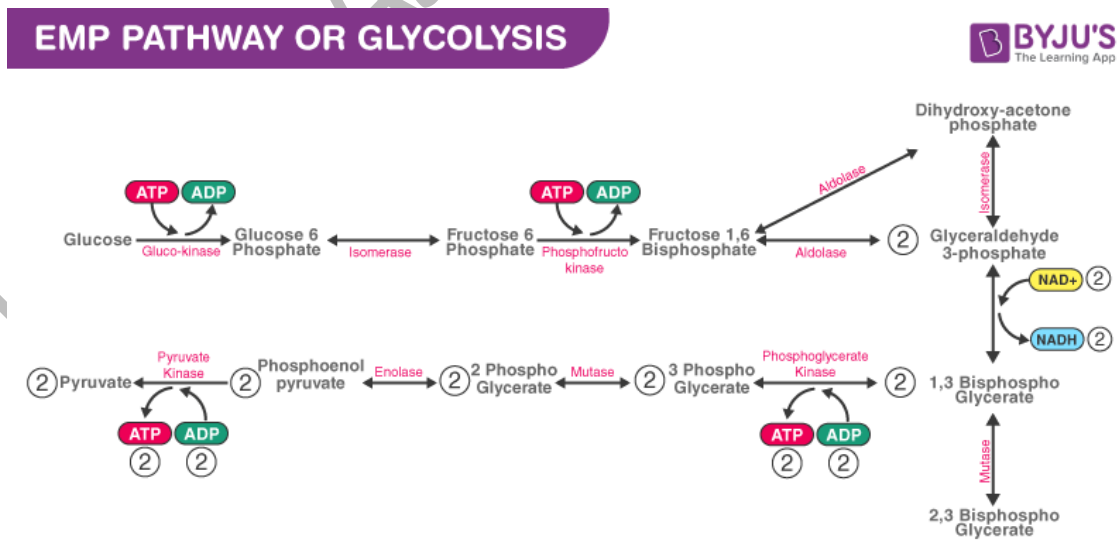
EMP pathway or glycolysis is the primary step of cellular respiration. Glucose is partially oxidised to pyruvate in this process. In aerobic organisms, it is followed by the Krebs cycle for the complete oxidation of glucose to CO2 and water. In anaerobic organisms, glycolysis is followed by fermentation.

Let us learn in detail about each step of the EMP pathway.

Steps of EMP Pathway

The EMP pathway occurs in the cytoplasm of the cell. It doesn't require oxygen. In plants, glucose is derived from sucrose formed during photosynthesis or from storage carbohydrates such as starch. The enzyme invertase converts sucrose into glucose and fructose that enter the EMP pathway.

- It is a series of ten enzyme-catalysed reactions, wherein a glucose molecule is broken down into two molecules of pyruvate.
- There is a net production of two ATP and 2 NADH also in this process.
- The first phase of the EMP pathway is the energy-requiring phase or preparatory phase, and the second half is the energy-yielding phase or pay-off phase.



The steps of glycolysis are as follows:

Step 1

Glucose is converted to Glucose 6-phosphate by the action of the enzyme hexokinase. An ATP is consumed in this process. The phosphate group of ATP is transferred to glucose.

Step 2

The second step is the conversion of G6P to Fructose 6-phosphate (F6P) by the enzyme phosphoglucoisomerase. It is an isomerisation reaction. Fructose can also enter the EMP pathway at this step by phosphorylation. After this step, all the steps are the same for glucose and fructose metabolism. It is a reversible reaction.

Step 3

F6P is converted to fructose 1,6-bisphosphate (FBP) by utilising another molecule of ATP. The enzyme phosphofructokinase catalyses the reaction. It is an irreversible reaction and is a rate-limiting step. During the gluconeogenesis process, which is an anabolic pathway, a different path is required for this step.

Step 4

The enzyme aldolase converts fructose 1,6-bisphosphate into two triose sugars, i.e. dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate, a ketose and aldose, respectively.

Step 5

The enzyme triosephosphate isomerase interconverts dihydroxyacetone phosphate (DHAP) with glyceraldehyde 3-phosphate (GADP) or 3-phosphoglyceraldehyde (PGAL).

Step 6

Now the pay-off phase starts. Since a molecule of glucose yields two molecules of triose sugar, each reaction from this step onwards occurs twice.

Here in this step, 3-phosphoglyceraldehyde (PGAL) is converted into 1, 3-bisphosphoglycerate (BPGA) by the action of the enzyme Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). NAD+ is reduced to NADH + H+. In this step, dehydrogenation and phosphorylation take place.

Step 7

1, 3-bisphosphoglycerate (BPGA) is then converted into 3-phosphoglycerate (3-PGA). Phosphate is transferred from BPGA to ADP, forming ATP. This is a substrate-level phosphorylation. Two molecules of ATP are produced in this step. The enzyme phosphoglycerate kinase catalyses this reaction.

Step 8

3-PGA is then converted into 2-PGA by the action of the enzyme phosphoglycerate mutase. The enzyme transfers a phosphate group from C-3 to C-2.

Step 9

It is an elimination reaction, wherein a molecule of water is removed from 2-PGA to produce phosphoenolpyruvate (PEP). The enzyme enolase catalyses this dehydration reaction.

Step 10

It is the last step of the EMP pathway or glycolysis. Pyruvate kinase transfers a phosphate group from PEP to ADP, forming ATP and pyruvate or pyruvic acid. 2 ATP molecules and 2 pyruvate molecules are produced. This is also a substrate-level phosphorylation.

Summary

To sum up, in the EMP pathway, partial oxidation of glucose takes place. The overall reaction in the EMP pathway is:

Glucose + 2 NAD+ + 2 ADP + 2 Pi → 2 pyruvate + 2 NADH + 2 H+ + 2 ATP

It occurs in two phases:

- **Preparatory Phase or Energy-requiring Phase:** The first 5 steps of the EMP pathway are known as the investment phase. Energy is consumed in this process to produce two molecules of triose sugar phosphates.
- **Pay-off Phase:** The second half of glycolysis produces ATP and NADH and pyruvate.

Location: Cytoplasm of living cells

ATP Utilisation: ATP is utilised in two steps. First in the conversion of glucose to G6P and then when F6P is converted into fructose 1,6-bisphosphate. Total 2 ATP molecules are utilised.

ATP Synthesis: A total of 4 ATP molecules are synthesised. ATP is synthesised in two steps. First, when 1,3-bisphosphoglyceric acid is converted into 3-phosphoglyceric acid, and then in the conversion of phosphoenolpyruvate to pyruvate.

NADH Synthesis: 2 NAD^+ is converted to 2 NADH + H^+ in the sixth step when glyceraldehyde-3-phosphate is converted to 1,3-bisphosphoglyceric acid.

Rate-limiting Step: The conversion of fructose-6-phosphate to fructose-1,6-bisphosphate by the enzyme phosphofructokinase.

End Products of EMP Pathway: Two molecules each of pyruvate, ATP and NADH.

Significance of EMP Pathway

- The EMP pathway is the universal pathway of glucose degradation, whether energy is derived in aerobic respiration or fermentation.
- It is the first step of cellular respiration.
- It is required by all tissues to derive energy in the form of ATP.
- Cells that perform anaerobic respiration derive energy from this process.
- Cells that lack mitochondria derive energy from this process. Krebs cycle and oxidative phosphorylation occur in mitochondria, where most of the energy is produced during cellular respiration.
- The intermediates formed during the EMP pathway are utilised in other metabolic pathways.
- The EMP pathway is connected to the various other metabolic pathways such as the Pentose phosphate pathway, glycogen synthesis, formation of triglycerides, fatty acid synthesis, cholesterol synthesis, amino acid synthesis, etc.

Entner–Doudoroff pathway

- Entner–Doudoroff pathway is an alternative pathway of Glycolysis. This pathway is found in Gram-negative bacteria, certain Gram-positive bacteria, and archaea.
- Entner–Doudoroff pathway is an alternative metabolic pathway of glycolysis where glucose is converted into Pyruvate with the help of a series of reactions and enzymes.
- Those bacteria are unable to perform glycolysis due to the absence of glycolytic enzymes they perform ED pathway to break down the glucose molecule into pyruvate.
- This pathway was first reported by Entner and Doudoroff (1952) and MacGee and Doudoroff (1954) in the bacterium Pseudomonas saccharophila.
- Recent evidence showed that this pathway also found in cyanobacteria, ferns, algae, mosses, and plants.
- In this pathway, two unique enzymes are involved such as 2-keto-deoxy-6-phosphogluconate (KDPG) aldolase and 6-phosphogluconate dehydratase aldolase.
- In ED pathway 1 ATP is formed per glucose molecule and as well as 1 NADH and 1 NADPH.

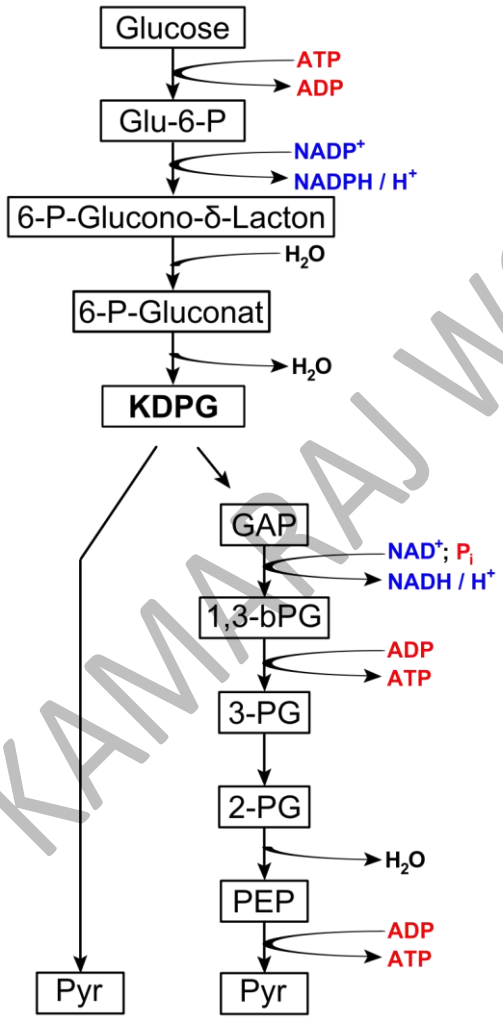
Entner–Doudoroff pathway containing Organisms

Those bacteria unable to metabolism glucose by using glycolysis follow this alternative or ED pathway to break the glucose into pyruvate. For example, Pseudomonas lacks the essential glycolytic enzyme phosphofructokinase, that's why they follow the ED pathway to form pyruvate from Glucose.

Only the aerobic and facultative anaerobes use the Entner–Doudoroff pathway and anaerobes use glycolysis due to its low energy yield.

Some example of bacteria those contain Entner–Doudoroff pathway are Pseudomonas, Azotobacter, Rhizobium, Agrobacterium, Escherichia coli, Enterococcus faecalis, Xanthomonas campestris, Zymomonas mobilis, Enterococcus faecalis. This pathway also found in Hordeum vulgare, Phaeodactylum tricornutum.

Entner–Doudoroff pathway Procedure



1. In the first step, glucose is converted into glucose 6 phosphate in the presence of enzyme hexokinase and Cofactors Mg2+. In this reaction, one molecule of ATP is consumed.
2. The glucose 6 phosphate is converted into 6-phosphoglucono δ lactone in presence of enzyme glucose 6 phosphate dehydrogenase. In this reaction, one NADP is reduced to NADPH.
3. The 6-phosphoglucono δ lactone is converted into 6-phosphogluconate in presence of enzyme lactonase. In this reaction, one molecule of H2O is required.
4. The 6-phosphogluconate is converted into 2-keto-3-deoxy-6-phosphoglucanate (KDPG) in presence of enzyme 6-phosphogluconate dehydrogenase. From this reaction, one H2O is released.
5. Now, KDPG is split into pyruvate and glyceraldehyde 3 phosphate in presence of enzyme KDPG aldolase. The pyruvate from this step enters into further metabolic pathways such as TCA cycle, ETC cycle, etc.
6. The glyceraldehyde 3 phosphate enters into the Glycolysis pathway and converts into pyruvate. The conversation of glyceraldehyde 3 phosphate to pyruvate is followed by the following steps;
    1. glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate
    2. 1,3-bisphosphoglycerate to 3-phosphoglycerate
    3. 3-phosphoglycerate to 2-phosphoglycerate
    4. 2-phosphoglycerate to phosphoenol pyruvate
    5. phosphoenol pyruvate to pyruvate

PENTOSE PHOSPHATE PATHWAY

Introduction

The pentose phosphate pathway is primarily catabolic and serves as an alternative glucose oxidizing pathway for the generation of NADPH that is required for reductive biosynthetic reactions such as those of cholesterol biosynthesis, bile acid synthesis, steroid hormone biosynthesis, and fatty acid synthesis. The pentose phosphate pathway can also function as an anabolic pathway that utilizes the six carbons of glucose to generate five carbon sugars, particularly ribose-5-phosphate (R5P) that is required for purine and pyrimidine nucleotide biosynthesis. The pentose phosphate pathway can, under certain conditions, completely oxidize glucose to CO2 and water. The primary functions of this pathway are:

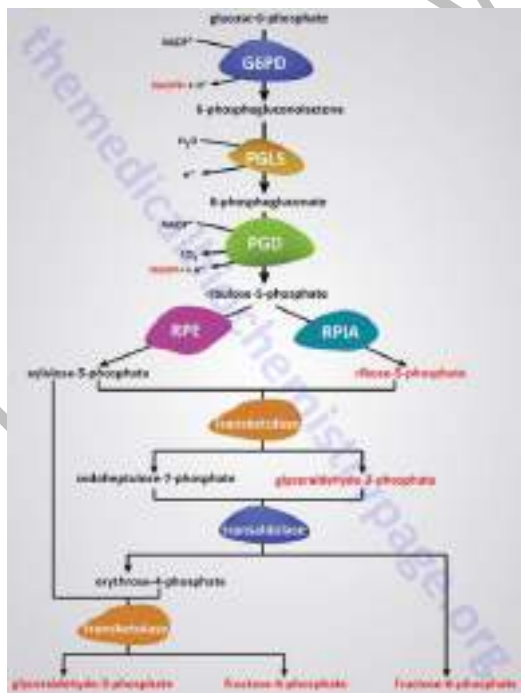
1. To generate reducing equivalents, in the form of NADPH, for reductive biosynthesis reactions within cells.

2. To provide the cell with ribose-5-phosphate (R5P) for the synthesis of the nucleotides and nucleic acids.

3. Although not a significant function of the PPP, it can operate to metabolize dietary pentose sugars derived from the digestion of nucleic acids as well as to rearrange the carbon skeletons of dietary carbohydrates into glycolytic and /or gluconeogenic intermediates.

Enzymes that function primarily in the reductive direction utilize the NADP+/NADPH co-factor pair as their co-factors as opposed to oxidative enzymes that utilize the NAD+/NADH co-factor pair. The reactions of fatty acid biosynthesis and steroid biosynthesis utilize large amounts of NADPH. As a consequence, cells of the liver, adipose tissue, adrenal cortex, testis and lactating mammary gland have high levels of the PPP enzymes. In fact 30% of the oxidation of glucose in the liver occurs via the PPP. Additionally, erythrocytes utilize the reactions of the PPP to generate large amounts of NADPH used in the reduction of glutathione (see below). The conversion of ribonucleotides to deoxyribonucleotides (through the action of ribonucleotide reductase) requires NADPH as the electron source, therefore, any rapidly proliferating cell needs large quantities of NADPH.

Although the PPP operates in all cells, with high levels of expression in the above indicated tissues, the highest levels of PPP enzymes (in particular glucose 6-phosphate dehydrogenase) are found in neutrophils and macrophages. These leukocytes are the phagocytic cells of the immune system and they utilize NADPH to generate superoxide radicals from molecular oxygen in a reaction catalyzed by the NADPH oxidase complex. Superoxide anion, in turn, serves to generate other reactive oxygen species (ROS) that kill the phagocytosed microorganisms. Following exposure to bacteria and other foreign substances there is a dramatic increase in O2 consumption by phagocytes. This phenomenon is referred to as the oxidative burst or respiratory burst.



Reactions of the pentose phosphate pathway:

The first three reactions of the PPP are referred to as the oxidative portion and includes the reactions that yield NADPH. The non-oxidative reactions result in the rearrangement of the carbon skeletons of numerous carbohydrates. G6PD: glucose-6-phosphate dehydrogenase. PGLS: 6-phosphogluconolactonase. PGD: 6-phosphogluconate dehydrogenase. RPE: ribulose-5-phosphate 3-epimerase. RPIA: ribose-5-phosphate isomerase

The reactions of the PPP operate exclusively in the cytoplasm. From this perspective it is understandable that fatty acid synthesis (as opposed to oxidation) takes place in the cytoplasm. The pentose phosphate pathway has both an oxidative and a non-oxidative arm. The oxidation steps, utilizing glucose-6-phosphate (G6P) as the substrate, occur at the beginning of the pathway and are the reactions that generate NADPH. The reactions catalyzed by glucose-6-phosphate dehydrogenase (G6PD; also identified ad G6PDH) and 6-phosphogluconate dehydrogenase (PGD) both generate one mole of NADPH for every mole of glucose-6-phosphate that enters the PPP. The conversion of 6-phosphogluconolactone to 6-phosphogluconate is catalyzed by 6-phosphogluconolactonase (PGLS).

Glucose-6-Phosphate Dehydrogenase: G6PD

The glucose-6-phosphate dehydrogenase gene (symbol: G6PD) is located on the X chromosome (Xq28) and is composed of 14 exons that generate three alternatively spliced mRNAs. These three mRNAs collectively encode the G6PD isoform a (545 amino acids) and isoform b (515 amino acids) enzymes.

The G6PD isoform a protein is inactive in the native full-length form but may undergo processing to the smaller 515 amino acid active form of the enzyme. The active G6PD enzyme is also referred to as the G form of glucose-6-phosphate dehydrogenase. The G6PD gene is ubiquitously expressed with high levels of expression in erythrocytes.

Hexose-6-Phosphate Dehydrogenase: H6PDH

Humans express a second glucose-6-phosphate dehydrogenase activity, referred to as the H form. This form of glucose-6-phosphate dehydrogenase activity is identified as hexose-6-phosphate dehydrogenase (encoded by the H6PD gene) and also as glucose-1-dehydrogenase. Whereas the G6PD encoded enzyme resides in the cytosol, the H6PD encoded enzyme resides within the endoplasmic reticulum (ER) and the sarcoplasmic reticulum (SR).

The H6PD gene is located on chromosome 1p36.22 and is composed of 7 exons that generate two alternatively spliced mRNAs encoding precursor proteins of 802 amino acids (isoform 1) and 791 amino acids (isoform 2). The H6PD gene is not expressed in erythrocytes.

Within the ER, hexose-6-phosphate dehydrogenase converts glucose-6-phosphate and $NADP^+$ to 6-phosphogluconate and NADPH in a single step, whereas this process in the cytosol requires two separate enzymes (G6PD and PGLS). In addition to glucose-6-phosphate, H6PD can metabolize other hexose-6-phosphates, as well as glucose-6-sulfate, and glucose.

One of the primary functions of the ER- and SR-localized NADPH is to maintain redox homeostasis within these organelles. Loss of ER redox homeostasis can lead to ER stress and induction of the unfolded protein response (UPR) which, if severe enough will trigger cell death via the apoptotic pathway.

Another principal function of the NADPH produced by ER-localized hexose-6-phosphate dehydrogenase is to provide the reducing energy to ER-localized reductases, specifically those involved in steroid hormone metabolism, with 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1; encoded by the HSD11B1 gene) being particularly important. The primary function of the HSD11B1 encoded enzyme is to reduce the 11-oxo groups in cortisone and 11-dehydrocorticosterone to the active glucocorticoids, cortisol and corticosterone, respectively. However, the enzyme can, under certain conditions, also inactivate cortisol and corticosterone by catalyzing the oxidation reactions converting cortisol to cortisone and corticosterone to 11-dehydrocorticosterone. Of clinical significance to the role of ER-localized NADPH is that mutations in the H6PD gene are associated with glucocorticoid deficiency.

6-Phosphogluconolactonase: PGLS

The product of the glucose-6-phosphate dehydrogenase reaction, 6-phosphogluconolactone, is converted to 6-phosphogluconate by the enzyme, 6-phosphogluconolactonase which is encoded by the PGLS gene. The PGLS gene is located on chromosome 19p13.11 and is composed of 5 exons that encode a 258 amino acid protein.

6-Phosphogluconate Dehydrogenase

The third enzyme of the PPP, 6-phosphogluconate dehydrogenase is encoded by the phosphogluconate dehydrogenase gene (symbol: PGD). The PGD gene is located on chromosome 1p36.33 and is composed of 13 exons that generate three alternatively spliced mRNAs, each of which encode different sized protein isoforms.

Non-Oxidative Reactions of the PPP

The non-oxidative reactions of the PPP are primarily designed to generate ribose-5-phosphate (R5P). Other reactions of the PPP that are of less physiological significance are designed to convert dietary five carbon sugars into both six (fructose-6-phosphate) and three (glyceraldehyde-3-phosphate) carbon sugars which can then be utilized by the pathways of glycolysis.

The primary enzymes involved in the non-oxidative steps of the PPP are transaldolase and transketolase. Transketolase functions to transfer two-carbon groups from substrates of the PPP thus, rearranging the carbon atoms that enter this pathway. Like other enzymes that transfer two-carbon groups, transketolase requires thiamine pyrophosphate (TPP) as a co-factor in the transfer reaction.

Two facts regarding transketolase make it a diagnostically useful enzyme. The enzyme is expressed at high levels in red blood cells, which are easy to isolate and analyze, and the only vitamin-

derived cofactor it requires is TPP. Therefore, assay for reduced activity of this enzyme, in red blood cell lysates, is highly diagnostic in cases of suspected thiamine deficiency.

Transaldolase transfers three-carbon groups and thus is also involved in a rearrangement of the carbon skeletons of the substrates of the PPP. The transaldolase reaction involves Schiff base formation between the substrate and a lysine residue in the enzyme.

Transketolase is encoded by the TKT gene. The TKT gene is located on chromosome 3p21.1 and is composed of 15 exons that generate three alternatively spliced mRNAs that collectively encode two distinct protein isoforms.

Transaldolase is encoded by the TALDO1 gene. The TALDO1 gene is located on chromosome 11p15.5 and is composed of 8 exons that encode a protein of 337 amino acids. A molecularly interesting fact about the TALDO1 gene is that exons 2 and 3 were derived as a result of the insertion of a retrotransposon into the gene.

There are two transketolase-related genes in the human genome. One is found on the X chromosome (Xq28) and is identified as the transketolase-like 1 gene (symbol: TKTL1). The other gene (symbol: TKTL2) is located on chromosome 4q32.2.

The TKTL1 encoded protein lacks 38 amino acids, compared to the TKT encoded protein, in the TPP-binding region. All TPP-dependent enzymes contain a highly similar TPP-binding domain and its lack in the TKTL1 encoded protein strongly suggests that it is unlikely that TKTL1 is a TPP-dependent protein capable of catalyzing the transketolase reaction. Indeed, biochemical evidence has indicated that the TKTL1 protein is not capable of catalyzing a transketolase reaction. Intense interest in the TKTL1 gene, and its encoded protein, was stimulated because it was shown that the level of TKTL1 expression correlated with poor patient outcomes and metastasis in many solid tumors. In addition, specific inhibition of TKTL1 mRNA has been shown to inhibit cancer cell proliferation in functional studies.

The net result of the PPP, if not used solely for R5P production, is the oxidation of G6P, a six-carbon sugar, into a five-carbon sugar. In turn, three moles of five-carbon sugar are converted, via the enzymes of the PPP, back into two moles of six-carbon sugars and one mole of three-carbon sugar. The six-carbon sugars can be recycled into the pathway in the form of G6P, generating more NADPH. The three-carbon sugar generated is glyceraldehyde-3-phosphate which can be shunted to glycolysis and oxidized to pyruvate. Alternatively, it can be utilized by the gluconeogenic enzymes to generate more six-carbon sugars, fructose-6-phosphate or glucose-6-phosphate.

TRICARBOXYLIC ACID CYCLE:

Introduction

The Krebs cycle or TCA cycle (tricarboxylic acid cycle) or Citric acid cycle is a series of enzyme catalysed reactions occurring in the mitochondrial matrix, where acetyl-CoA is oxidised to form carbon dioxide and coenzymes are reduced, which generate ATP in the electron transport chain.

Krebs cycle was named after Hans Krebs, who postulated the detailed cycle. He was awarded the Nobel prize in 1953 for his contribution.

It is a series of eight-step processes, where the acetyl group of acetyl-CoA is oxidised to form two molecules of CO_2 and in the process, one ATP is produced. Reduced high energy compounds, NADH and $FADH_2$ are also produced.

Two molecules of acetyl-CoA are produced from each glucose molecule so two turns of the Krebs cycle are required which yields four CO_2, six NADH, two $FADH_2$ and two ATPs.

Krebs Cycle is a part of Cellular Respiration

Cellular respiration is a catabolic reaction taking place in the cells. It is a biochemical process by which nutrients are broken down to release energy, which gets stored in the form of ATP and waste products are released. In aerobic respiration, oxygen is required.

Cellular respiration is a four-stage process. In the process, glucose is oxidised to carbon dioxide and oxygen is reduced to water. The energy released in the process is stored in the form of ATPs. 36 to 38 ATPs are formed from each glucose molecule.

The four stages are:

1. Glycolysis: Partial oxidation of a glucose molecule to form 2 molecules of pyruvate. This process takes place in the cytosol.

2. Formation of Acetyl CoA: Pyruvate formed in glycolysis enters the mitochondrial matrix. It undergoes oxidative decarboxylation to form two molecules of Acetyl CoA. The reaction is catalysed by the pyruvate dehydrogenase enzyme.

$$2Pyruvate + 2NAD^+ + 2CoA \xrightarrow{Pyruvate\ dehydrogenase} 2AcetylCoA + 2NADH + CO_2$$

3. Krebs cycle (TCA cycle or Citric Acid Cycle): It is the common pathway for complete oxidation of carbohydrates, proteins and lipids as they are metabolised to acetyl coenzyme A or other intermediates of the cycle. The Acetyl CoA produced enters the Tricarboxylic acid cycle or Citric acid cycle. Glucose is fully oxidized in this process. The acetyl CoA combines with 4-carbon compound oxaloacetate to form 6C citrate. In this process, 2 molecules of CO_2 are released and oxaloacetate is recycled. Energy is stored in ATP and other high energy compounds like NADH and $FADH_2$.

4. Electron Transport System and Oxidative Phosphorylation: ATP is generated when electrons are transferred from the energy-rich molecules like NADH and $FADH_2$, produced in glycolysis, citric acid cycle and fatty acid oxidation to molecular O_2 by a series of electron carriers. O_2 is reduced to H_2O. It takes place in the inner membrane of mitochondria.

Krebs Cycle Steps

It is an eight-step process. Krebs cycle or TCA cycle takes place in the matrix of mitochondria under aerobic condition.

Step 1: The first step is the condensation of acetyl CoA with 4-carbon compound oxaloacetate to form 6C citrate, coenzyme A is released. The reaction is catalysed by citrate synthase.

Step 2: Citrate is converted to its isomer, isocitrate. The enzyme aconitase catalyses this reaction.

Step 3: Isocitrate undergoes dehydrogenation and decarboxylation to form 5C **α**-ketoglutarate. A molecular form of CO_2 is released. Isocitrate dehydrogenase catalyses the reaction. It is an NAD+ dependent enzyme. NAD+ is converted to NADH.

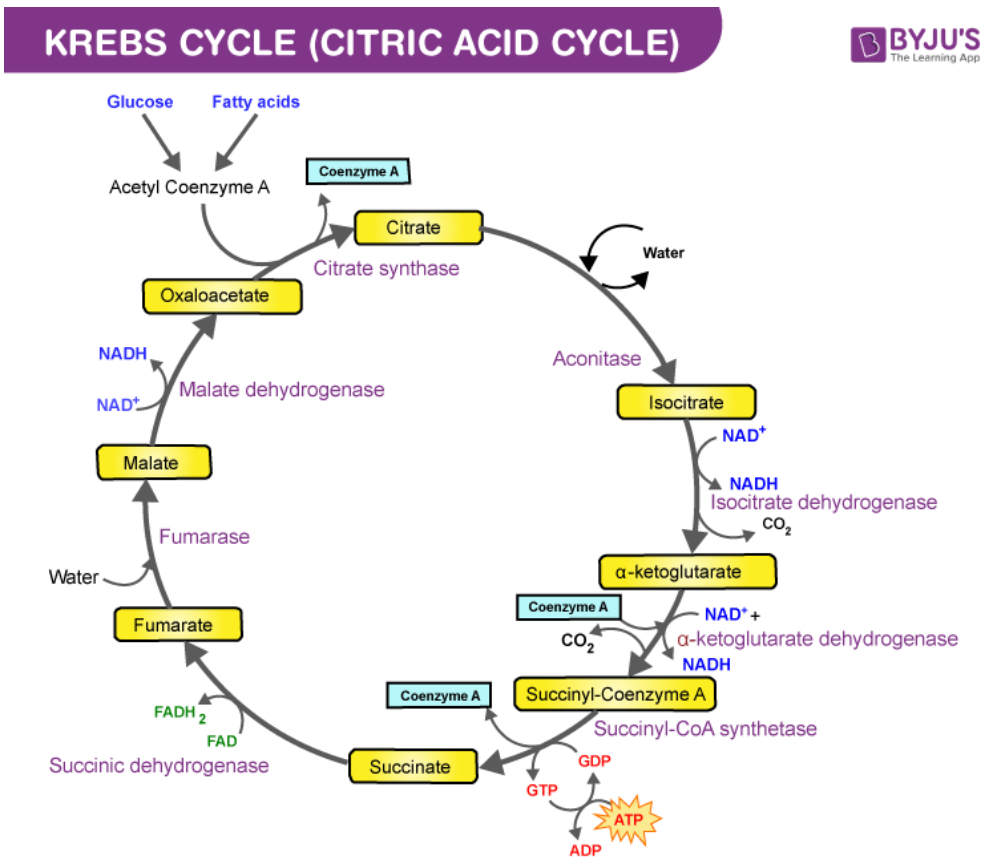
Step 4: **α**-ketoglutarate undergoes oxidative decarboxylation to form succinyl CoA, a 4C compound. The reaction is catalyzed by the **α**-ketoglutarate dehydrogenase enzyme complex. One molecule of CO_2 is released and NAD+ is converted to NADH.

Step 5: Succinyl CoA forms succinate. The enzyme succinyl CoA synthetase catalyses the reaction. This is coupled with substrate-level phosphorylation of GDP to get GTP. GTP transfers its phosphate to ADP forming ATP.

Step 6: Succinate is oxidised by the enzyme succinate dehydrogenase to fumarate. In the process, FAD is converted to $FADH_2$.

Step 7: Fumarate gets converted to malate by the addition of one H_2O. The enzyme catalysing this reaction is fumarase.

Step 8: Malate is dehydrogenated to form oxaloacetate, which combines with another molecule of acetyl CoA and starts the new cycle. Hydrogens removed, get transferred to NAD+ forming NADH. Malate dehydrogenase catalyses the reaction.



Krebs Cycle Summary

Location: Krebs cycle occurs in the mitochondrial matrix

Krebs cycle reactants: Acetyl CoA, which is produced from the end product of glycolysis, i.e. pyruvate and it condenses with 4 carbon oxaloacetate, which is generated back in the Krebs cycle

Krebs cycle products

Each citric acid cycle forms the following products:

- 2 molecules of CO2 are released. Removal of CO2 or decarboxylation of citric acid takes place at two places:
    1. In the conversion of isocitrate (6C) to **α**-ketoglutarate (5C)
    2. In the conversion of **α**-ketoglutarate (5C) to succinyl CoA (4C)
- 1 ATP is produced in the conversion of succinyl CoA to succinate
- 3 NAD+ are reduced to NADH and 1 FAD+ is converted to FADH2 in the following reactions:
    1. Isocitrate to **α**-ketoglutarate → NADH
    2. **α**-ketoglutarate to succinyl CoA → NADH

3. Succinate to fumarate → FADH2
4. Malate to Oxaloacetate → NADH

Note that 2 molecules of Acetyl CoA are produced from oxidative decarboxylation of 2 pyruvates so two cycles are required per glucose molecule.

To summarize, for complete oxidation of a glucose molecule, Krebs cycle yields 4 CO2, 6NADH, 2 FADH2 and 2 ATPs.

Each molecule of NADH can form 2-3 ATPs and each FADH2 gives 2 ATPs on oxidation in the electron transport chain.

Krebs cycle equation

$$2Acetyl\ CoA + 6NAD^{+} + 2\,FAD + 2\,ADP + 2\,P_i + 2\,H_2O \rightarrow 4\,CO_2 + 6\,NADH + 2\,FADH_2 + 2\,ATP + 2\,CoA$$

Significance of Krebs Cycle

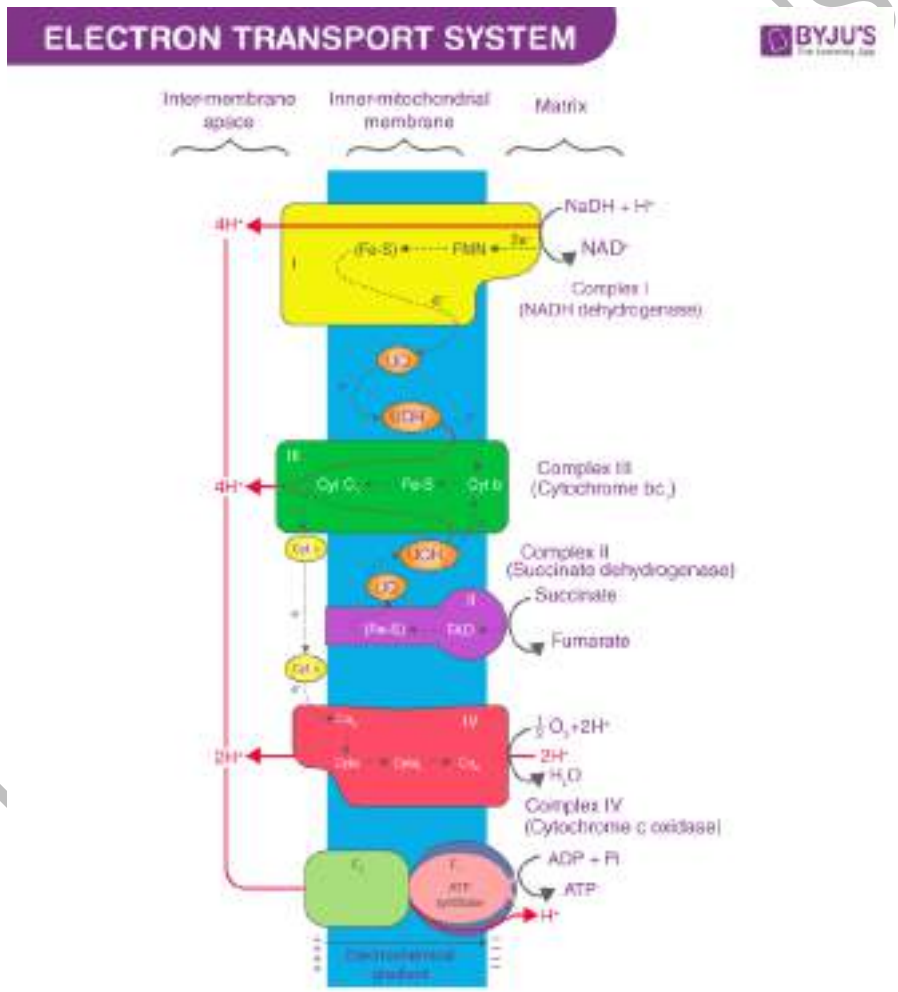
- Krebs cycle or Citric acid cycle is the final pathway of oxidation of glucose, fats and amino acids
- Many animals are dependent on nutrients other than glucose as an energy source
- Amino acids (metabolic product of proteins) are deaminated and get converted to pyruvate and other intermediates of the Krebs cycle. They enter the cycle and get metabolised e.g. alanine is converted to pyruvate, glutamate to **α**-ketoglutarate, aspartate to oxaloacetate on deamination
- Fatty acids undergo $\boldsymbol{\beta}$-oxidation to form acetyl CoA, which enters the Krebs cycle
- It is the major source of ATP production in the cells. A large amount of energy is produced after complete oxidation of nutrients
- It plays an important role in gluconeogenesis and lipogenesis and interconversion of amino acids
- Many intermediate compounds are used in the synthesis of amino acids, nucleotides, cytochromes and chlorophylls, etc.
- Vitamins play an important role in the citric acid cycle. Riboflavin, niacin, thiamin and pantothenic acid as a part of various enzymes cofactors (FAD, NAD) and coenzyme A
- Regulation of Krebs cycle depends on the supply of NAD+ and utilization of ATP in physical and chemical work
- The genetic defects of the Krebs cycle enzymes are associated with neural damage

- As most of the biological processes occur in the liver to a significant extent, damage to liver cells has a lot of repercussions. Hyperammonemia occurs in liver diseases and leads to convulsions and coma. This is due to reduced ATP generation as a result of the withdrawal of **α**-ketoglutarate and formation of glutamate, which forms glutamine.

ELECTRON TRANSPORT CHAIN:

Electron Transport Chain is a series of compounds where it makes use of electrons from electron carrier to develop a chemical gradient. It could be used to power oxidative phosphorylation. The molecules present in the chain comprises enzymes that are protein complex or proteins, peptides and much more.

Large amounts of ATP could be produced through a highly efficient method termed oxidative phosphorylation. ATP is a fundamental unit of metabolic process. The electrons are transferred from electron donor to the electron acceptor leading to the production of ATP. It is one of the vital phases in the electron transport chain. Compared to any other part of cellular respiration the large amount of ATP is produced in this phase.



Electron transport is defined as a series of redox reaction that is similar to the relay race. It is a part of aerobic respiration. It is the only phase in glucose metabolism that makes use of atmospheric oxygen. When electrons are passed from one component to another until the end of the chain the electrons reduce molecular oxygen thus producing water. The requirement of oxygen in the final phase could be witnessed in the chemical reaction that involves the requirement of both oxygen and glucose.

Electron Transport Chain in Mitochondria

A complex could be defined as a structure that comprises a weak protein, molecule or atom that is weakly connected to a protein. The plasma membrane of prokaryotes comprises multi copies of the electron transport chain.

Complex 1- NADH-Q oxidoreductase: It comprises enzymes consisting of iron-sulfur and FMN. Here two electrons are carried out to the first complex aboard NADH. FMN is derived from vitamin B2.

Q and Complex 2- Succinate-Q reductase: FADH2 that is not passed through complex 1 is received directly from complex 2. The first and the second complexes are connected to a third complex through compound ubiquinone (Q). The Q molecule is soluble in water and moves freely in the hydrophobic core of the membrane. In this phase, an electron is delivered directly to the electron protein chain. The number of ATP obtained at this stage is directly proportional to the number of protons that are pumped across the inner membrane of the mitochondria.

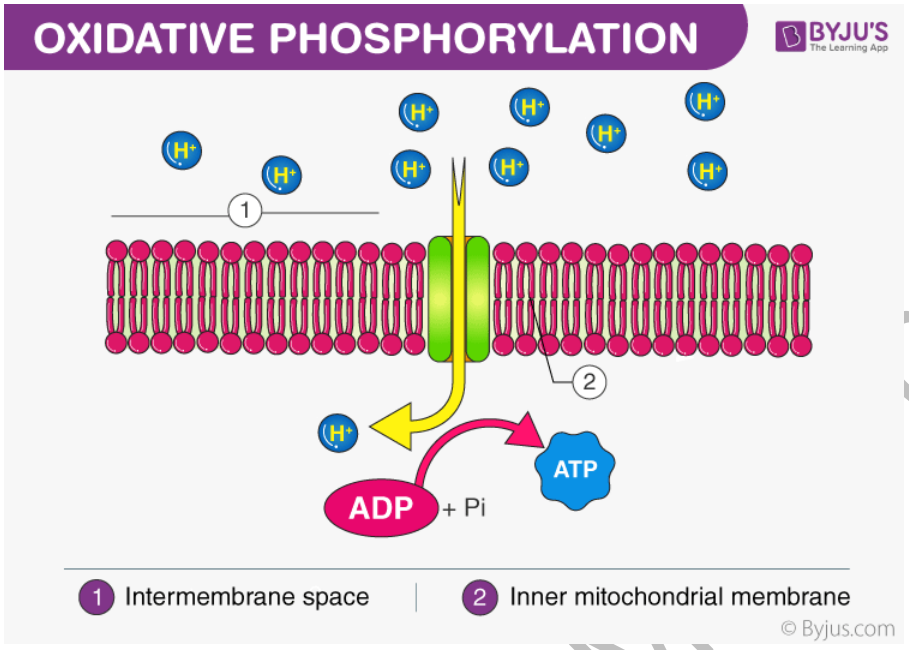
Complex 3- Cytochrome c reductase: The third complex is comprised of Fe-S protein, Cytochrome b, and Cytochrome c proteins. Cytochrome proteins consist of the heme group. Complex 3 is responsible for pumping protons across the membrane. It also passes electrons to the cytochrome c where it is transported to the 4th complex of enzymes and proteins. Here, Q is the electron donor and Cytochrome C is the electron acceptor.

Complex 4- Cytochrome c oxidase: The 4th complex is comprised of cytochrome c, a and a3. There are two heme groups where each of them is present in cytochromes c and a3. The cytochromes are responsible for holding oxygen molecule between copper and iron until the oxygen content is reduced completely. In this phase, the reduced oxygen picks two hydrogen ions from the surrounding environment to make water.

OXIDATIVE PHOSPHORYLATION:

Definition

"Oxidative phosphorylation is the process of ATP formation, when electrons are transferred by electron carriers from NADH or FADH2 to oxygen"



What is Oxidative Phosphorylation?

Oxidative phosphorylation is the final step in cellular respiration. It occurs in the mitochondria. It is linked to a process known as electron transport chain. The electron transport system is located in the inner mitochondrial membrane. The electrons are transferred from one member of the transport chain to another through a series of redox reactions.

Also Read: Amphibolic Pathway

Oxidative Phosphorylation Steps

The major steps of oxidative phosphorylation in mitochondria include:

Delivery of Electrons by NADH and FADH2

Reduced NADH and FADH2 transfer their electrons to molecules near the beginning of the transport chain. After transferring the electrons, they get oxidised to NAD+ and FAD and are utilised in other steps of cellular respiration.

Electron Transport and Proton Pumping

The electrons move from a higher energy level to a lower energy level, thereby releasing energy. Some of the energy is used to move the electrons from the matrix to the intermembrane space. Thus, an electrochemical gradient is established.

Splitting of Oxygen to form Water

The electrons are then transferred to the oxygen molecule which splits into half and uptakes H+ to form water.

ATP Synthesis

The H+ ions pass through an enzyme called ATP synthase while flowing back into the matrix. This controls the flow of protons to synthesize ATP.

Chemiosmosis

Oxidative phosphorylation uses the chemical reactions that release energy to drive a chemical reaction that requires energy. These 2 sets of reactions are coupled and interrelated.

The electrons that flow through electron transport chain is an exergonic process and the synthesis of ATP is an endergonic process. These two processes are ingrained within a membrane. As a result, energy will be transmitted from the electron transport chain to ATP synthase by the movement of proteins. This process is termed as chemiosmosis.

Endergonic Process is a chemical reaction in which energy is absorbed. There will be a change in free energy and it is always positive. Exergonic Process is a chemical reaction in which there will be a positive flow of energy from the system to the surrounding environment. Chemical reactions are also considered exergonic when they are spontaneous.

Electron Transport Chain

Most of the biochemical catabolic processes like the citric acid cycle, glycolysis, beta-oxidation, etc. produce the coenzyme NADH. It consists of electrons having high transfer potential.

These reactions release a huge amount of energy on oxidation. These reactions are also known to be the uncontrollable reactions since the energy within the cells is not released at once.

The electrons are separated from the NADH and then passed to the oxygen with a series of enzymes releasing a small amount of energy. All these series of enzymes having complexes is known as electron transport chain.

This chain can be seen in the inner layer or membrane of mitochondria. The salts of succinic acid are also oxidized by this electron chain transport system.

In the case of eukaryotes, the enzymes make use of energy that has been released in the electron transport system from the oxidation of NADH that pumps protons across the inner membrane of the mitochondria. This results in the generation of the electrochemical gradient across the membrane. This can be considered as one of the best examples to understand the concept of oxidative phosphorylation.

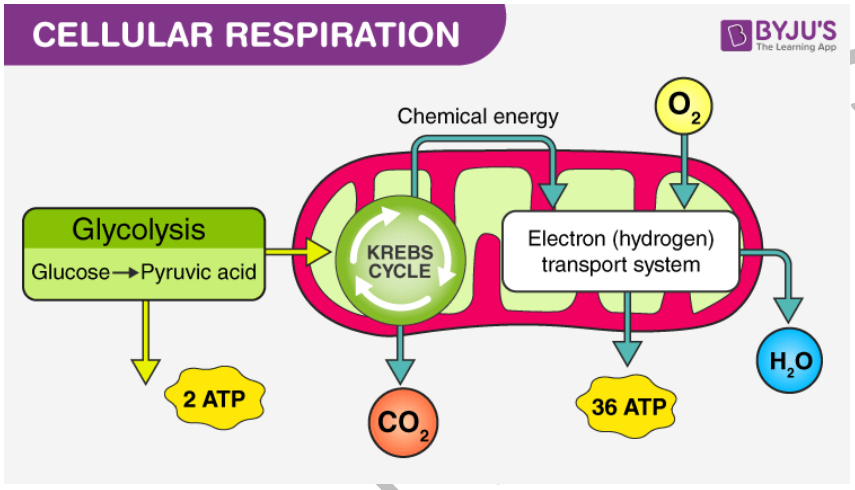
ATP SYNTHESIS: Synthesis

ATP or adenosine triphosphate is the energy currency of cells or living organisms. It is required for various cellular activities such as active transport of ions, muscle contraction, cell signalling, synthesis of biomolecules, etc.

ATP is primarily synthesised in the cellular respiration process. ATP is synthesised by the oxidation of respiratory substrates such as carbohydrates, lipids, proteins, etc. The oxidation of these results in energy production, which is stored in the form of high energy bonds in ATP.

Glucose is the main energy source in living organisms. During the process of aerobic respiration, the catabolism of glucose takes place in three steps, which are glycolysis, Krebs Cycle or TCA cycle and oxidative phosphorylation.

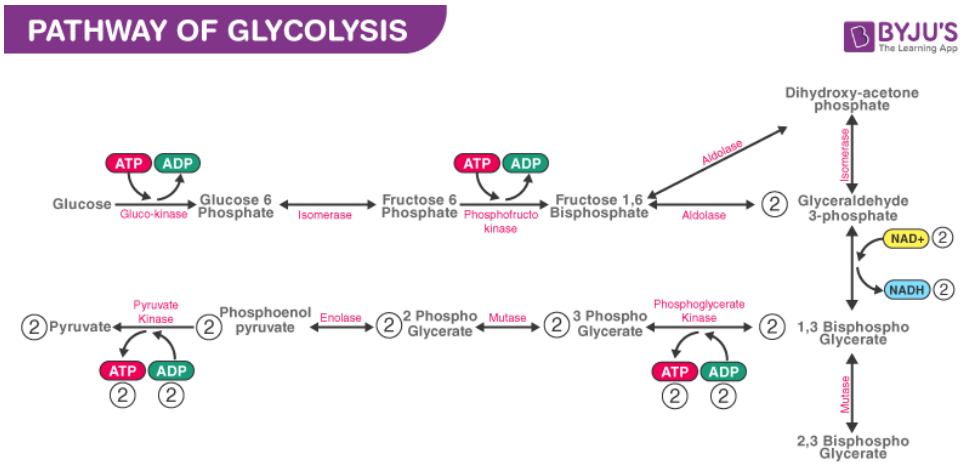
Most ATP formation takes place in the electron transport chain by oxidative phosphorylation. The enzyme ATP synthase catalyses the synthesis of ATP.



ATP Synthesis Pathways

Glycolysis

Glycolysis is the first step of cellular respiration occurring in all living organisms. Some anaerobic organisms and also some mammalian cells depend only on this process for fulfilling their energy needs.



It is a multi-step enzyme catalysed process, where glucose is converted into two molecules of pyruvate. Glycolysis does not require oxygen. It occurs in two phases, the first phase is the preparatory phase where 2 molecules of ATPs are utilised and the second phase is the payoff phase where ATPs are produced.

In glycolysis, there is a net gain of 2 molecules of ATP and 2 molecules of NADH per glucose molecule. It occurs in the cytosol.

Also Check: Significance Of Glycolysis

Under aerobic conditions, the pyruvate is completely oxidised in the mitochondria. Pyruvate enters mitochondria and after oxidative decarboxylation, it is converted to acetyl CoA, which then enters the TCA cycle and undergoes complete oxidation.

During anaerobic conditions as in muscles during strenuous exercise, the oxygen supply is not sufficient enough, then pyruvate is reduced to lactate and NAD+ is regenerated to continue the glycolysis. Lactic acid fermentation is catalysed by lactate dehydrogenase (LDH).

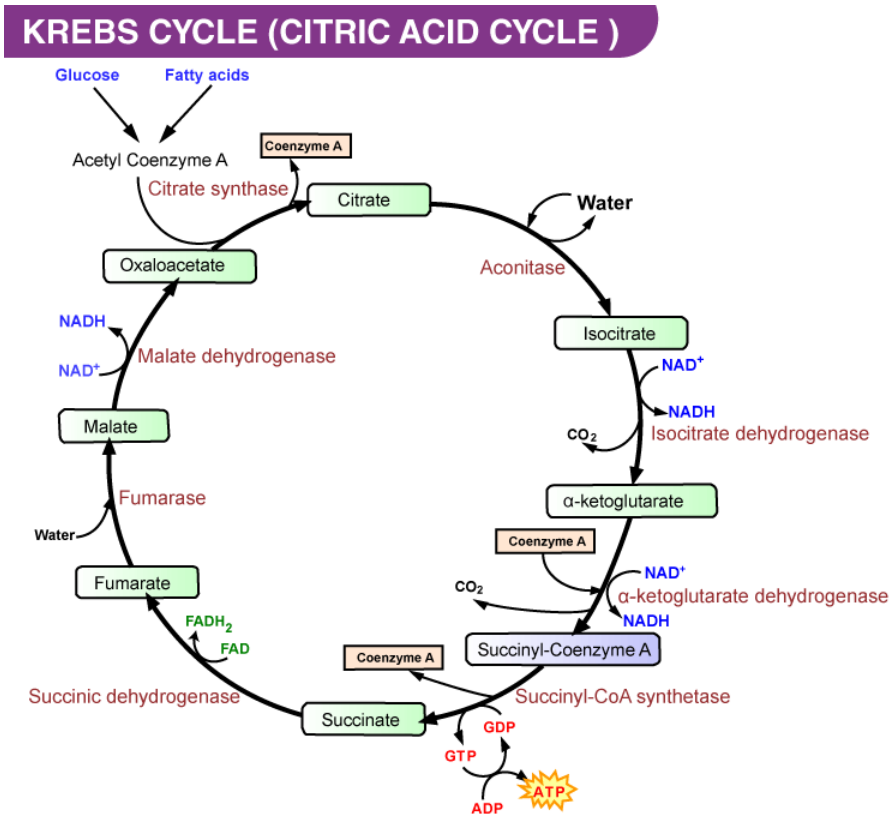
ATP Synthesis in Mitochondria

Pyruvate produced by glycolysis enters mitochondria. Pyruvate is completely oxidised to three molecules of CO_2 in mitochondria.

Pyruvate undergoes oxidative decarboxylation in the mitochondrial matrix by the enzyme pyruvate dehydrogenase complex. NADH is produced in this reaction. The acetyl CoA thus produced enters the TCA cycle. Acetyl CoA is also produced in lipid metabolism.

TCA Cycle

The Tricarboxylic acid cycle (TCA) was described by Sir Hans Krebs. It is a multi-step enzymatic process, wherein acetyl CoA is oxidised and coenzymes are reduced. It occurs in the mitochondrial matrix.

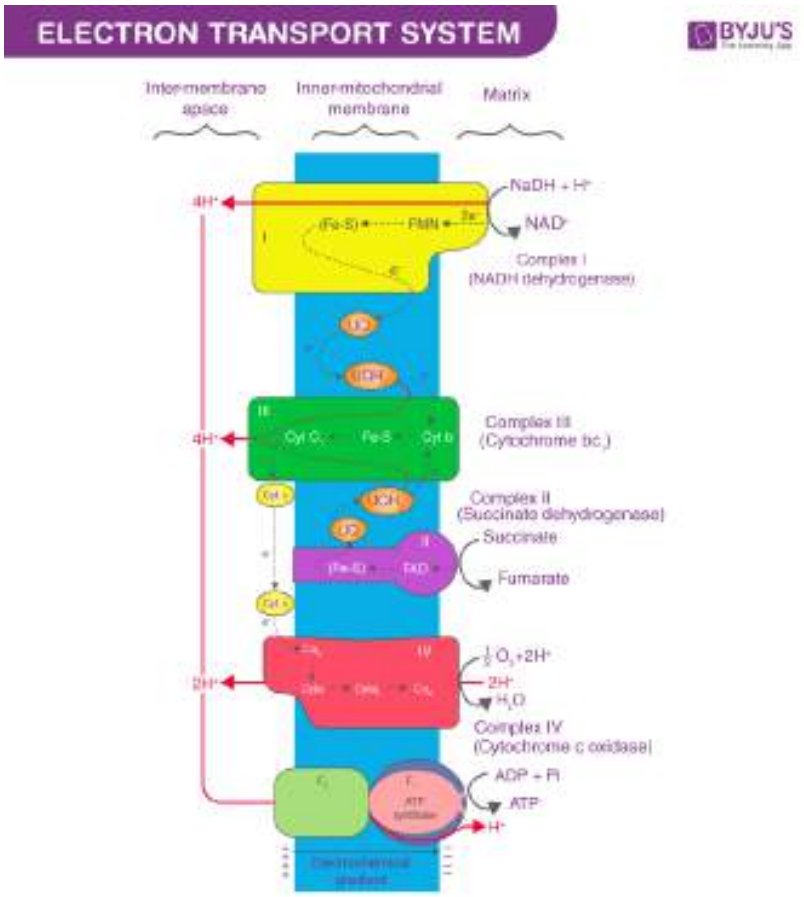


In the TCA cycle, only one molecule of ATP (GTP) is produced but three molecules of NADH and one molecule of FADH2 (per cycle) are produced, which provide electrons for the electron transport chain and facilitate a large amount of ATP synthesis.

Oxidative Phosphorylation and Electron Transport Chain

Electron transport system and ATP synthase complex are present in the inner mitochondrial membrane of eukaryotes and plasma membrane of prokaryotes. It comprises a series of enzyme complexes. The electron transport results in the formation of a proton gradient across the membrane, which is utilised in the formation of ATP. Here oxygen acts as the terminal electoral acceptor.

In the electron transport system, electron transport is coupled with ATP synthesis by oxidative phosphorylation. The enzyme ATP synthase catalyses the reaction.



The electron transport system comprises four complexes. They are:

- Complex I – NADH dehydrogenase
- Complex II – Succinate dehydrogenase
- Complex III – Cytochrome bc1
- Complex IV – Cytochrome c oxidase

The transport of electrons in the respiratory chain is coupled to the synthesis of ATP by ADP and inorganic phosphate by the enzyme ATP synthase.

Electron transport through these complexes induces the pumping of protons from the matrix to the intermembrane space leading to the production of a proton gradient across the membrane. This energy is utilised in the synthesis of ATP by the ATP Synthase through chemiosmosis.

The amount of ATP synthesised in the respiratory chain depends on the electron donor oxidised. The oxidation of NADH produces 3 molecules of ATP, whereas the oxidation of FADH2 produces two molecules of ATP.

Read more: High energy compounds

ATP Synthase

ATP synthase is an enzyme complex that catalyses the synthesis of ATP. It is a type of ligase as it catalyses the formation of phosphodiester bonds.

The reaction is as follows:

ADP + Pi + 2H+(out) ⇌ ATP + H2O + 2H+(in)

The formation of ATP is energetically unfavourable, therefore it is coupled to an electrochemical gradient created during cellular respiration in the electron transport chain. The electrochemical gradient is formed by the difference in proton concentration across the inner mitochondrial membrane.

The facilitated diffusion of protons through the transmembrane channel of ATP synthase releases energy that causes conformational changes in the enzyme and leads to the formation of ATP molecules.

The ATP synthase is also sometimes referred to as complex V of the electron transport system.

It consists of two components:

1. A transmembrane protein complex known as F0
2. A peripheral protein complex known as F1

The F1 headpiece present peripherally contains the site of ATP synthesis. F0 is a channel protein and allows the diffusion of protons through it, down the electrochemical gradient.

ATP synthesis by chemiosmosis requires a membrane, proton pump, a proton gradient and the ATP Synthase. The transmembrane channel of ATP synthase facilitates the diffusion of protons back to the mitochondrial matrix. The energy released in the process activates ATP synthase and it catalyses ATP synthesis.

Two protons pass through the F0 channel from intermembrane space to the matrix of mitochondria for each ATP molecule synthesised.

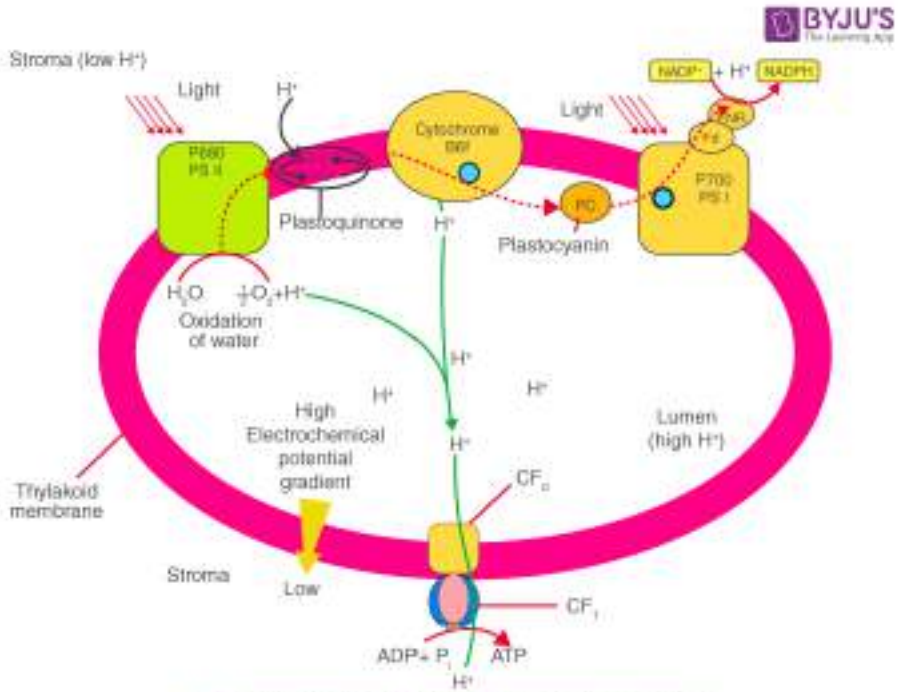
On complete oxidation of one glucose molecule, theoretically, there is a net gain of 38 ATP molecules. There are various factors that influence this, such as many pathways occurring simultaneously, withdrawal of substrates from the pathway, alternative substrates entering the pathway at intermediary stages, ATP utilisation whenever required, etc. Hence, this calculation is based on assumptions and is not very valid for living systems.

ATP Synthesis in Chloroplast

During photosynthesis, ATP is synthesised in the plants by the ATP Synthase. ATP synthesis occurs through chemiosmosis. A proton gradient is created across the thylakoid membrane. Protons are accumulated in the thylakoid lumen as opposed to intermembrane space in the mitochondria.

A proton gradient is created by the following steps:

- The splitting of water in PSII leads to the accumulation of protons in the lumen.
- As a result of the movement of electrons through the photosystems. Protons are moved from the stroma to the lumen side of the membrane.
- Protons are used from the stroma to reduce NADP+ by the enzyme NADP reductase, which is present on the stroma side.



ATP synthesis through chemiosmosis

The above-mentioned steps create a proton gradient across the thylakoid membrane. The breakdown of this gradient drives the synthesis of ATP.

The protons move from the thylakoid lumen to the stroma through the transmembrane channel of ATP synthase known as CF0. Protons move through facilitated diffusion. The other component known as CF1 is the peripheral unit and is present on the stroma side. The movement of protons causes conformational changes in CF1 and catalyses the synthesis of ATP molecules.

The ATP produced this way is utilised in the dark reaction for the synthesis of sugars.

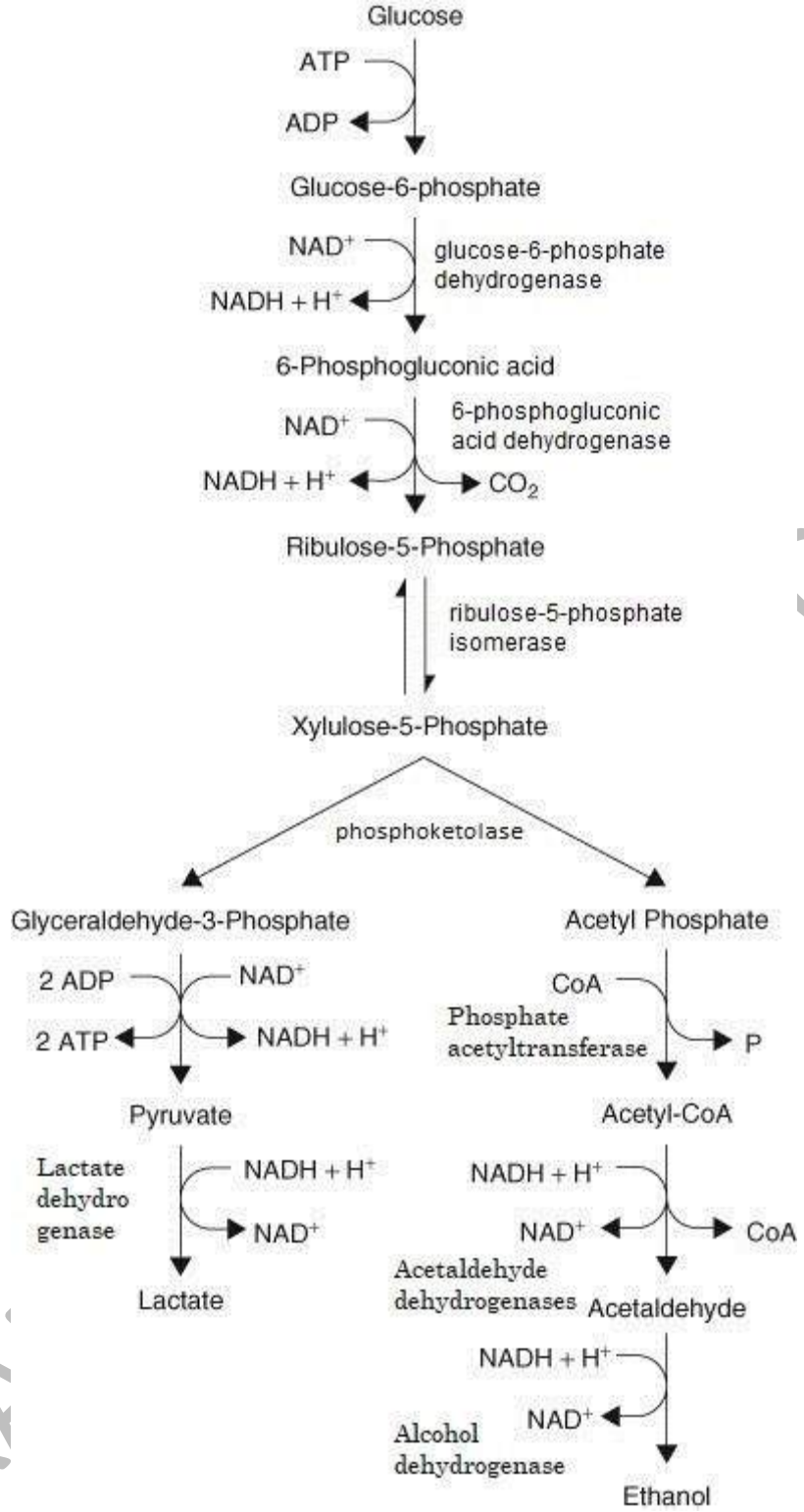
HOMOLACTIC FERMENTATION:

Refer EMBDEN- MEYERHOF PATHWAY for homolactic fermentation

HETEROLACTIC FERMENTATION:

Heterolactic (Phosphoketolase) Pathway

- The phosphoketolase pathway is distinguished by the key cleavage enzyme, phosphoketolase, which cleaves pentose phosphate into glyceraldehyde-3-phosphate and acetyl phosphate.
- As a fermentation pathway, it is employed mainly by the heterolactic acid bacteria, which include some species of Lactobacillus and Leuconostoc.
- In hetero lactic fermentation, end product is ethanol and CO2 in addition to lactic acid.
- In this reaction glucose is first metabolized to pyruvate, acetic acid and CO2 by Pentose phosphate pathway.
- Pyruvate is then reduced to lactic acid whereas acetic acid is reduced to ethanol and CO2.



Steps:

- In this pathway, glucose-phosphate is oxidized to 6- phosphogluconic acid, which becomes oxidized and decarboxylated to form pentose phosphate.

- Unlike the Embden-Meyerhof pathway, NAD-mediated oxidations take place before the cleavage of the substrate being utilized.
- Pentose phosphate is subsequently cleaved to glyceraldehyde-3- phosphate (GAP) and acetyl phosphate.
- GAP is converted to lactic acid by the same enzymes as the EM pathway.
- This branch of the pathway contains oxidation coupled to a reduction while 2 ATP are produced by substrate-level phosphorylation.
- Acetyl phosphate is reduced in two steps to ethanol, which balances the two oxidations before the cleavage but does not yield ATP.
- The overall reaction is Glucose———->1 lactic acid + 1 ethanol +1 CO_2 with a net gain of 1 ATP.
- The efficiency is about half that of the EM pathway.

Application:

- Heterolactic species of bacteria are occasionally used in the fermentation industry.
- For example, kefir, a type of fermented milk to yogurt, is produced by is produced using a heterolactic Lactobacillus species.
- Likewise, sauerkraut fermentations use Leuconostoc, a heterolactic bacterium, to complete the fermentation.

Heterolactic bacteria: Leuconostoc mesenteroides, Lactobacillus bifermentous, Leconostoc lactis

Mixed Acid Fermentation:

Fermentation is an anaerobic process of breaking down molecules like glucose and other carbohydrates. The fermentation process is usually helpful in alcohol production. Bacteria follow different fermentation pathways.

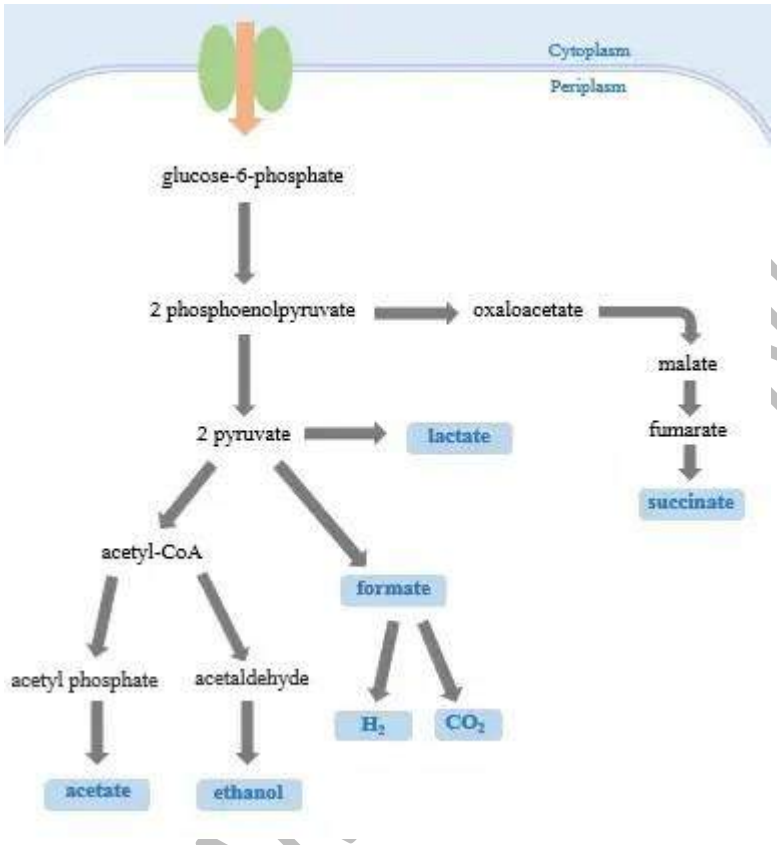
Among them, mixed acid fermentation is a characteristic feature of the family Enterobacteriaceae, especially the genera Citrobacter, Proteus, Shigella, Salmonella, Escherichia, Aeromonas, Yersinia, Vibrio, and some species of Aeromonas. Some anaerobic fungi also follow this pathway.

These microorganisms ferment monosaccharides, disaccharides, polyalcohol, and frequently polysaccharides. The glycolytic pathway of this type of fermentation produces lactic acid, succinic acid, formic acids, acetic acids, and ethanol.

Reactions Involved in Mixed Acid Fermentation

In mixed acid fermentation reactions, two stages are present. The first stage of mixed acid fermentation is glycolysis, converting glucose to pyruvate. Here two NADH molecules are produced.

The second stage of mixed acid fermentation follows the following reactions the conversion of the pyruvate produced after glycolysis to one or more end products. The two NADH molecules produced in the first stage are reoxidized to NAD+. The reactions in the second stage are discussed below.



Mixed acid fermentation in E coli

Lactate Production

The enzyme lactate dehydrogenase catalyzes the formation of lactate/lactic acid. Here, glycolysis generates two molecules of pyruvate. Each molecule converts to lactate in the presence of a NADH+H+ molecule.

The overall reaction of lactate production is as follows:

Pyruvate → Lactate; in presence of lactate dehydrogenase and NADH+H+, which converts to NAD+

Acetate Production

In this reaction, pyruvate converts to acetyl CoA with the enzyme pyruvate dehydrogenase and NADH catalysts. The acetyl CoA now converts to acetate, which produces ATP by substrate-level phosphorylation. The conversion of acetyl CoA to acetate is a two-step process requiring two separate enzymes; phosphate acetyltransferase and acetate kinase.

The overall reaction of this reaction is as follows;

1. Pyruvate → Acetyl CoA in the presence of pyruvate dehydrogenase
2. Acetyl CoA + Phosphate → Acetyl phosphate + CoA in the presence of phosphate acetyltransferase.
3. Acetyl phosphate + ADP → Acetate + ATP.

Ethanol Production

The reduction of Acetyl CoA with the help of NADH forms the third end product of mixed acid fermentation; ethanol. This ethanol production is a two-step reaction and requires the enzyme alcohol dehydrogenase.

The overall reaction of this step of fermentation is;

1. Acetyl CoA + NADH +H+ → Acetaldehyde + NAD+ + CoA
2. Acetaldehyde + NADH + H+ → Ethanol + NAD+

Formate Production

The cleavage of pyruvate helps in the production of Formate. The enzyme pyruvate-formate-lyase catalyzes this production reaction. The enzyme pyruvate-formate-lyase also plays an essential role in regulating anaerobic fermentation in Enterobacteriaceae.

The overall reaction of the formate production is as follows;

Pyruvate + CoA → Acetyl CoA + Formate; catalyzed by pyruvate-formate-lyase.

Succinate Production

The production of succinate is a multi-step process. The glycolytic pathway intermediate, phosphoenol pyruvate, is the first substrate for carboxylation to form oxaloacetate, with the enzyme phosphoenol pyruvate carboxylase catalyzing this step.

In the second step, the oxaloacetate converts to malate in the presence of malate dehydrogenase. The third step is the formation of fumarate from the dehydration of malate in the fact of fumarate hydratase.

Phosphoenol pyruvate + HCO3 → Oxaloacetate +Phosphate

Oxaloacetate + NADH + H+ → Malate + NAD+

Malate → Fumarate + H2O

The final step is succinate production by reducing formate catalyzed by the enzyme fumarate reductase.

Fumarate + NADH + NAD+ → Succinate + NAD+

The reduction is the anaerobic respiration reaction that uses electrons in NADH dehydrogenase and the electron transport chain. ATP is produced using electrochemical balance and ATP synthetase—this step of fermentation produces ATP, not through substrate-level phosphorylation.

Hydrogen and Carbon Dioxide Production

The enzyme formate hydrogen lyase catalyzes the conversion of formate to hydrogen carbon dioxide gas. The production of these gases helps in preventing acidic condition inside the cells.

End Products of Mixed Acid Fermentation

The end products of mixed acid fermentation are as follows:

1. Lactate
2. Acetate
3. Formate
4. Succinate
5. Ethanol

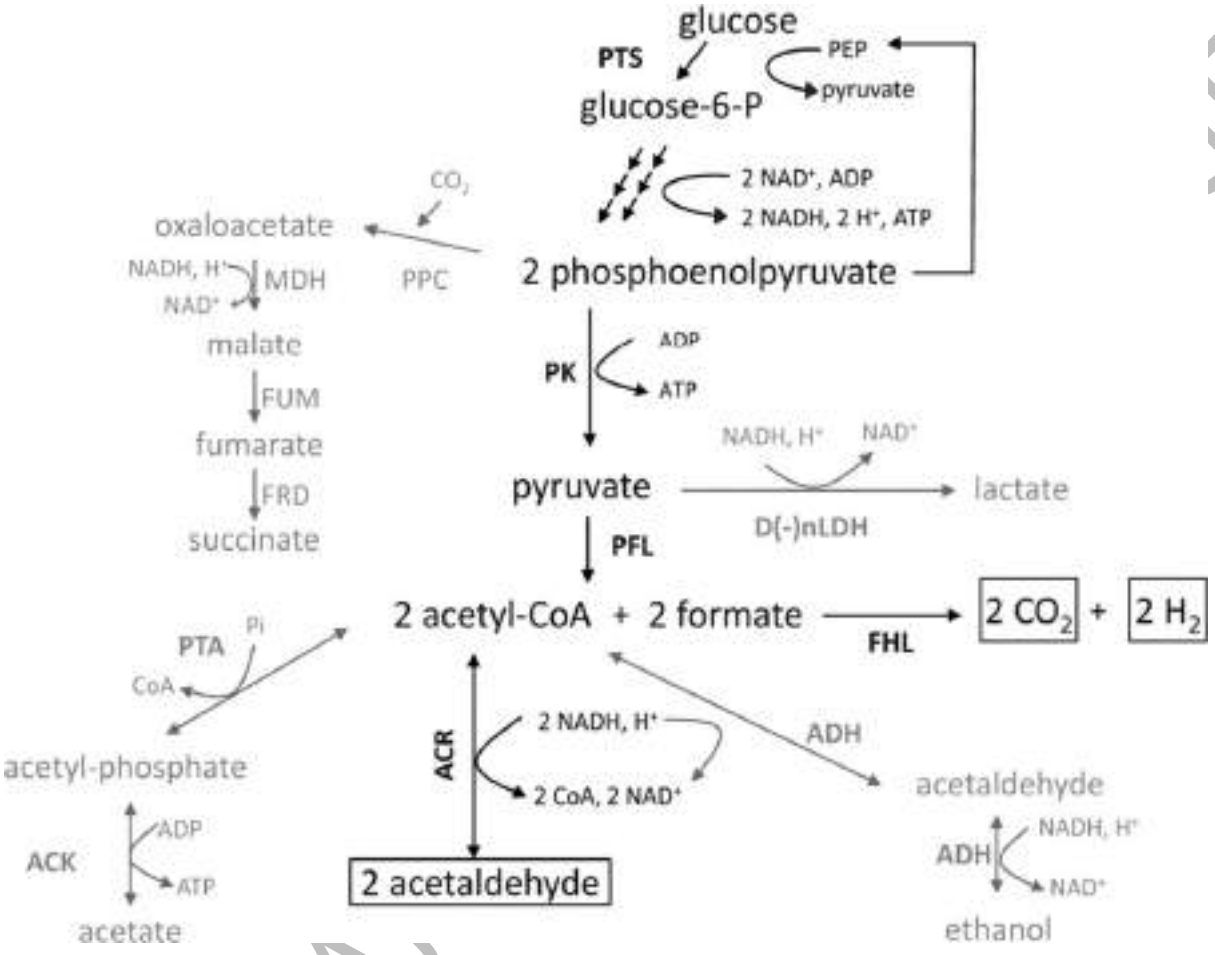
The concentration of these end products of fermentation vary depending on the environment and microorganism but it may be at the 4:1 ratio of neutral to acid products. Hydrogen and carbon dioxide is formed in bacteria with formate hydrogen lyase complex.

Application of Mixed Acid Fermentation

Mixed Acid fermentation is applicable in various fields of science, especially for producing many helpful end products. The applications of mixed acid fermentation are as follows:

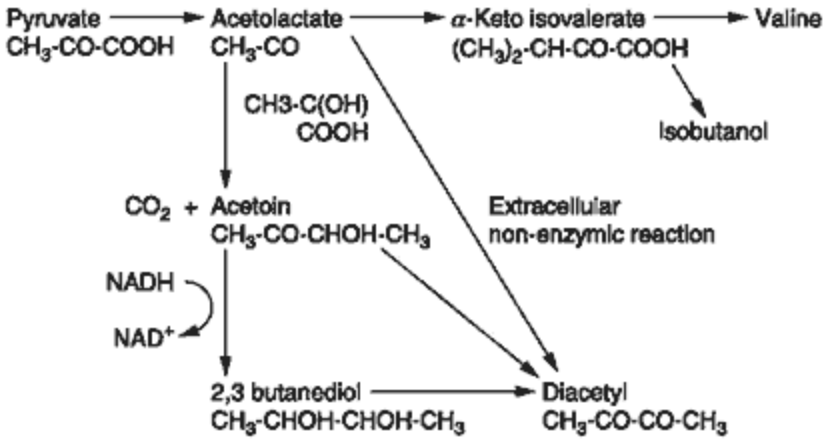
1. The use of single bacteria can help produce various products in biotechnology and the food industry.
2. Likewise, many different strains of bacteria have been metabolically engineered in the laboratory to increase the yield of the specific end product.

3. This fermentation method is applied to identify bacteria in the laboratory. Methyl red test is standard for detecting the bacteria following the mixed acid fermentation reaction. Here, the test solution turns red if the pH drops below 4.4 in the presence of those microorganisms that follows the mixed acid fermentation pathway.



2,3-Butanediol fermentation:

- In this pathway 2,3-butanediol is the end product.
- Some Pyruvate produced during glycolysis is metabolized as in mixed acid fermentation but most of the pyruvate is condensed to form α-acetolactate.
- α-acetolactate undergoes decarboxylation in the presence of enzyme pyruvate decarboxylase to produce Acetoin (acetyl methylcarbainol) which is reduced by NADH2 to form 2,3-butanediol.



- This pathway is followed by some member of Enterobacteriaceae famil. Eg Klebsiella
- This fermentative pathway is the basis of VP test.

UNIT – IV

OVERVIEW OF CHLOROPLAST STRUCTURE

Plants form the basis of all life on earth and are known as producers. Plant cells contain structures known as plastids which are absent in animal cells. These plastids are double-membraned cell organelles which play a primary role in the manufacturing and storing of food. There are three types of plastids –

- Chromoplasts- They are the colour plastids, found in all flowers, fruits and are mainly responsible for their distinctive colours.
- Chloroplasts- They are green coloured plastids, which comprise green-coloured pigments within the plant cell and are called chlorophyll.
- Leucoplasts- They are colourless plastids and are mainly used for the storage of starch, lipids and proteins within the plant cell.

Chloroplast Definition

"Chloroplast is an organelle that contains the photosynthetic pigment chlorophyll that captures sunlight and converts it into useful energy, thereby, releasing oxygen from water. "

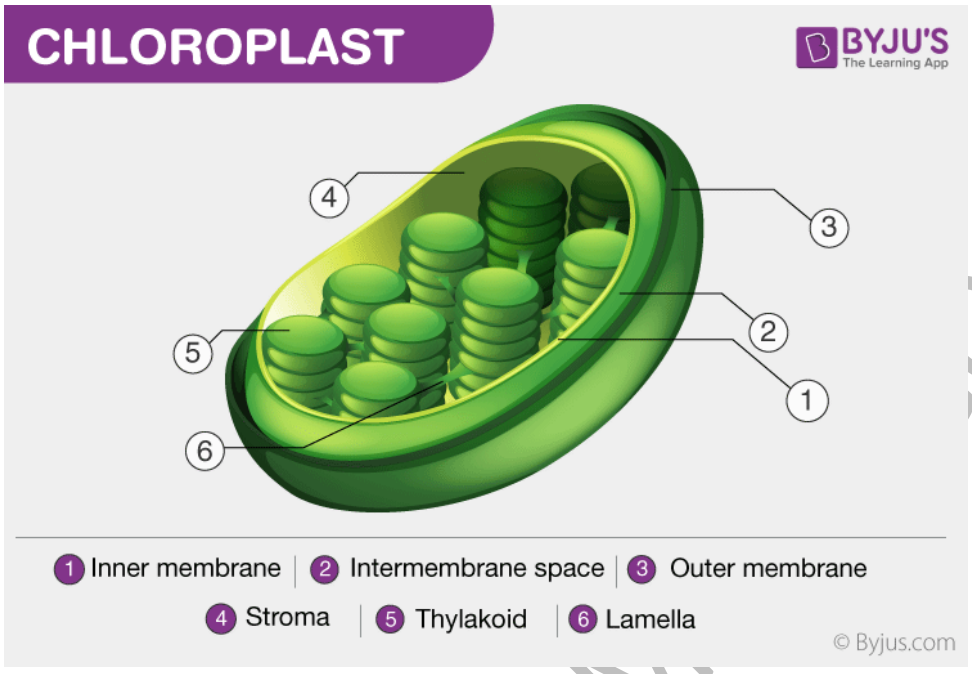
INTRODUCTION:

Chloroplasts are found in all green plants and algae. They are the food producers of plants. These are found in mesophyll cells located in the leaves of the plants. They contain a high concentration of chlorophyll that traps sunlight. This **cell organelle** is not present in animal cells.

Chloroplast has its own extra-nuclear DNA and therefore are semiautonomous, like mitochondria. They also produce proteins and lipids required for the production of chloroplast membrane.

Diagram of Chloroplast

The chloroplast diagram below represents the chloroplast structure mentioning the different parts of the chloroplast. The parts of a chloroplast such as the inner membrane, outer membrane, intermembrane space, thylakoid membrane, stroma and lamella can be clearly marked out.



Chloroplast Diagram representing Chloroplast Structure

Structure of Chloroplast

Chloroplasts are found in all higher plants. It is oval or biconvex, found within the mesophyll of the plant cell. The size of the chloroplast usually varies between 4-6 µm in diameter and 1-3 µm in thickness. They are double-membrane organelle with the presence of outer, inner and intermembrane space. There are two distinct regions present inside a chloroplast known as the grana and stroma.

- Grana are made up of stacks of disc-shaped structures known as thylakoids or lamellae. The grana of the chloroplast consists of chlorophyll pigments and are the functional units of chloroplasts.
- Stroma is the homogenous matrix which contains grana and is similar to the cytoplasm in cells in which all the organelles are embedded. Stroma also contains various enzymes, DNA, ribosomes, and other substances. Stroma lamellae function by connecting the stacks of thylakoid sacs or grana.

The chloroplast structure consists of the following parts:

Membrane Envelope

It comprises inner and outer lipid bilayer membranes. The inner membrane separates the stroma from the intermembrane space.

Intermembrane Space

The space between inner and outer membranes.

Thylakoid System (Lamellae)

The system is suspended in the stroma. It is a collection of membranous sacs called thylakoids or lamellae. The green coloured pigments called chlorophyll are found in the thylakoid membranes. It is the sight for the process of light-dependent reactions of the photosynthesis process. The thylakoids are arranged in stacks known as grana and each granum contains around 10-20 thylakoids.

Stroma

It is a colourless, alkaline, aqueous, protein-rich fluid present within the inner membrane of the chloroplast present surrounding the grana.

Grana

Stack of lamellae in plastids is known as grana. These are the sites of conversion of light energy into chemical energy.

Chlorophyll

It is a green photosynthetic pigment that helps in the process of photosynthesis.

Functions of Chloroplast

Following are the important chloroplast functions:

- The most important function of the chloroplast is to synthesise food by the process of photosynthesis.
- Absorbs light energy and converts it into chemical energy.
- Chloroplast has a structure called chlorophyll which functions by trapping the solar energy and is used for the synthesis of food in all green plants.
- Produces NADPH and molecular oxygen (O2) by photolysis of water.
- Produces ATP – Adenosine triphosphate by the process of photosynthesis.
- The carbon dioxide (CO2) obtained from the air is used to generate carbon and sugar during the Calvin Cycle or dark reaction of photosynthesis.

Photosynthetic Pigments

Pigments are compounds that give colour to materials around us. The substances that give this colour are also termed as biochromes or biological pigments. They are usually insoluble in water and are of two types – organic (natural) and inorganic (synthetic). Photosynthetic pigments are a type of natural pigment that helps in the process of photosynthesis. Here, let's discuss more about the different types of photosynthetic pigments. Photosynthetic pigments are the most important coloured components of the chloroplast lamellae. These pigments are molecules that strongly absorb visible light. They interact with sunlight to alter the wavelengths that are either reflected or transmitted by the plant tissue. These pigments are also found in cyanobacteria and algae.

Groups of Pigments

The major groups of photosynthetic pigments are:

Chlorophyll

Carotenoids

Phycobilins

Flavonoids

Chlorophyll

Chlorophyll – Chemical Formula – $C_{55}H_{72}O_5N_4Mg$

The most widespread pigment in photosynthetic plants is chlorophyll. They are cyclic tetrapyrrole pigments chelated with magnesium. They share structural features with the haem and bile pigments of animals. These chlorophyll pigments can be found in fruits, flowers as well as leaves.

Chlorophyll a and chlorophyll b are the major types of chlorophylls found in plants. The former is a blue-green pigment and the latter is a yellow-green pigment. They give their characteristic green colour due to the strong absorbance of red and blue light. The other types of chlorophyll include chlorophyll c1, c2, c3, d, e and chlorophyll f.

Achlorophyllous – Achlorophyllous is a term used to describe an organism without chlorophyll and thus not able to take part in photosynthesis.

Carotenoids

Carotenoids are also associated with the process of photosynthesis. Additionally, they give a bright colour to the fruits and flowers. They are terpenoid pigments present in all photosynthetic plants and they also occur in photosynthetic bacteria such as Rhodobacter and Erwinia. Carotenoids are orange, red and yellow pigments that usually occur in the roots, tubers, leaves, fruits, seeds and flowers.

This group includes the xanthophylls (yellow pigments) and carotenes (orange pigments).

- Zeaxanthin (xanthophyll) – yellow of corn seeds
- β carotene – orange peel

Phycobilins

It is a light-capturing bile pigment found in the chloroplast of red algae and cyanobacteria. They have chromatophores that are primarily responsible for their colour. These are unique pigments as they are bonded to water-soluble phycobiliproteins which pass sunlight to chlorophyll and thus help in the process of photosynthesis.

Flavonoids

Flavonoids are another type of commonly-found pigment, which are phenylpropanoid compounds. There are several classes of flavonoids out of which only a few provide pigments to plants like the proanthocyanidins (condensed tannins) and anthocyanins. Flavonoids occur in almost all tissues. Apart from providing floral pigmentation, they are also involved in nitrogen fixation, physiological regulation, UV filtration, etc.

Pigment	Common Types	Occurrence
Chlorophyll	Chlorophyll	All photosynthetic plants
Flavonoids	Aurones Anthocyanins Flavonols Chalcones Proanthocyanidins	Common in plants including gymnosperms, angiosperms, bryophytes and ferns.
Carotenoids	Carotenes Xanthophylls	Photosynthetic plants and bacteria.
Phycobilins	Phycoerythrobilin Phycocyanobilin Phycourobilin	Red algae, cryptomonads, glaucophytes and cyanobacteria.

Functions of Photosynthetic Pigments

Chlorophylls and carotenoids are required for photosynthesis. Chlorophylls are essential for the capture of light energy and are the primary electron donors. Carotenoids are essential structural components of the photosynthetic apparatus, where they protect against photo-oxidation. Plant pigments are also involved in other interactions of plants with light, in particular, the response to UV radiation.

With the exception of chlorophyll, the most obvious function of plant pigments is to provide colour to fruits and flowers and aid in pollination. Anthocyanins often occur in vegetative tissues. They contribute to the autumn colour in leaves of many deciduous species, which they generate in combination with the retention of carotenoids and loss of chlorophyll. In some cases, anthocyanin production is induced in response to stress like pests, pathogens, cold, nutrient deficiency, etc.

CALVIN'S CYCLE:

Introduction

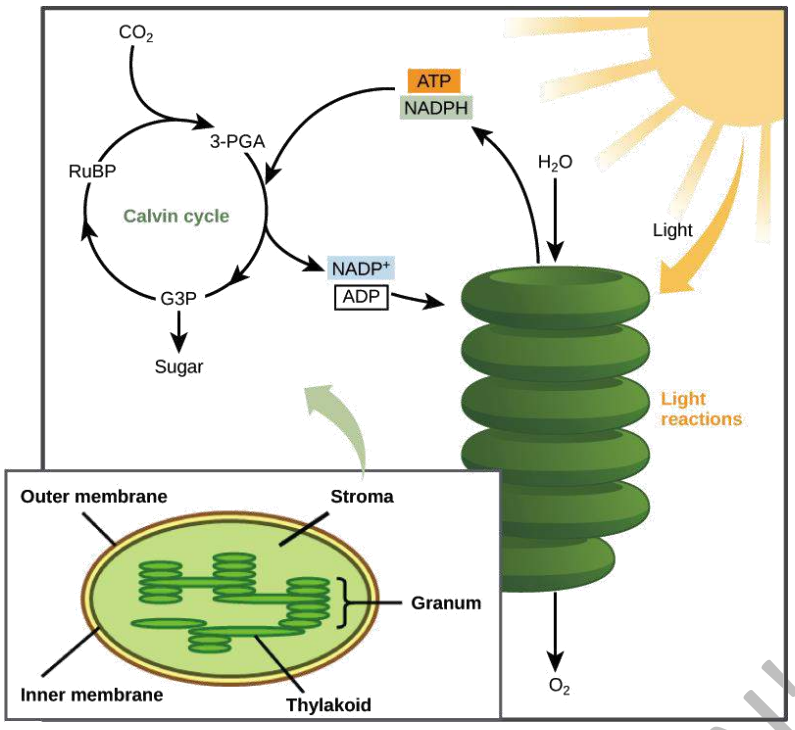
You, like all organisms on Earth, are a carbon-based life form. In other words, the complex molecules of your amazing body are built on carbon backbones. You might already know that you're carbon-based, but have you ever wondered where all of that carbon comes from?

As it turns out, the atoms of carbon in your body were once part of carbon dioxide (\[\text{CO}_2\]) molecules in the air. Carbon atoms end up in you, and in other life forms, thanks to the second stage of photosynthesis, known as the Calvin cycle (or the light-independent reactions).

Overview of the Calvin cycle

In plants, carbon dioxide (\[\text{CO}_2\]) enters the interior of a leaf via pores called stomata and diffuses into the stroma of the chloroplast—the site of the Calvin cycle reactions, where sugar is synthesized. These reactions are also called the light-independent reactions because they are not directly driven by light.

In the Calvin cycle, carbon atoms from \[\text{CO}_2\] are fixed (incorporated into organic molecules) and used to build three-carbon sugars. This process is fueled by, and dependent on, ATP and NADPH from the light reactions. Unlike the light reactions, which take place in the thylakoid membrane, the reactions of the Calvin cycle take place in the stroma (the inner space of chloroplasts).



This illustration shows that ATP and NADPH produced in the light reactions are used in the Calvin cycle to make sugar.

Reactions of the Calvin cycle

The Calvin cycle reactions can be divided into three main stages: carbon fixation, reduction, and regeneration of the starting molecule.

Here is a general diagram of the cycle:

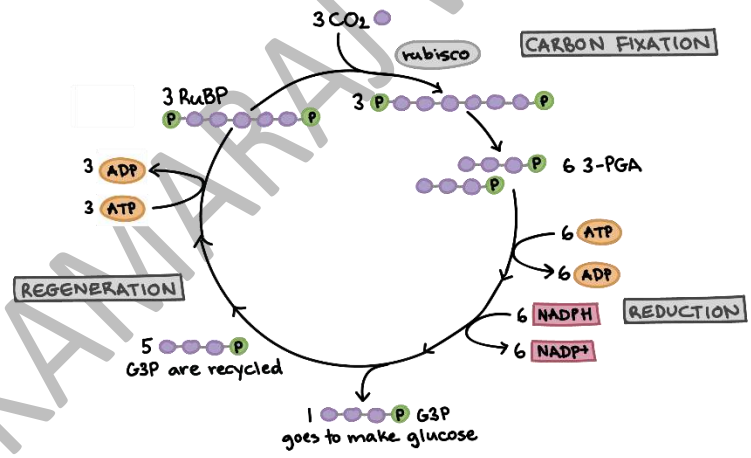


Diagram of the Calvin cycle, illustrating how the fixation of three carbon dioxide molecules allows one net G3P molecule to be produced (that is, allows one G3P molecule to leave the cycle).

3 {CO}_2\] molecules combine with three molecules of the five-carbon acceptor molecule (RuBP), yielding three molecules of an unstable six-carbon compound that splits to form six molecules of a three-carbon compound (3-PGA). This reaction is catalyzed by the enzyme rubisco.

In the second stage, six ATP and six NADPH are used to convert the six 3-PGA molecules into six molecules of a three-carbon sugar (G3P). This reaction is considered a reduction because NADPH must donate its electrons to a three-carbon intermediate to make G3P.

3.Regeneration. One G3P molecule leaves the cycle and will go towards making glucose, while five G3Ps must be recycled to regenerate the RuBP acceptor. Regeneration involves a complex series of reactions and requires ATP.

1. **Carbon fixation.** A \[\text{CO}_2\] molecule combines with a five-carbon acceptor molecule, ribulose-1,5-bisphosphate (RuBP). This step makes a six-carbon compound that splits into two molecules of a three-carbon compound, 3-phosphoglyceric acid (3-PGA). This reaction is catalyzed by the enzyme RuBP carboxylase/oxygenase, or rubisco.
2. **Reduction.** In the second stage, ATP and NADPH are used to convert the 3-PGA molecules into molecules of a three-carbon sugar, glyceraldehyde-3-phosphate (G3P). This stage gets its name because NADPH donates electrons to, or reduces, a three-carbon intermediate to make G3P.
3. **Regeneration.** Some G3P molecules go to make glucose, while others must be recycled to regenerate the RuBP acceptor. Regeneration requires ATP and involves a complex network of reactions, which my college bio professor liked to call the "carbohydrate scramble."

In order for one G3P to exit the cycle (and go towards glucose synthesis), three {CO}_2\] molecules must enter the cycle, providing three new atoms of fixed carbon. When three \[\text{CO}_2\] molecules enter the cycle, six G3P molecules are made. One exits the cycle and is used to make glucose, while the other five must be recycled to regenerate three molecules of the RuBP acceptor.

Summary of Calvin cycle reactants and products

Three turns of the Calvin cycle are needed to make one G3P molecule that can exit the cycle and go towards making glucose. Let’s summarize the quantities of key molecules that enter and exit the Calvin cycle as one net G3P is made. In three turns of the Calvin cycle:

- **Carbon.** [3CO2] combine with [3] RuBP acceptors, making [6] molecules of glyceraldehyde-3-phosphate (G3P).
- \[1\] G3P molecule exits the cycle and goes towards making glucose.
- \[5\] G3P molecules are recycled, regenerating [3] RuBP acceptor molecules.

- **ATP.** \[9\] ATP are converted to \[9\] ADP (\[6\] during the reduction step, \[3\] during the regeneration step).
- **NADPH.** [6] NADPH are converted to \[6\] NADP (during the reduction step).

A G3P molecule contains three fixed carbon atoms, so it takes two G3Ps to build a six-carbon glucose molecule. It would take six turns of the cycle, or \[6\] {CO}_2\], \[18\] ATP, and \[12\] NADPH, to produce one molecule of glucose.

CYCLIC AND NON-CYCLIC PHOTOPHOSPHORYLATION:

Photosynthesis is the biological process of converting light energy into chemical energy. In this process, light energy is captured and used for converting carbon dioxide and water into glucose and oxygen gas. The complete process of photosynthesis is carried out through two processes:

Light reaction

The light reaction takes place in the grana of the chloroplast. Here, light energy gets converted to chemical energy as ATP and NADPH. In this very light reaction, the addition of phosphate in the presence of light or the synthesizing of ATP by cells is known as photophosphorylation.

Dark reaction

While in the dark reaction, the energy produced previously in the light reaction is utilized to fix carbon dioxide into carbohydrates. The location where this happens is the stroma of the chloroplasts.

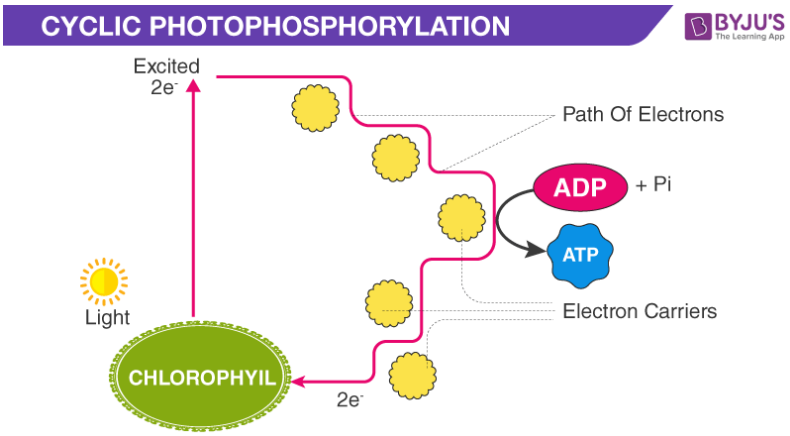
Photophosphorylation

Photophosphorylation is the process of utilizing light energy from photosynthesis to convert ADP to ATP. It is the process of synthesizing energy-rich ATP molecules by transferring the phosphate group into ADP molecule in the presence of light.

Photophosphorylation is of two types:

- Cyclic Photophosphorylation
- Non-cyclic Photophosphorylation

Cyclic Photophosphorylation



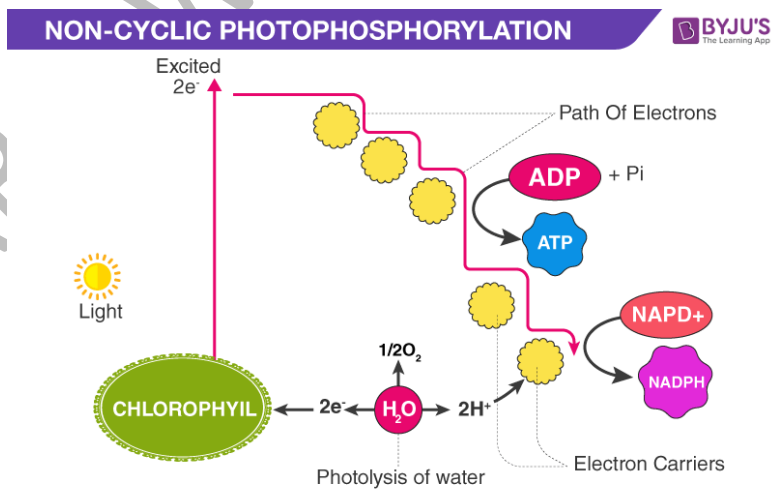
Cyclic Photophosphorylation

The photophosphorylation process which results in the movement of the electrons in a cyclic manner for synthesizing ATP molecules is called cyclic photophosphorylation.

In this process, plant cells just accomplish the ADP to ATP for immediate energy for the cells. This process usually takes place in the thylakoid membrane and uses Photosystem I and the chlorophyll P700.

During cyclic photophosphorylation, the electrons are transferred back to P700 instead of moving into the NADP from the electron acceptor. This downward movement of electrons from an acceptor to P700 results in the formation of ATP molecules.

Non-Cyclic Photophosphorylation



Non-cyclic Photophosphorylation

The photophosphorylation process which results in the movement of the electrons in a non-cyclic manner for synthesizing ATP molecules using the energy from excited electrons provided by photosystem II is called non-cyclic photophosphorylation.

This process is referred to as non- cyclic photophosphorylation because the lost electrons by P680 of Photosystem II are occupied by P700 of Photosystem I and are not reverted to P680. Here the complete movement of the electrons is in a unidirectional or in a non- cyclic manner.

During non-cyclic photophosphorylation, the electrons released by P700 are carried by primary acceptor and are finally passed on to NADP. Here, the electrons combine with the protons – H+ which is produced by splitting up of the water molecule and reduces NADP to NADPH2.

Difference between Cyclic and Non-Cyclic Photophosphorylation

Following are the important differences between cyclic and non-cyclic photophosphorylation:

Cyclic Photophosphorylation	**Non-Cyclic Photophosphorylation**
Only Photosystem I is involved.	Both Photosystem I and II are involved.
P700 is the active reaction centre.	P680 is the active reaction centre.
Electrons travel in a cyclic manner.	Electrons travel in a non – cyclic manner.
Electrons revert to Photosystem I	Electrons from Photosystem I are accepted by NADP.
ATP molecules are produced.	Both NADPH and ATP molecules are produced.
Water is not required.	Photolysis of water is present.
NADPH is not synthesized.	NADPH is synthesized.
Oxygen is not evolved as the by-product	Oxygen is evolved as a by-product.
This process is predominant only in bacteria.	This process is predominant in all green plants.

UNIT – V

BINARY FISSION

Definition

Binary fission is the process through which asexual reproduction happens in bacteria. During binary fission, a single organism becomes two independent organisms. Binary fission also describes the duplication of organelles in eukaryotes. Mitochondria and other organelles must reproduce via binary fission before mitosis so each cell has ample organelles.

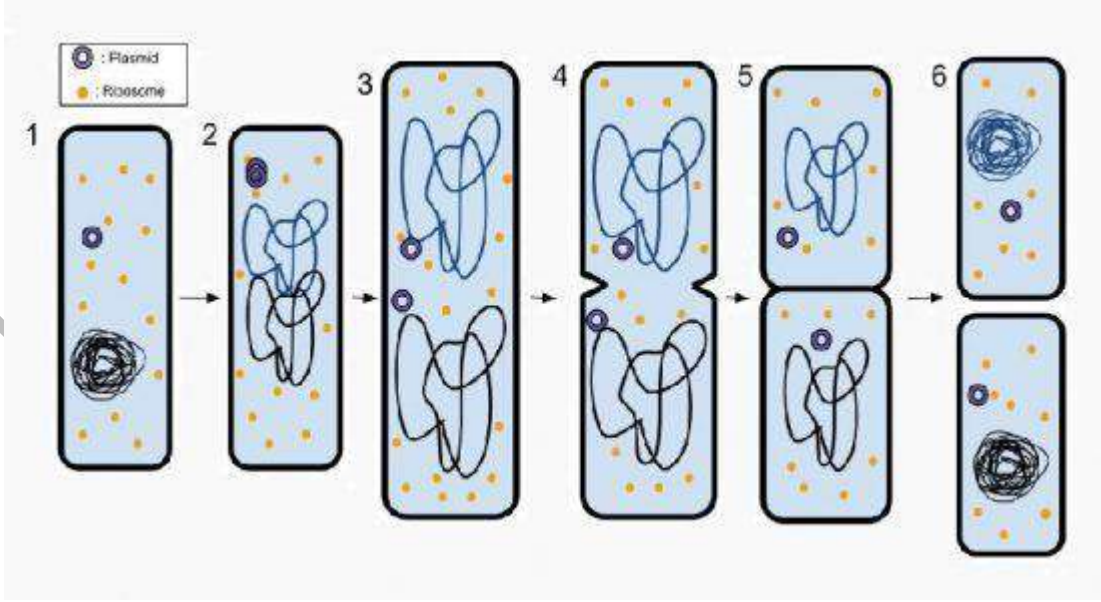
OVERVIEW OF BINARY FISSION:

Binary fission is a relatively simple process, compared to mitosis, because binary fission does not involve reproducing organelles or complex chromosomes. The process starts with the replication of the DNA within the cell. Mitochondria must also replicate their DNA before binary fission, though other organelles have no DNA.

Then, the DNA is separated into alternate ends of the single cell. The plasma membrane pinches the cell apart, and one cell becomes two. With a fully-functioning DNA molecule, each cell is then capable of all the functions of life. Therefore, the cells become independent organisms.

Organelles, though they are not independent organisms, separate in this way as well. Endosymbiotic theory says that mitochondria and chloroplasts were once independent organisms that have evolved to live within other cells. As such, they still replicate via binary fission.

Binary Fission Steps

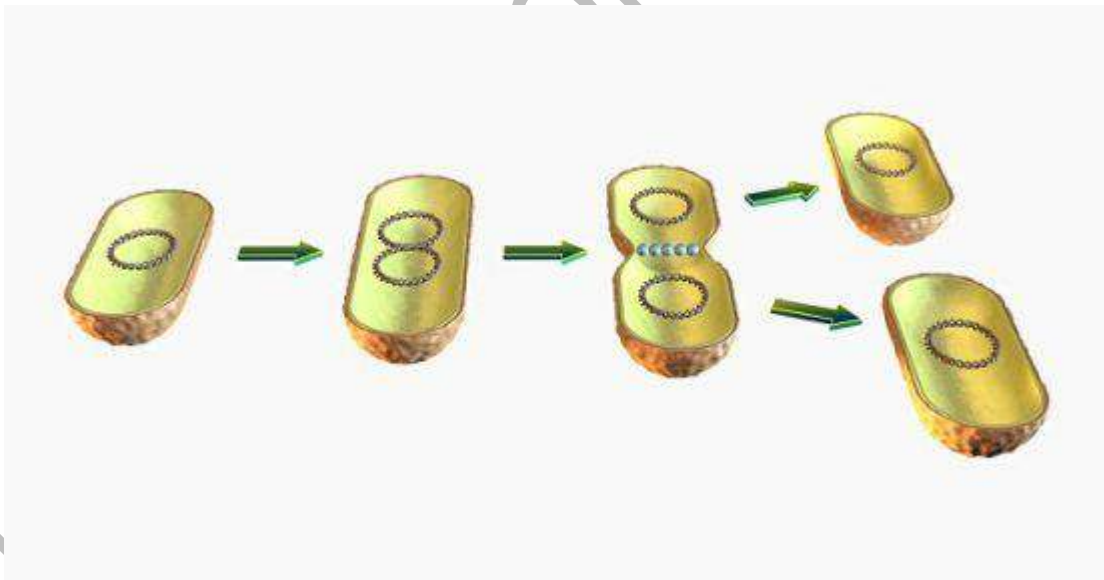


Before binary fission of a prokaryote, as seen in step 1 of the above graphic, a prokaryote's DNA is tightly wound. Sometimes, the prokaryote will carry small plasmids, which are small rings of DNA that carry extra genetic information. During the second step of binary fission, the DNA is unraveled. As it is unraveled, specialized proteins gain access to the DNA, which then works to replicate the ring of DNA. The same proteins work on the plasmids in the cell, duplicating them as well. By step 3, both the DNA and plasmids have been duplicated. The individual copies of DNA attach themselves to different parts of the cell membrane. As the cell elongates in preparation for division, the DNA molecules are pulled to different sides of the cell.

At step 4, a cleavage furrow appears in the cell membrane, as the cell wall and membrane start to pinch off and create two new cells. Finally, as seen in step 5, the cells become completely separated from one another as a new bacterial cell wall forms. The final step includes breaking any additional proteins or other molecules that still connect the two cells. Each cell now has everything it needs to continue the functions of life independently.

Binary Fission in Bacteria

All of the organisms in the domains Archaea and Bacteria reproduce asexually through binary fission. By far, bacteria account for the most populous organisms on the planet. The process of binary fission is a very stable one, and because bacteria have a very simple genome, there are relatively few mutations in prokaryotes as compared to eukaryotes. Eukaryotes must undergo many cell divisions before gametes can be produced for sexual reproduction, therefore many more mutations can be introduced before offspring are created.



Bacteria will go through the steps listed above as they proceed through binary fission. However, there are many variations of this scheme that have evolved in the different lines of bacteria. For instance, the bacteria Bacillus subtilis is a bacteria that exists in the soil and in the gut of some mammals, including humans. **This bacteria can divide equally, creating two relatively identical cells, or it can create a much smaller division, which acts as a spore.**

This *endospore* is much more resilient than its larger counterpart and can travel through an animal or the environment to new locations or simply survive until favourable conditions return. Bacteria also exhibit variations in the ways in which they elongate to divide. Some bacteria extend at the far end, while others grow from the middle outward. Even the timing with which the bacteria divide differs and is directed by genetics. Some bacteria can divide in as little as 20 minutes, while others take many hours.

Binary Fission in Organelles

Although the process of mitosis in eukaryotes is similar to binary fission, it is much more complex because eukaryotes have larger genomes and many organelles to duplicate. However, the organelles of eukaryotes replicate using binary fission. Many organelles even harbour their own DNA, which directs their functions and growth. Mitochondria, for example, the energy centre of the cell, must make many copies of itself to provide a dividing cell with enough energy. Mitochondrial DNA is replicated, and the organelle divides in the same sequence described above.

Throughout the cell, each organelle must be replicated at least once, if the resulting cells are to have the proper amount of organelles. As the organelles undergo binary fission, they are also moved by the directions of the spindle apparatus and microtubules to opposite ends of the cells. Thus, when the cell divides through cytokinesis after mitosis, each cell is ready to operate independently immediately.

BUDDING:

Budding is a form of asexual reproduction in which a new organism develops from an outgrowth or "bud" on the parent organism, eventually detaching to live independently.

Budding is a specialized form of asexual reproduction in which a new organism develops from a specific generative anatomical point on the parent organism. This process is not universal but is confined to certain organisms, both unicellular and multicellular. The fundamental principle behind budding is the formation of a new organism from a growth, known as a "bud", which eventually detaches from the parent organism to live independently.

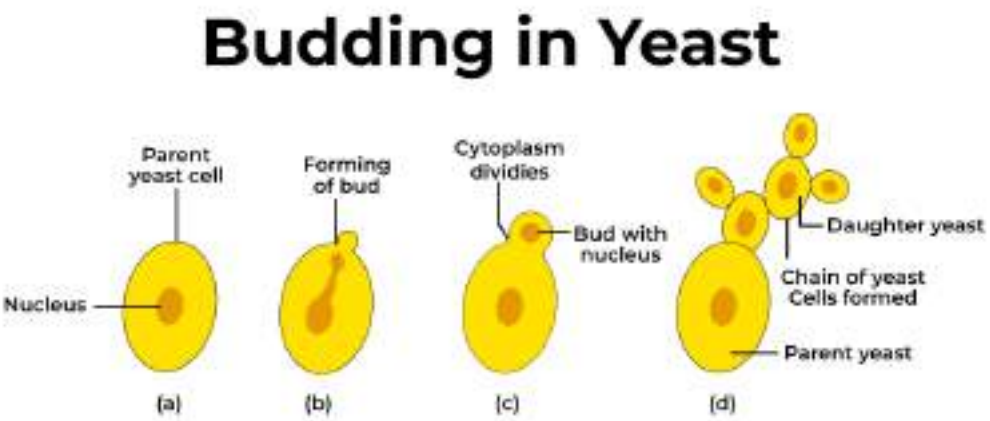
- In the realm of biology, budding is observed in a diverse range of organisms, from fungi and algae to more complex organisms like the hydra and certain coral species. The mechanism of budding varies among these organisms. For instance, in fungi, the new organism, once formed, separates from the parent. In contrast, in the hydra, the budding offspring remains attached, leading to the growth of a colony.
- The process of budding holds significance in the life cycles of certain animal and plant species. It facilitates rapid reproduction, enabling these species to colonize new environments swiftly. This method of reproduction ensures that the offspring are genetically identical to the parent, given that there's no involvement of gametes or their fusion, which could introduce genetic variations.

- Furthermore, budding has implications beyond natural reproduction. In the field of biotechnology, budding principles are harnessed to engineer new microbial strains or to produce genetically modified plants. The process underscores the ability of certain organisms to reproduce without the need for a counterpart, using only a fragment of the parent's body to give rise to a new, genetically identical individual.
- In summary, budding is an intricate form of asexual reproduction, pivotal to the life cycles of specific organisms. It exemplifies nature's ability to reproduce and sustain life in diverse ways, ensuring the continuity of species across various environments.

Types of Budding

Budding is a form of asexual reproduction wherein a new organism develops from an outgrowth or "bud" on the parent organism. Based on the site of bud formation, budding can be classified into two primary types:

1. **Exogenous Budding:**
    - In exogenous budding, the bud forms externally on the parent body. As the bud matures, it evolves into a distinct organism and may eventually separate from the parent. This type of budding is observed in organisms such as bacteria, yeast, protozoans, and cnidarians.
    - A classic example of exogenous budding is observed in yeast. Here, an unequal division leads to the creation of a small bud that remains attached to the parent cell. Over time, the bud may detach and develop into a new yeast organism. Yeast, a member of the fungi kingdom, is a single-celled, achlorophyllous microorganism. It is larger than bacteria, typically measuring 3-4 µm in diameter. In certain scenarios, the newly formed buds may remain attached to the parent cell for an extended period, leading to a chain of buds known as pseudomycelium. These buds, in due course, separate from the parent and mature into individual organisms.
2. **Endogenous Budding:**
    - Also known as internal budding, endogenous budding is a unique process where the new organism or bud develops internally within the parent organism or cell. This budding type is predominantly observed in sponges from the phylum Porifera, especially in freshwater and marine environments.



Budding in yeast cell

- Yeast, a unicellular eukaryotic organism, predominantly employs budding as its primary mode of asexual reproduction, especially when thriving in nutrient-abundant environments.
- The process initiates with the appearance of a soft protrusion or "bud" on the yeast cell wall. Concurrently, the nucleus of the mother cell undergoes mitotic division, resulting in the formation of daughter nuclei. One of these nuclei migrates into the budding outgrowth.
- As the bud continues to develop, a constriction or narrow zone emerges at the junction between the mother cell and the bud. Eventually, this constriction becomes more pronounced, leading to the detachment of the bud from the mother cell.
- This newly separated bud matures into an independent yeast cell, identical to the original mother cell, and is capable of undergoing its own budding process under favorable conditions. This method ensures the rapid propagation of yeast populations, allowing them to flourish and adapt to their surroundings.

ADVANTAGES OF BUDDING:

Budding, as a mode of asexual reproduction, offers several distinct advantages that contribute to the rapid proliferation and survival of organisms:

1. **Efficient Population Growth:** Given conducive environmental conditions, budding can lead to the swift generation of a substantial number of offspring, ensuring the continuity of the species.
2. **No Need for Mating Partners:** One of the primary benefits of budding is that it requires only a single parent for reproduction. This eliminates the need for finding a mating partner, thereby simplifying the reproductive process.

3. **Rapid Reproductive Cycle:** Budding is a time-efficient process. Since it bypasses the complexities of mating and fertilization, organisms can reproduce in a relatively short duration, leading to quicker population expansion.

4. **Consistency in Offspring:** As budding is an asexual mode of reproduction, the offspring produced are genetically identical to the parent. This genetic consistency ensures that advantageous traits of the parent are preserved in the progeny.

In summary, budding provides a streamlined and efficient reproductive strategy, especially beneficial for organisms in stable and resource-rich environments.

DISADVANTAGES OF BUDDING:

While budding offers several advantages as a mode of asexual reproduction, it also presents certain limitations:

1. **Limited Genetic Diversity:** One of the primary drawbacks of budding is the production of genetically identical offspring. This uniformity in genetics means that the population lacks genetic variation, which is essential for adaptability and evolution. A lack of genetic diversity can hinder the potential for adaptation to changing environmental conditions.

2. **Vulnerability to Environmental Stresses:** Since the offspring produced through budding are genetically identical, they share the same strengths and weaknesses. As a result, a single environmental stressor or pathogen can potentially affect the entire population. This makes the population more susceptible to diseases, pests, or any other adverse conditions that might arise.

3. **Reduced Evolutionary Potential:** The absence of genetic recombination in budding limits the potential for evolutionary advancements. Genetic recombination, which occurs during sexual reproduction, introduces new genetic combinations, fostering adaptability and evolution. In contrast, budding does not provide this benefit.

REPRODUCTION THROUGH CONIDIA:

Conidia formation takes place in filamentous bacteria like Streptomyces etc., by the formation of a transverse septum at the apex of the filament (Fig. 2.21 A). The part of this filament which bears conidia is called conidiophore. After detachment from the mother and getting contact with suitable substratum, the conidium germinates and gives rise to new mycelium.

Cysts:

Cysts are formed by the deposition of additional layer around the mother wall. These are the resting structure and during favourable condition they again behave as the mother, e.g., many members of Azotobacter.

Endospore:

Spores are formed during unfavourable environmental condition like desiccation and starvation. As the spores are formed within the cell, they are called endospores. Only one spore is formed in a bacterial cell. On germination, it gives rise to a bacterial cell.

A. Some endospore forming bacteria:

1. Gram-positive

(a) Bacilli

(i) Obligate aerobes, e.g., Bacillus subtilis, B. anthracis.

(ii) Obligate anaerobes, e.g., Clostridium tetani, C. botulinum.

(b) Cocci, e.g., Sporosarcina.

2. Gram-negative

(i) Bacillus, e.g., Coxiella burnetii

(ii) Cocci, e.g., Escherichia coli

B. Some non-sporing anaerobic bacteria:

1. Gram-positive

(a) Bacilli

(i) Lactobacillus

(ii) Propionibacterium

(iii) Bifidobacterium

(b) Cocci

(i) Peptococcus

(ii) Sarcina

(iii) Peptostreptococcus

2. Gram-negative

(a) Bacilli

(i) Fusobacterium

(ii) Leptotrichia

(iii) Bacteroides

(b) Cocci

(i) Acidoaminococcus

(ii) Veillonella

C. Shape and position of endospore:

Spores may be oval or spherical in shape. The position, relative size and shape remain constant in a particular species. The position of spore may be central, subterminal or terminal (Fig. 2.22). In diameter, it may be the same or wider (Clostridium) or less (Bacillus) than the width of the specific bacterial cell.

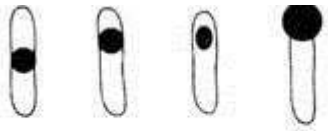


Fig. 2.22 : Different position of endospore in bacterial cell

D. Structure:

Endospore consists of a central protoplast, the core (Fig. 2.23). The core is mainly composed of DNA, ribosome, t-RNA, enzymes etc. The core is covered by a thin membrane, called core membrane or inner membrane or germ cell membrane, from which the cell wall of future vegetative bacterium develops.

It is covered by a thick layer, the cortex and then a multilayered thin and tough outer spore coat, which may be differentiated into outer and inner coat layer. In some species (Bacillus thuringiensis), it is covered by an additional covering, called exosporium or exosporium basal layer, which is apparently loose.

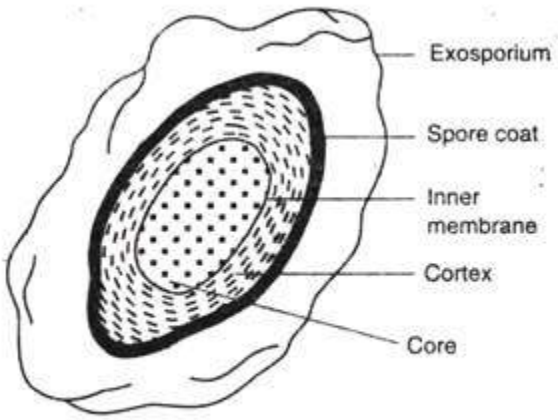


Fig. 2.23 : Bacterial endospore (diagrammatic representation)

E. Formation of endospore:

The endospore formation does not take place during active phase of growth. The sporulation starts in conditions unfavourable for the growth due to starvation, desiccation, high temperature etc. The sporulation can also be induced by depleting S, C, N, Fe and PO4 from culture medium.

During sporulation, the first detectable change is the conversion of compact nucleoid into an axial chromatin filament (Fig. 2.24). Then a transverse septum is laid down towards one pole, which separates into small and large portion. The small portion with its cytoplasm and DNA forms the fore spore, which later on develops into a spore.

The membrane of large portion gradually grows around the fore spore. The fore spore increases in size, which becomes opaque and highly refractive, called the endospore. The entire process of sporulation takes place within 16 to 20 hours. The cell in which spore is formed, called sporangium, which remains viable for short period of time after maturation of spore. The spore is liberated by autolysis of sporangium.

The spores can survive in different adverse conditions like heat, drying, freezing, toxic chemicals and radiations. Some bacilli can resist a temperature higher than 150°C.

The endospores germinate under favourable condition which consists of three stages:

(i) Activation:

It takes place by the induction of one or more factors such as acidic pH, heat (60°C for 1 hour), compounds containing free SH groups or through abrasion.

(ii) Initiation:

Binding of any effector substance like L-alanine, adenosine etc. of the medium with the spore coat activates to form an autolysin. The autolysin destroys the peptidoglycan of the cortex. Thereafter, water is taken up and calcium dipicolonate is released. Dipicolonic acid helps to stabilise the spore protein, and both dipicolonic acid and Ca ions provide resistance to heat.

(iii) Outgrowth:

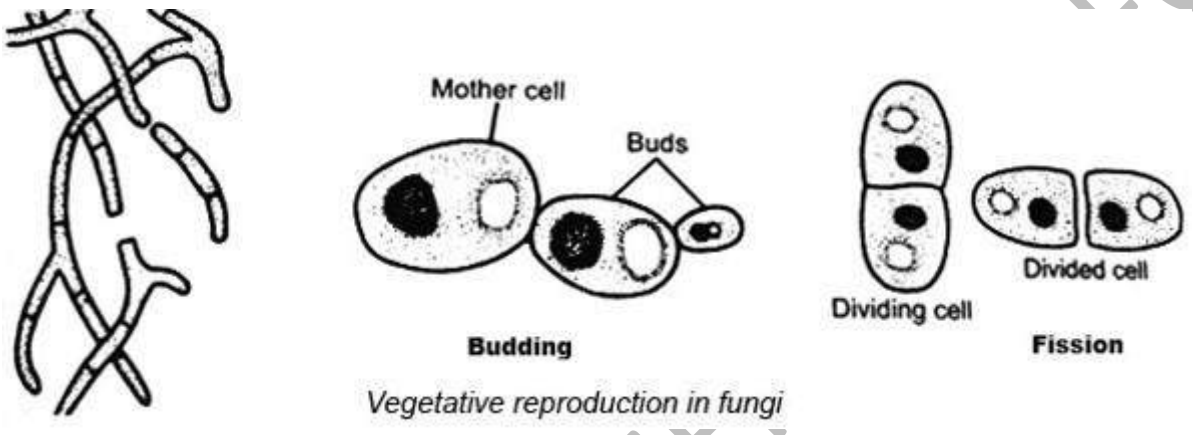
The swelling of spore wall and disintegration of the cortex help to emerge a germ cell after breaking the spore coat, which behaves like a vegetative cell.

Each cell forms only one endospore and persists during unfavourable condition. During favourable condition, it germinates and gives rise to a single bacterial cell. So the spore is a perennating organ and the process is called perennation rather than multiplication.

Reproduction in fungi: asexual and sexual methods

Asexual reproduction in fungi:

1. fission of somatic cell
2. Budding of somatic cell
3. Fragmentation or disjoining of hyphae
4. Asexual spore formation



Vegetative reproduction in fungi

1. Fission:

- In binary fission a mature cell elongates and its nucleus divides into two daughter nuclei.
- The daughter nuclei separates, cleaves cytoplasm centripetally in the middle till it divides parent protoplasm into two daughter protoplasm.
- A double cross wall is deposited in the middle to form two daughter cell.
- Ultimately the middle layer of double cross wall degenerates and daughter cells are separated.
- Examples: Saccharomyces pobbe, Psygosaccharomyces

2. Budding:

- The cell wall bulge out and softens in the area probably by certain enzymes brought by vesicles.
- The protoplasm also bulge out in this region as small protuberance.
- The parent nucleus also divides into two, one of the daughter nucleus migrates into bud, the cytoplasm of bud and mother remain continuous for some time

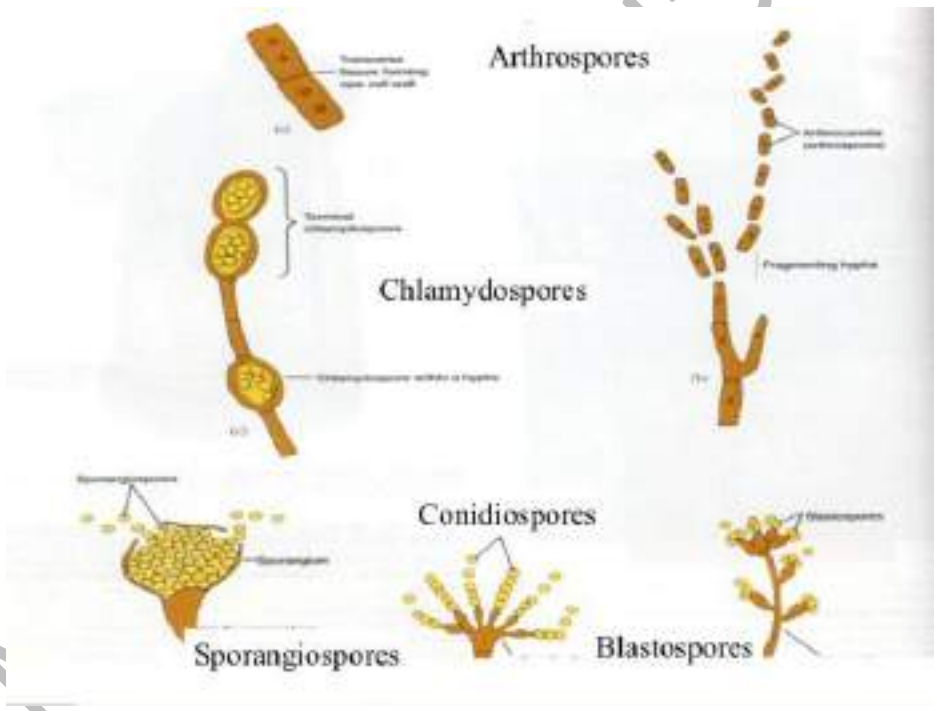
- As the bud enlarges, a septum is laid down at the joining of bud with mother cell. Then bud separates and leads independent life.
- Some time, bud starts reproducing while still attached with mother cell. This gives branching appearance.
- Budding is the typical reproductive characteristics of Ascomycetes.
- Examples: yeast

3. Fragmentation:

- In some fungi, fragmentation or disjoining of hyphae occurs and each hyphae become a new organism

4. Asexual spore of fungi:

- Spore formation is the characteristic feature of fungi.
- Different fungi forms different types of spore,



Types of asexual spore:

i. Sporangiospore:

- These asexual spore are produced in a sac like structure called sporangia (singular;saprangium).
- Sporangium are produced at the end of special aerial hyphae called sporangiophore

- Sporangium contains large numbers of haploid spores, which are released by rapture of sporangial wall
- Examples: Rhizopus

ii. Conidiospore:

- Conidiospore or conidia are single celled, bicelled or multicelled structure born on the tip or side of aerial hyphal structure called conidiophore
- Conidia are different from sporangiospore as these are not produced inside sporangium or any sac like structure.
- Conidia are born singly or in chain
- Examples: Penicillium, Apergillus

iii. Arthrospore:

- Arthrospore are very primitive type of spore formed by the breaking up of fungal mycelium
- A spore is formed by separation followed by fragmentation of hyphae
- Examples: Trichosporium, Geotrichum, Coccididious imitis

iv. Chlamydospore:

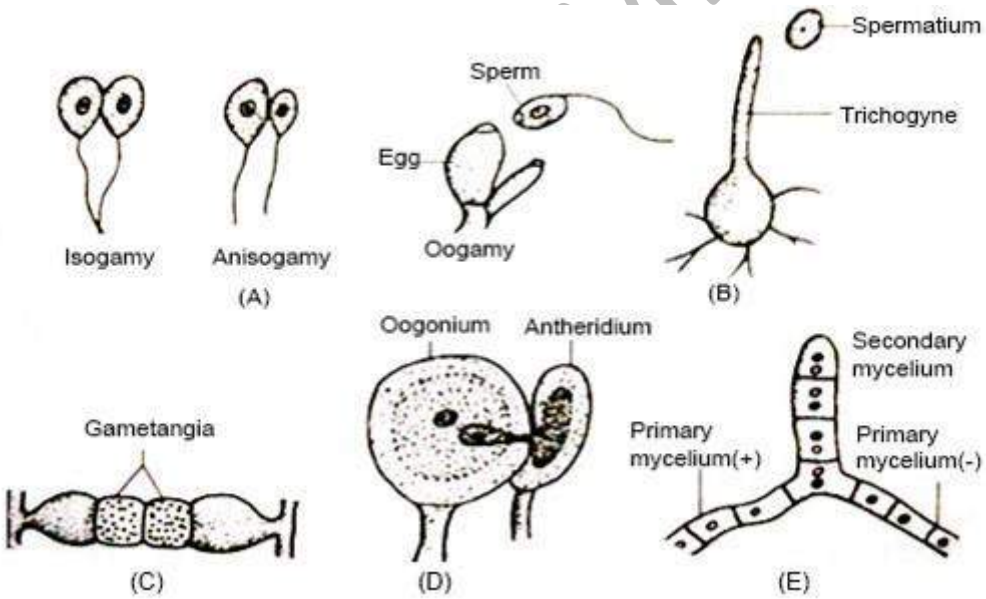
- These are usually formed during unfavorable condition and are thick walled single celled spore, which are highly resistant to adverse condition.
- Hyphal cell or portion of hyphae contracts, loose water, round up and develops into thick walled chalmydospore.
- When favorable condition returns, each chlamydospore give rise to a new individual fungi.
- Examples: ascomycetes, basidiomycetes, zygomycetes,
- Histoplasma capsulatum, Candida albicans

v. Blastospore:

- It is a budding spores usually formed at the terminal end of hyphae.
- These spore may remains attached to hyphae and bud further to gibe branching chain of blastospores
- Examples: ascomycetes, basidiomycetes, zygomycetes

Sexual reproduction in fungi:

- Sexual reproduction is carried out by diffusion of compatable nuclei from two parent at a definite state in the life cycle of fungi.
- The process of sexual reproduction involves three phases:
    - Plasmogamy: fusion of protoplasm
    - Karyogamy: fusion of nucleus
    - Meiosis: reductional nuclear division
- Various methods by which compatible nuclei are brought together in plasmogamy. Some are:
    - Gametic copulation
    - Gamete-gametangial copulation
    - Gametangial copulation
    - Somatic copulation
    - Spermatization



1. Gametic copulation:

- Fusion of two naked gametes, one or both of them are motile
    - Isogamous
    - Anisogamous

- Oogamous

2. Gamete-gametangial copulation:

- Male and female gametangia comes into contact but do not fuse.
- A fertilization tube formed from where male gametangium enters the female gametangium and male gamate passes through this tube

3. Gametangial copulation;

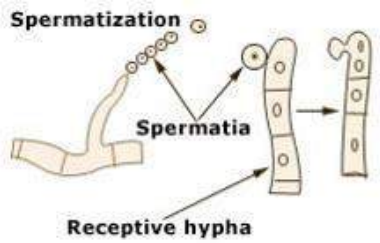
- Two gametangia or their protoplast fuse and give rise to zygospore

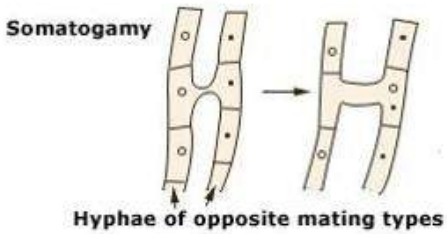
4. Somatic copulation:

- Also known as somatogamy.
- In this process fusion of somatic cell occurs
- This sexual fusion of undifferentiated vegetative cell results in dikaryotic hyphae, so the process is also called dikarotization

5. Spermetization:

- It is an union of special male structure called spertatium with a female receptive structure.
- Spermatium empties its content into receptive hyphae during plasmogamy.





Sexual spores of fungi

- As a result of sexual reproduction sexual sores are produced.
- Sexual spores are fewer in number than asexual spores.

Types of sexual spores

i. Ascospore:

- It is usually single celled produced in a sac called ascus (plural;asci) and usually there are 4-8 ascospore in an ascus but the number may vary from species to species
- The ascospore are usually arranged in a linear order. In some case ascospores are long, narrow and are arranged in parallel order.

ii. Basidiospore:

- It is a reproductive spore produced by basidiomycetes.
- This single celled spores are born in a club shaped structure called basidium
- These basidiospore aerves as main air dispersal unit for the fungi.

iii. Zygospore:

- Zygospores are thick walled spores formed when two sexually compatible hyphae or gametangia of certain fungi fuse together.
- In suitable condition, zygospore germinates to produce a single vertical hyphae which forms a aporangium and releases its spores

iv. Oospore:

- These are formed within a special female structure called Oogonium.
- Fertilization of egg by male gamete in female sex organ give rise to oospoes.
- There are one or more oospores in each oogonium.

Asexual Reproduction in Protozoa:

The mode of reproduction in which there is no union of gametes. In such a case, only one animal can produce new individuals. Protozoa usually reproduces asexually by binary fission and multiple fission.

I. Binary Fission:

The animal divides and two individuals are produced from one:

1. The micronucleus divides into two by a simplified form of mitosis.

2. The macronucleus divides into two by amitosis.

3. The cytoplasm divides into two equal halves by a constriction.

4. The daughter individuals can reconstruct the wanting structures which it does not obtain from the parent. Asymmetrical structures like gullet, peristome of Paramoecim cannot be equally shared by both the daughter individuals.

Binary fission is again of three types:

a. Transverse fission. The animal divides transversely into two. Examples: Amoeba, Paramoecium, etc.

b. Longitudinal fission. The animal splits into two along the long axis of the body. Examples: Euglena, Vorticella, etc.

c. Oblique binary fission. The plane of fission is oblique. Examples: Dinoflagellata, Ceratium, Cochliodinium,. etc.

II. Multiple Fission or Sporulation:

Many individuals are produced from one at a time. Examples: Some Amoebae, Euglena, Polystomella, etc.

1. The animal becomes encysted, the nucleus divides repeatedly and a large number of minute daughter nuclei are produced.

2. The cytoplasm fragments and a small bit of it surrounds each daughter nucleus and, thus, many minute animals are formed.

3. Under favourable circumstances the cyst bursts and these small animals come out and grow to the adult stage.

Multiple fission is common in sarcomastigophorans and apicomplexans. The process has been differently named according to the period and time of occurrence.

Following types of multiple fission are found in protozoa:

a. Gamogony:

Products are gametes. Examples: Monocytes.

b. Scizogony or agamogony occurs in asexual stages:

The resulting individuals are known as agametes or merozoites. Example: Plasmodium.

ADVERTISEMENTS:

c. Sporogony:

It occurs following sexual fusion. The products are surrounded by a cyst or a resistant covering and termed as spores. Motile spores are known as swarmer’s or swarmospores.

The swarmers are of two types:

i. Flagellospore. Spores bearing flagella.

ii. Pseudopodiospore or amoebospore. Spores beaning pseudopodia.

III. Plasmotomy:

The multinucleate individual divides into many small multinucleate offspring's by simple division of cytoplasm independent of nuclear division. The daughter individuals regain the normal size and the number of nuclei is restored by further nuclear division.

IV. Budding:

New individuals are produced by separation of a portion of the cytoplasm of the parent organism with a daughter nucleus. It may be simple or multiple, exogenous or endogenous. Budding is common in suctorians. Examples: Noctiluca, Tokophrya, etc.

Sexual Reproduction in Protozoa:

The modes of reproduction in which two gametes unite to form a new individual is known as sexual reproduction. The two units (male and female gametes) from two separate individuals unite by fusion of their cytoplasm, followed by the union of their nuclei. Most protists (protozoa) can continue to live, multiplying asexually for prolonged periods and may undergo sexual reproduction only at irregular intervals.

However, there are many protozoans in which sexual reproduction is of regular occurrence. Sexual reproduction involves meiotic division reducing the chromosomes to haploid number. In majority, reduction division occurs shortly before syngamy. This is called gametic meiosis, in which gametes become haploid.

But in some protozoans reduction division occurs in one of the subsequent divisions after formation of zygote. This is termed as zygotic meiosis, in which only zygote is diploid but rest of the life cycle is haploid. Of different types of sexual reproduction in protozoans syngamy, conjugation, automyxis are important.

I. Syngamy or Sexual Fusion:

Syngamy is the complete and permanent union or fusion of two specialised protozoan individuals or gametes resulting in the formation of a fertilized cell or zygote or oospore. The nuclei of the gametes fuse to form the zygote nucleus or synkaryon. The zygotes develop into adult, either directly or through encystment and fission of various types.

Depending upon the degree of differentiation of the fusing gametes syngamy may be of the following types:

a. Autogamy:

The gametes derived from the same parent cell fuse. Examples: Actinophrys, Actinosphaerium, Paramoecium aurelia, etc.

b. Paedogamy:

The fusing individuals are young. Example: Actinophrys

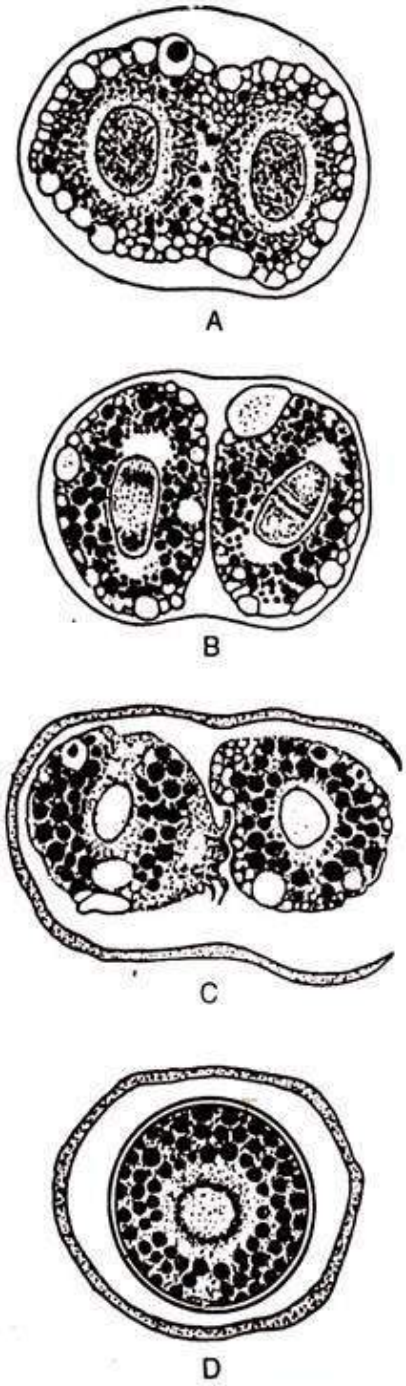


Fig. 18.15. Paedogamy in Actinophrys sp. A. Encysted-individual divides. B. Each daughter undergoes maturation division. C. Left daughter individual acts as male, right as female. D. Zygote

c. Hologamy:

The two mature individuals behave as gametes and fuse. Example: Copromonas (18.16).

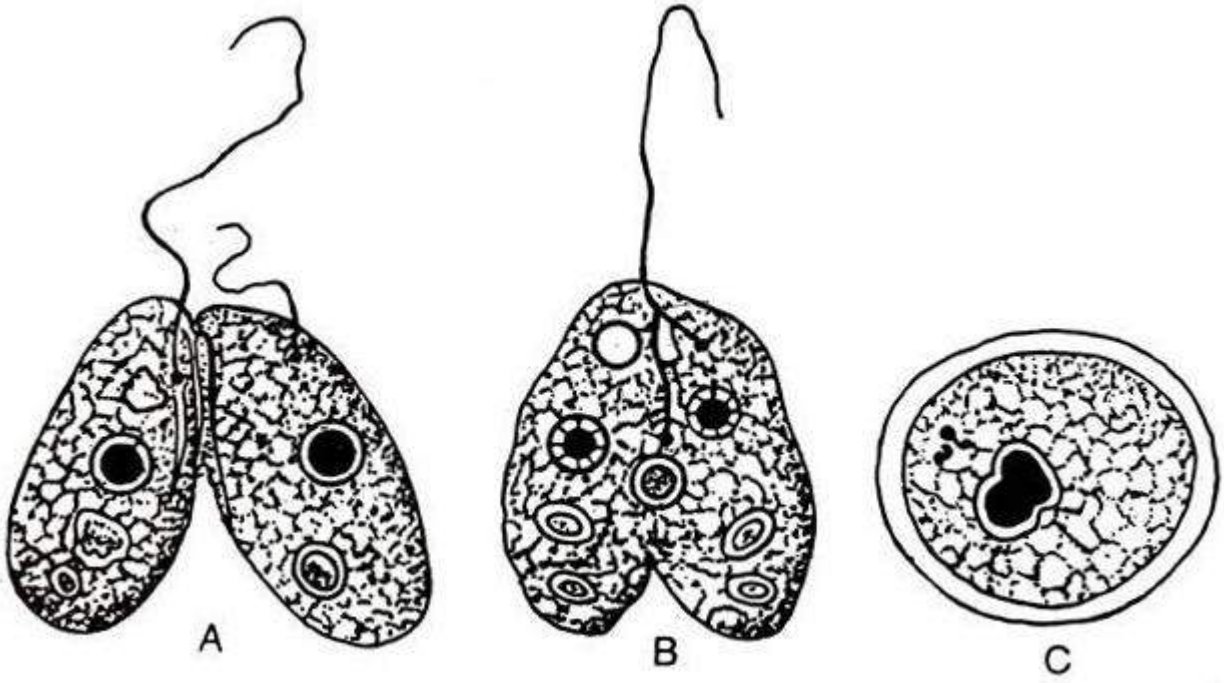


Fig. 18.16. Hologamy in a flagellate capromonas sp. A-B. Fusion of two individuals. C. Encysted zygote

d. Merogamy:

The uniting individuals are smaller than the ordinary vegetative individuals, called merogametes.

e. Isogamy:

Union of the gametes of similar size and shape. The isogametes are produced by multiple or repeated binary fission. Isogamy has been reported in Foraminifera (Elphidium), Phytomonadina (Chlamydomonas, Copromonas) and Gregarinida (Monocystis).

f. Anisogamy:

The two fusing gametes differ in size, shape and behaviour. The gametes are termed as heterogametes or anisogametes and their fusion is known as anisogamy or heterogamy. The formation of morphologically different gametes, is the first indication of sex differentiation in Protozoa.

The smaller gametes, the microgametes, or male gametes, are active, motile, generally flagellated and more numerous. They are produced by multiple or repeated fissions.

The fusion of two microgametes is called Micro-gamy. Example: Foraminifera, Arcella, etc. The larger gametes, macrogametes, are immotile, voluminous, and referred to as female gametes.

The fusion of two macrogametes is called Macro-gamy. Examples: Plasmodium, Eimeria, Volvox, etc.

The syngamy brings about a combination of two different lines of hereditary characters. It increases the external differences in offspring's. It also renews the vigour which is lost due to repeated binary fissions. The fusion of two nuclei initiates the development of eggs.

II. Conjugation:

The conjugation is the temporary union of two mating types of individuals of the same species to facilitate exchange of nuclear materials. They retain their distinct individuality and separate out after nuclear exchange. The pairing gametes are known as conjugants. The conjugants may be either isogamous (Paramecium) or anisogamous (Vorticella).

Conjugation is considered to be an episode in reproduction and not a mode of multiplication. In conjugation (i) reorganization of a fresh meganucleus occurs to accelerate the metabolic activities, (ii) rejuvenation and revival of lost vigour, (iii) new nuclear combinations and new hereditary combinations arise.

III. Automixis:

Automixis is the fusion of two gametic nuclei originating by the division of the single nucleus of an individual.

Automixis may be of the following types:

a. Autogamy:

The fusing nuclei come from the same cell as in Paramecium. All the steps in nuclear changes are similar to conjugation but the union occurs between the pronuclei of the same individual.

b. Paedogamy:

The fusion occurs between two nuclei coming from two different cells of a parent. A single organism encysts and then divides into two or more gametocytes. The nuclei of these gametocytes undergo meiosis and the gametes thus produced unite in pairs forming the zygotes. Examples: Actinosphaerium, Actinophrys, myxosporidians, etc.

c. Cytogamy:

In a number of species of Paramoecium the two individuals fuse with their oral surfaces. The nuclear changes occur as in conjugation but no nuclear exchange occurs. The two gametic nuclei in each individual fuse to form synkaryon. Cytogamy is said to be intermediate between conjugation and autogamy.

d. Hemixis:

Other modes of reproduction:

1. Plasmogamy:

Two or more individuals may fuse by their cytoplasm to form a plasmodium and separate out unchanged with their distinct nuclei. This sexual phenomenon is known as Plasmogamy and occurs in certain Rhizopoda and Mycetozoa.

2. Regeneration:

The regeneration and replacement of lost parts among free-living and few parasitic protists is widespread. A proper proportion of cytoplasm and nucleus can regenerate into an entire individual.

3. Parthenogenesis:

The gametes which fail to fertilize start their development parthenogenetically. Examples: Actinophrys, Chlamydomonas, etc.

MICROALGAE REPRODUCTION:

Reproduction in algae can be vegetative, asexual, or sexual. Vegetative reproduction occurs through fragmentation, asexual occurs through formation of spores and binary fission, whereas sexual reproduction takes place by fusion of two haploid gametes. Some algal species can reproduce by more than one means depending upon the environmental conditions. Here's more about the reproductive process in algae.

Algae (singular: alga) are autotrophic organisms that can carry out the process of photosynthesis. As of now, more than 30,000 species of algae are identified. Though algae possess chlorophyll similar to the green plants, they lack true roots, rhizoids, and leaves. Hence, they are not categorized as plants; rather they are considered as a different organism altogether.

The structure of algae can vary from simple unicellular (for example, Micromonas) to complex multicellular (for example, Kelps) forms. Usually, algae are found in any type of habitat: freshwater, marine water, swampy areas, moist soil and rocks. Based on the characteristic features, there are four major types of algae, namely cyanobacteria, green algae, red algae, and brown algae.

Procreation in Algae

The reproduction of algae can be discussed under two types, namely asexual reproduction and sexual reproduction. The former type refers to reproduction in which a new organism is generated from a single parent. In case of the sexual type, two haploid sex cells are fused to form a diploid zygote that develops into an organism. Let's discuss in brief about the vegetative, asexual, and sexual reproduction in algae along with examples.

Vegetative Reproduction

Vegetative breeding in algae is quite diverse. Some unicellular forms of algae like Euglena reproduce by binary fission, in which the parent cell divides (longitudinal or transverse) into two similar parts. These two cells develop as organisms and are similar to the parent cell. Fragmentation is a process that is classified under vegetative reproduction in algae. It occurs in Sargassum and other colonial algae, whereby the parent cell divides into two or more fragments that grow into new organisms.

Asexual Reproduction

Asexual reproduction occurs by the formation of spores; the algal species Chlamydomonas and Chlorella reproduce by this method. Depending upon the algal species, the spores can be produced in normal or specialized cells. They are either motile or non-motile. Different types of spores are zoospores, synzoospores, aplanospores, hypnospores, autospores, and tetraspores.

Sexual Reproduction

As mentioned earlier, sexual reproduction takes place by the union of male and female gametes. The gametes may be identical in shape, size, and structure (isogamy) or different (heterogamy). Some of the simplest forms of algae like Spirogyra reproduce by the conjugation method of sexual reproduction. In the process of conjugation, two filamentous strands (or two organisms) of the same algae species exchange genetic material through the conjugation tube. Among two strands, one acts as a donor and another behaves as a receiver. After exchanging the genetic material, two strands separate from each other. The receiver then gives rise to a diploid organism.

In the higher forms of algae, for example Ulva and Laminaria, an alternation of generation is usually observed. Both asexual and sexual reproduction occur in such organisms. Thus, the mature forms of haploid organisms called gametophyte and diploid organisms called sporophyte are present in the life cycle. If gametophyte and sporophyte organisms are similar in appearance, then they are referred to as isomorphic, whereas algae with different gametophyte and sporophyte forms are called heteromorphic.

The gametophyte produces haploid gametes by mitosis cell division, which unite to form diploid zygote that develops into a sporophyte. The sporophyte then undergoes meiotic cell division to give rise to haploid spores, which grow into gametophytes. This way, the gametophyte and sporophyte generations alter with each other.