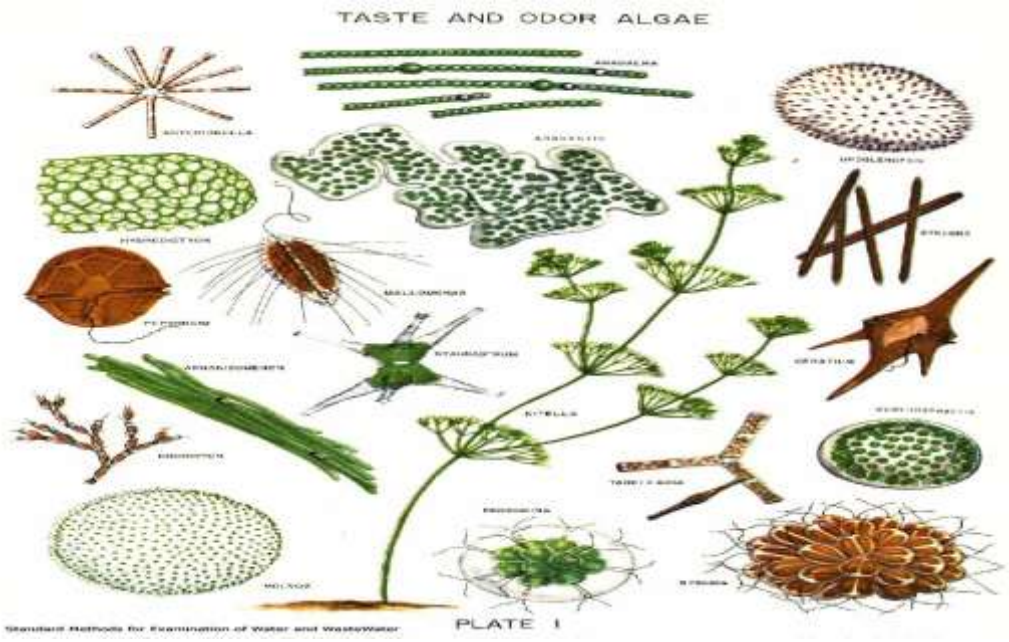
ALGAL PHYSIOLOGY



For 3rd year
Chemistry & Microbiology Students
Faculty of Science

Dr. Eman Abdel-Aty Hassan Alwaleed

VISION AND MISSION OF THE FACULTY

VISION

The faculty of science seeks to achieve academic community and student dominated by science, realization, culture and challenge, where all aspects are in continuing dialogue, graduating alumni equipped with information that qualifies them to be productive and creative. .+

MISSION

The faculty aims to excel at local level and regional throughout:

- Providing distinguished educational service to provide the market labor with graduates of high efficiency.
- Cooperating with universities and scientific institutions, regional and international.
- Academic research studies, and purposeful applied

- Providing community services and distinguished scientific consulting for South Valley community

- Training and continuous improvement in the academic field to keep pace with scientific progress

Course Syllabus :

- **INTRODUCTION**
- **PLASTID STRUCTURE IN ALGAE**
- **ALGAL PRODUCTION:**
  - Algae cultures of limited volume (Batch culture)
  - Algal Growth in Continuous Culture
  - Microalgae Isolation Techniques
  - Indices of growth of algae
  - Inorganic Nutrients of Algae
  - Algal Nutrition
- **Nitrogen Fixation In Algal Cell**
- **Photosynthesis**
- **Respiration**
- **ECONMIC IMPORTANTS OF ALGAE.**
- **References**
-

Course Goals

Dear student, by the end of this course you should be able to:

- Be aware with cultivation of algae in laboratory.
- Become acquainted with different kinds of algae cultures.
- Gain knowledge about methods of media preparation.
- Familiarize with different steps of batch culture.
- Know different kinds of continuous cultures.
- Gain knowledge about Principles of photosynthesis of algae.
- Become acquainted with different kinds of pigments involved in photosynthesis.
- Gain knowledge about structure of different pigments.
- Familiarize methods of extraction of pigments.
- Familiarize methods of estimation of pigments.
- Be aware with morphological structures of plastids in algae.
- Become acquainted with arrangement of thylakoids in algae.
- Know difference between plastid structures in algae divisions.
- Be aware with principles of carbon dioxide fixation in algae.

- Know difference between autotrophic and heterotrophic carbon dioxide fixation by algae.
- Familiarize with different sources of inorganic carbon available for algae.
- Be aware with principles of nitrogen fixation in algae.
- Become acquainted with mechanism of nitrogen fixation.
- Familiarize with factor influencing algal nitrogen fixation

INTRODUCTION

Plants and animals are separated by about 1.5 billion years of evolutionary history. First, plants get their energy from sunlight, not by ingesting other organisms. This dictates a body plan different from that of animals. Second, their cells are encased in semi rigid cell walls and cemented together, preventing them from moving as animal cells do. This dictates a different set of mechanisms for shaping the body and different developmental processes to cope with a changeable environment.

Physiology, as a term, was derived from the Greek words physis, meaning nature and logos, meaning discourse. Algal physiology is then, "the discourse about the nature of algae". From the physiological perspective, plants are viewed as machines that take inorganic molecules and energy from their surrounding environment and use them to assemble chemical structures.

In principle, algal physiology describes how algae work and focus on how algae use the energy of the sun to assimilate carbon, and how they convert that carbon to the stuff of which they are made. It also deals with several processes such as nutrient uptake and distribution, algae response to

their environment, reaction to stress and finally, the mode of algae reproduction.

This course was designed to provide skills to cultivate algae in laboratory. The importance to know the nutrients required for algal growth in order to cultivate algae and to exploit these algae in production of mass culture to be utilized in different aspects of life such as pharmaceuticals and biofertilizers and biodiesel. The need to know the indices to measure algae growth in order to monitor the different algae cultures such as batch cultures and continuous cultures. This course includes six units. Batch culture and Continuous culture, Growth media, Growth of Alga Pigments involved in Photosynthesis, Plastid structure in Algae divisions, Source of inorganic carbon, Factors influencing algal nitrogen fixation by cyanophyta and Vitamin requirements by algae.

With my best wishes Dr: Eman Abdel-Aty Hassan Alwaleed

Botany Department, Faculty of Science, South Valley University.

PLASTID STRUCTURE IN ALGAE

A chloroplast is one of three types of plastids, characterized by its high concentration of chlorophyll. (The other two types, the leucoplast and the chromoplast, contain little chlorophyll and do not carry out photosynthesis. Chloroplasts are highly dynamic they circulate and are moved around within plant cells, and occasionally pinch in two to reproduce. Their behavior is strongly influenced by environmental factors like light color and intensity. Chloroplasts, like mitochondria, contain their own DNA, which is thought to be inherited from their ancestor a photosynthetic cyanobacterium that was engulfed by an early eukaryotic cell.

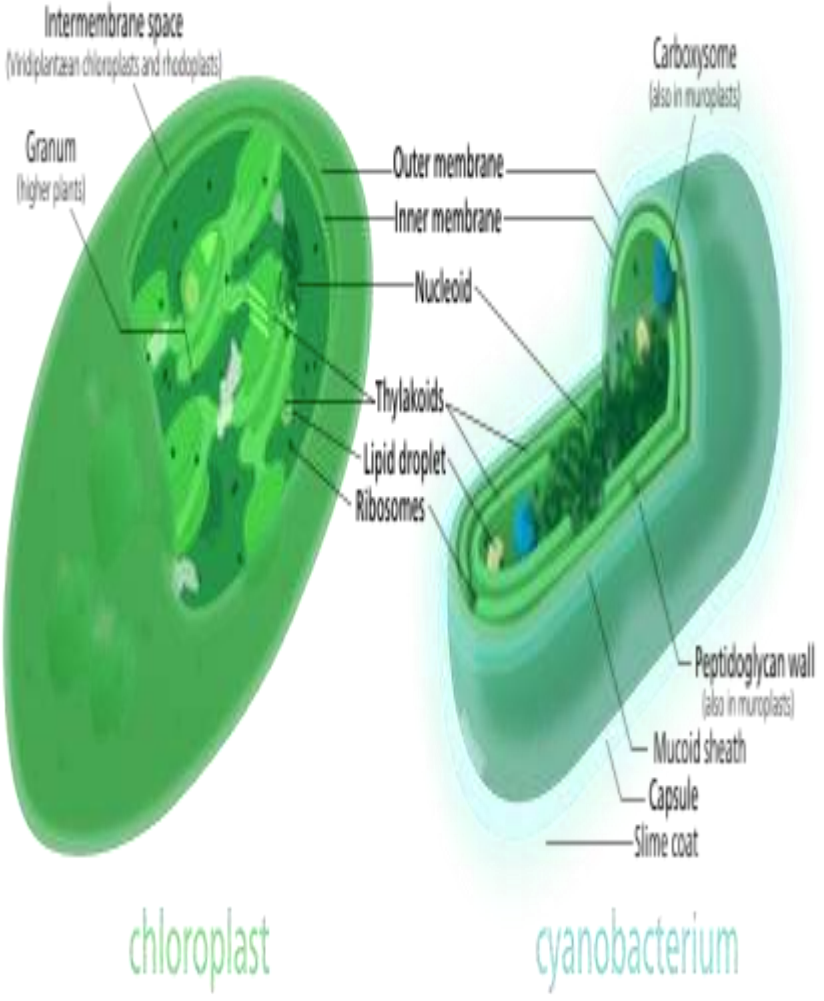
With one exception, all chloroplasts can probably be traced back to a single endosymbiotic event (the cyanobacterium being engulfed by the eukaryote). Despite this, chloroplasts can be found in an extremely wide set of organisms, some not even directly related to each other—a consequence of many secondary and even tertiary endosymbiotic events. . [The word chloroplast is derived from the Greek words **chloros,** which means green, and **plastes,** which means "the one who forms"

- **Chloroplast lineages and evolution**

Chloroplasts are one of many types of organelles in the plant cell. They are considered to have originated from cyanobacteria through endosymbiosis—when eukaryotic cell engulfed a photosynthesizing cyanobacterium which remained and became a permanent resident in the cell. Mitochondria are thought to have come from a similar event, where an ærobic prokaryote was engulfed. This origin of chloroplasts was first suggested by Russian biologist in 1905 after Andreas Schimper observed that chloroplasts closely resemble cyanobacteria in 1883. Chloroplasts are only found in plants and algae.

CYANOBACTERIA

Cyanobacteria are considered the ancestors of chloroplasts. They are sometimes called blue-green algae even though they are prokaryotes. They are a diverse phylum of bacteria capable of carrying out photosynthesis, and are gram-negative, meaning they have two cell membranes. They also contain a peptidoglycan cell wall, which is thicker than in other gram-negative bacteria, and which is located between their two cell membranes. Like chloroplasts, they have thylakoids inside of them. On the thylakoid membranes are photosynthetic pigments, including chlorophyll a.

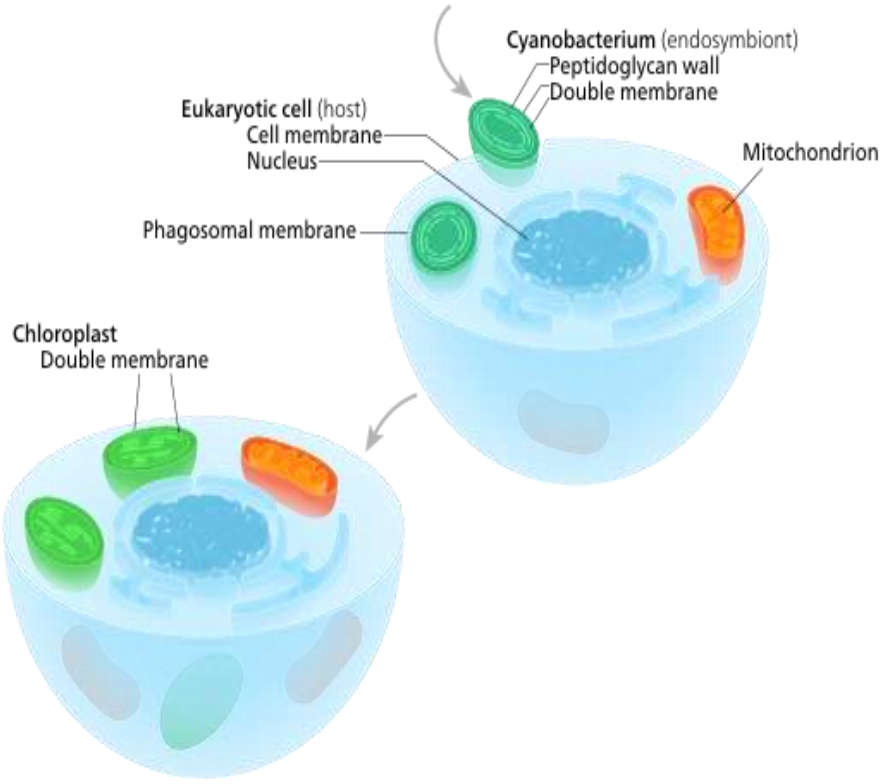


Both chloroplasts and cyanobacteria have a double membrane, DNA, ribosomes, and thylakoids. Both the chloroplast and cyanobacterium depicted are idealized versions (the chloroplast is that of a higher plant)—a lot of diversity exists among chloroplasts and cyanobacteria.

- **Primary endosymbiosis**

A eukaryote with mitochondria engulfed a cyanobacterium in an event of serial primary endosymbiosis, creating a lineage of cells with both organelles. It is important to note that the cyanobacterial endosymbiont already had a double membrane

Somewhere around a billion years ago, a free-living cyanobacterium entered an early eukaryotic cell, either as food or an internal parasite, and managed to escape the phagocytic vacuole it was contained in. The new cellular resident quickly became an advantage, providing food for the eukaryotic host, which allowed it to live within it. Over time, the cyanobacterium was assimilated, and many of its genes were lost or transferred to the nucleus of the host. Some of its proteins were then synthesized in the cytoplasm of the host cell, and imported back into the chloroplast.



This event is called **endosymbiosis,** or "cell living inside another cell". The cell living inside the other cell is called the **endosymbiont;** the endosymbiont is found inside **the host cell.**

Chloroplasts are believed to have arisen after mitochondria, since all eukaryotes contain mitochondria, but not all have chloroplasts. This is called serial endosymbiosis—an early eukaryote engulfing the mitochondrion ancestor, and some descendants of it then engulfing the chloroplast ancestor, creating a cell with both chloroplasts and mitochondria.

These chloroplasts, which can be traced back directly to a cyanobacterial ancestor are known as primary plastids

("plastid" in this context means the almost the same thing as chloroplast. All primary chloroplasts belong to one of three chloroplast lineages—the **glaucophyte chloroplast lineage**, the rhodophyte, or **red algal chloroplast lineage**, or the chloroplastidan, or **green chloroplast lineage.**

The second two are the large stand the green chloroplast lineage is the one that contains the land plants.

GLAUCOPHYTA

The alga Cyanophora, a glaucophyte, is thought to be one of the first organisms to contain a chloroplast. The glaucophyte chloroplast group is the smallest of the three primary chloroplast lineages, being found in only 13 species, and is thought to be the one that branched off the earliest. Glaucophytes have chloroplasts that retain a peptidoglycan wall between their double membranes, like their cyanobacterial parent. For this reason, glaucophyte chloroplasts are also known as muroplasts. Glaucophyte chloroplasts and cyanobacteria keep their carbon fixation enzyme rubisco in. The starch that they synthesize collects outside the chloroplast. Like cyanobacteria, glaucophyte chloroplast thylakoids are studded with light collecting structures called phycobilisomes. For these reasons, glaucophyte chloroplasts are considered a primitive intermediate between cyanobacteria and the more evolved chloroplasts in red algae and plants.

RHODOPHYCEÆ (RED ALGAE)

The rhodophyte, or red algal chloroplast group is another large and diverse chloroplast lineage. Rhodophyte chloroplasts are also called rhodoplasts literally "red chloroplasts.

Rhodoplasts have a double membrane with an intermembrane space and phycobilin pigments organized into phycobilisomes on the thylakoid membranes, preventing their thylakoids from stacking.

Rhodoplasts have chlorophyll a and phycobilins for photosynthetic pigments; the phycobilin phycoerytherin is responsible for giving many red algae their distinctive red color. However, since they also contain the blue-green chlorophyll a and other pigments, many are reddish to purple from the combination. The red phycoerytherin pigment is an adaptation to help red algae catch more sunlight in deep water as such, some red algae that live in shallow water have less phycoerytherin in their rhodoplasts, and can appear more greenish. Rhodoplasts synthesize a form of starch called floridean, which collects into granules outside the rhodoplast, in the cytoplasm of the red alga.

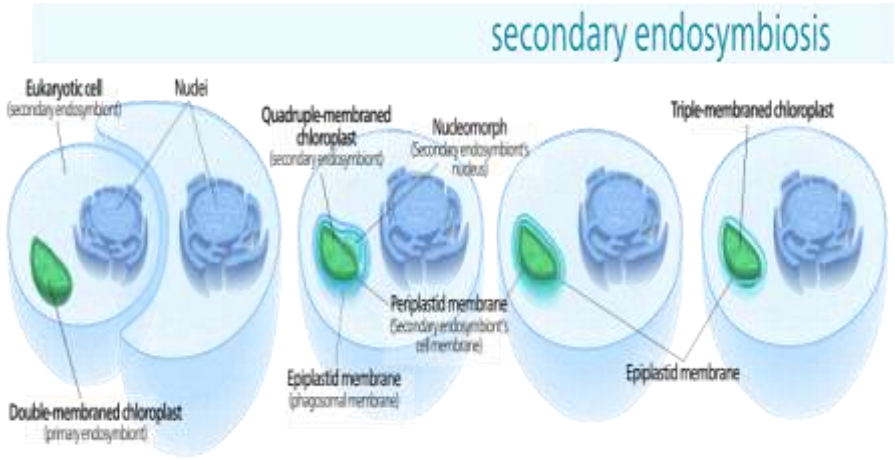
CHLOROPLASTIDA (GREEN ALGAE AND PLANTS)

The chloroplastidan chloroplasts, or green chloroplasts, are another large, highly diverse primary chloroplast lineage. Their host organisms are commonly known as the green algae and land plants. They differ from glaucophyte and red algal chloroplasts in that they have lost their phycobilisomes, and contain chlorophyll b instead. Most green chloroplasts are (obviously) green, though some aren't, Chloroplastidan chloroplasts have lost the peptidoglycan wall between their double membranes, and have replaced it with an intermembrane space. Some plants seem to have kept the genes for the synthesis of the

peptidoglycan layer, though they've been repurposed for use in chloroplast division instead.

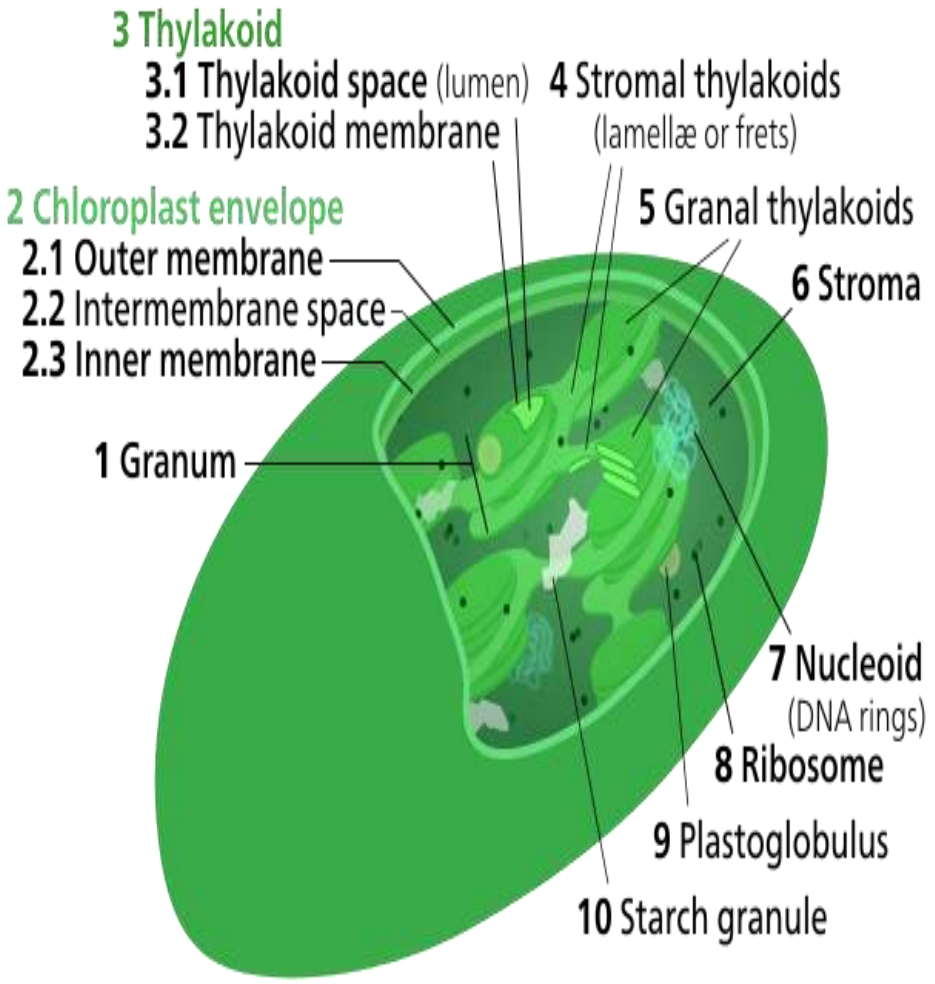
SECONDARY AND TERTIARY ENDOSYMBIOSIS

Many other organisms obtained chloroplasts from the primary chloroplast lineages through secondary endosymbiosis—engulfing a red or green alga that contained a chloroplast. These chloroplasts are known as secondary plastids.



In land plants, chloroplasts are generally lens-shaped, 5–8 μm in diameter and 1–3 μm thick. Greater diversity in chloroplast shapes exists among the algae, which often contain a single chloroplast that can be shaped like a net (e.g., Oedogonium), a cup (e.g., Chlamydomonas), a ribbon-like spiral around the edges of the cell (e.g.,

Spirogyra), or slightly twisted bands at the cell edges (e.g., Sirogonium). Some algae have two chloroplasts in each cell; they are star-shaped in Zygnema, or may follow the shape of half the cell in order Desmidiales. In some algae, the chloroplast takes up most of the cell, with pockets for the nucleus and other organelles (for example some species of Chlorella have a cup-shaped chloroplast that occupies much of the cell).



Components of a typical chloroplast

1) **Granum**
2) **Chloroplast envelope**
   - Outer membrane
   - Intermembrane space
   - Inner membrane
3) **Thylakoid**

- Thylakoid space (lumen)-Thylakoid membrane

4) Stromal thylakoid
5) Stroma
6) Nucleoid
7) Ribosome
8) Starch granule

All chloroplasts have at least three membrane systems the outer chloroplast membrane, the inner chloroplast membrane, and the thylakoid system. Chloroplasts that are the product of endosymbiosis may have additional membranes surrounding these three. Inside the outer and inner chloroplast membranes is the chloroplast stroma, a semi-gel-like fluid that makes up much of a chloroplast's volume, and in which the thylakoid system floats.

Outer chloroplast membrane

The outer chloroplast membrane is a semi-porous membrane that small molecules and ions can easily diffuse across. However, it is not permeable to larger proteins

Intermembrane space and peptidoglycan wall

Instead of an intermembrane space, glaucophyte algae have a peptidoglycan wall between their inner and outer chloroplast membranes.

Usually, a thin intermembrane space about 10–20 nanometers thick exists between the outer and inner chloroplast membranes

Glaucophyte algal chloroplasts have a peptidoglycan layer between the chloroplast membranes. It corresponds to the peptidoglycan cell wall of their cyanobacterial ancestors, which is located between their two cell membranes. These chloroplasts are called muroplasts (from Latin "mura", meaning "wall"). Other chloroplasts have lost the cyanobacterial wall

Inner chloroplast membrane

The inner chloroplast membrane borders the stroma and regulates passage of materials in and out of the chloroplast. After passing through the outer chloroplast membrane, polypeptides must pass through the inner chloroplast membrane

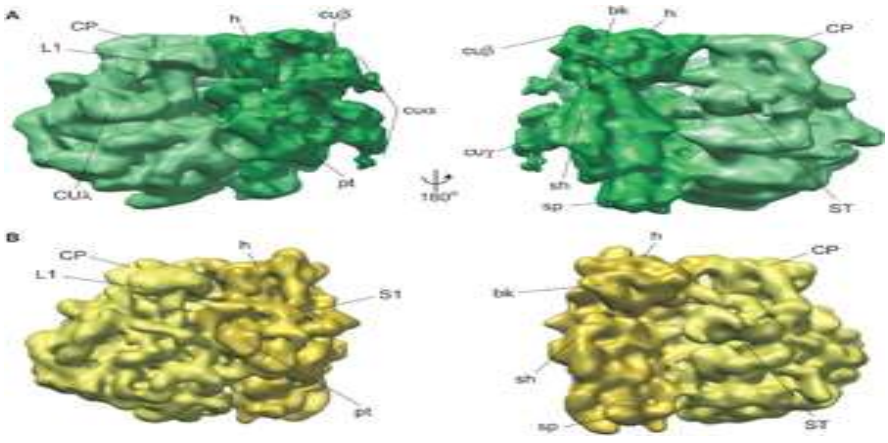
In addition to regulating the passage of materials, the inner chloroplast membrane is where fatty acids, lipids, and carotenoids are synthesized

Stroma

The protein-rich, alkaline, aqueous fluid within the inner chloroplast membrane and outside of the thylakoid space is called the stroma, which corresponds to the cytosol of the original cyanobacterium. Nucleoids of chloroplast DNA, chloroplast ribosomes, the thylakoid system, starch granules, and many proteins can be found floating around in it. The Calvin cycle, which fixes CO_2 into sugar takes place in the stroma

Chloroplast ribosomes

Chloroplasts have their own ribosomes, which they use to synthesize a small fraction of their proteins



Chloroplast ribosomes Comparison of a chloroplast ribosome (green) and a bacterial ribosome (yellow). Important features common to both ribosomes and chloroplast-unique features are labeled.

Starch granules

Starch granules are very common in chloroplasts, typically taking up 15% of the organelle's volume, though in some other plastids they can be big enough to distort the shape of the organelle. Starch granules are simply accumulations of starch in the stroma, and are not bounded by a membrane

Starch granules appear and grow throughout the day, as the chloroplast synthesizes sugars, and are consumed at night to fuel respiration and continue sugar export into the phloem, though in mature chloroplasts, it is rare for a starch granule to be completely consumed or for a new granule to accumulate.

Starch granules vary in composition and location across different chloroplast lineages. In red algae, starch granules are found in the cytoplasm rather than in the chloroplast.

Rubisco

The chloroplast stroma contains many proteins, though the most common and important is Rubisco, which is probably also the most abundant

protein on the planet. Rubisco is the enzyme that fixes CO_2 into sugar molecules.

Pyrenoid

The chloroplasts of some algae contain structures called pyrenoids.

Pyrenoids are roughly spherical and highly refractive bodies which are a site of starch accumulation in plants that contain them. They consist of a matrix opaque to electrons, surrounded by two hemispherical starch plates. The starch is accumulated as the pyrenoids mature. In algae with carbon concentrating mechanisms, the enzyme rubisco is found in the pyrenoids. Starch can also accumulate around the pyrenoids when CO_2 is scarce.

Thylakoid system

Suspended within the chloroplast stroma is the thylakoid system, a highly dynamic collection of membranous sacks called thylakoids where chlorophyll is found and the light reactions of photosynthesis happen. In most vascular plant chloroplasts, the thylakoids are arranged in stacks called grana

Granal structure

Using a light microscope, it is just barely possible to see tiny green granules—which were named grana.

Pigments and chloroplast colors

Inside the photosystems embedded in chloroplast thylakoid membranes are various photosynthetic pigments, which absorb and transfer light energy. The types of pigments found are different in various groups of chloroplasts, and are responsible for a wide variety of chloroplast colorations.

Chlorophylls

Chlorophyll a is found in all chloroplasts, as well as their cyanobacterial ancestors. Chlorophyll a is a blue-green pigment partially responsible for

giving most cyanobacteria and chloroplasts their color. Other forms of chlorophyll exist, such as the accessory pigments chlorophyll b, chlorophyll c, chlorophyll d, and chlorophyll f.

Chlorophyll b is an olive green pigment found only in the chloroplasts of plants, green algae, any secondary chloroplasts obtained through the secondary endosymbiosis of a green alga, and a few cyanobacteria.[9] It is the chlorophylls a and b together that make most plant and green algal chloroplasts green.

Chlorophyll c is mainly found in secondary endosymbiotic chloroplasts that originated from a red alga, although it is not found in chloroplasts of red algae themselves. Chlorophyll c is also found in some green algae and cyanobacteria

Chlorophylls d and f are pigments found only in some cyanobacteria in addition to chlorophylls, another group of yellow–orange pigments called carotenoids are also found in the photosystems. There are about thirty dissipate excess energy, and their bright colors sometimes override the chlorophyll green, like during the fall, when the leaves of some land plants change color. β-carotene is a bright red-orange carotenoid found in nearly all chloroplasts, like chlorophyll a. Xanthophylls, especially the orange-red zeaxanthin, are also common. Many other forms of

carotenoids exist that are only found in certain groups of chloroplasts

Phycobilins: Phycobilins are a third group of pigments found in cyanobacteria, and red algal, Phycobilins come in all colors, though phycoerytherin is one of the pigments that makes many red algae red. Cryptophytic chloroplasts and some cyanobacteria don't have their phycobilin pigments

ALGAL PRODUCTION

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, salinity and temperature

Culture medium/nutrients: Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients Macronutrients include nitrate, phosphate (in an approximate ratio of 6:1), and silicate. Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium and the Guillard's F/2 medium.

Light: As with all plants, micro-algae photosynthesize, i.e. they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination.

pH: The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7.

Aeration/mixing: Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients and to improve gas exchange between the culture medium and the air

Temperature: The optimal temperature for growth between 20 and 24°C

Salinity : Marine phytoplankton are extremely tolerant to changes in salinity.

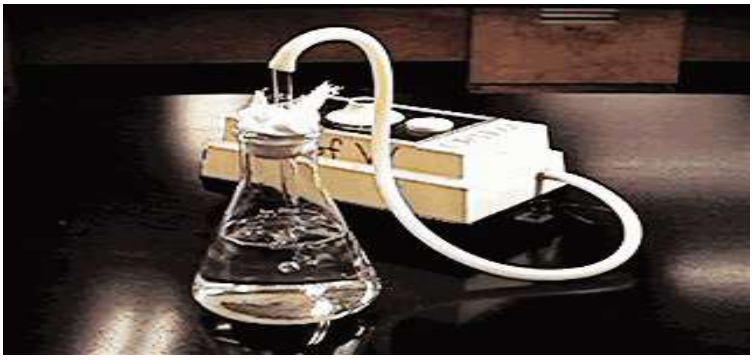
ALGAL CULTURE MEDIA

In order to grow algae in the classroom you will need to make up some growth media. In their natural habitats algae obtain all the nutrients, minerals and vitamins they require from the water in which they live. To grow them in the lab you must provide them with all of these essential resources. A culture can be defined as an artificial environment in which the algae grow in theory culture condition should resemble the alga's natural environment as far as possible.

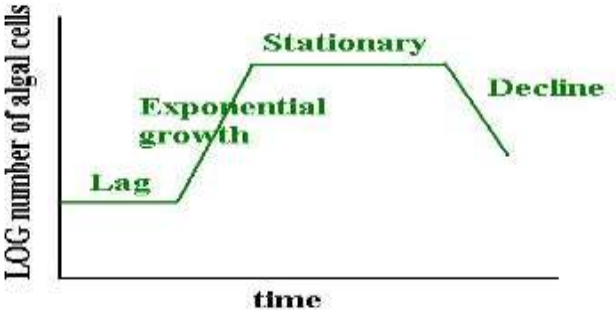
Algae cultures of limited volume (Batch culture)

In this method algal cells are allowed to grow and reproduce in a closed container. The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase. They have a finite amount of nutrient, and when that is exhausted, their growth stops and eventually they die. These types of cultures typically last for about one week. The most common culture system is the batch culture, due to its simplicity and low cost. This is a closed system in which there is no input or output of materials.

The photo below shows a typical batch culture set-up.



Limited volume of medium containing the necessary nutrient when inoculated with algae cells and then exposed to suitable conditions of light, temperature and aeration. Increase in cell number follows a characteristic course as:



Phases in the growth curve illustrated a typical algal batch culture

There are five phases of algal growth, lag phase, exponential growth phase, Declining growth, stationary phases and death phase.

The Lag (induction) phase is the time where the alga is not reproduction, this lasts for about 4-6 days. This phase, during which little increase in cell density occurs.

After a while, the algae multiplies super-fast in a short period of time. This is called the **Exponential growth phase** during the second phase, the cell density increases.

Later, the algae reach a point where there is not enough space for growth and there are no more nutrients in the water so the algae stop reproducing and the growth rate are balanced, which results in a relatively constant cell density. This is called **the Stationary phase**. In the middle of this phase is the optimal time to harvest the algae.

Phase of **Declining growth** rate; cell division slows down when nutrients, light, pH, carbon dioxide light intensity, auto inhibition or other physical and chemical factors begin to limit growth.

If the algae are not harvested in the stationary phase, they will move to **the Death phase.** There is no more space and nutrients to grow so cell density decreases rapidly and the culture eventually collapses.

In practice, culture crashes can be caused by a variety of reasons, including the depletion of a nutrient, oxygen deficiency, overheating, pH disturbance, or contamination. The key to the success of algal production is maintaining all cultures in the exponential phase of growth

Continuous Culture

This method of culturing algae differs from the batch culture method in that fresh medium is added to the culture at a

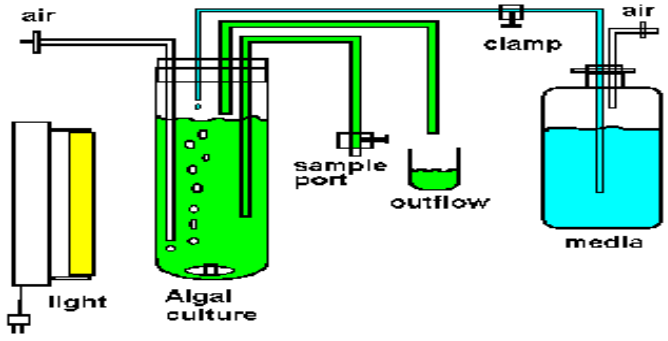
constant rate and old media (and some of the algae cells) is removed at the same rate. Two categories of continuous cultures can be distinguished:

Turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.

Chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

The diagram and photographs below show the parts of a continuous culture system.

First, fresh growth medium is stored in the large vessel. Air is pumped into the airspace in this medium vessel. This air pressure will push the medium through a tube which is connected to the culture vessel. By opening and closing the clamp on this medium line one can add medium to the culture vessel.



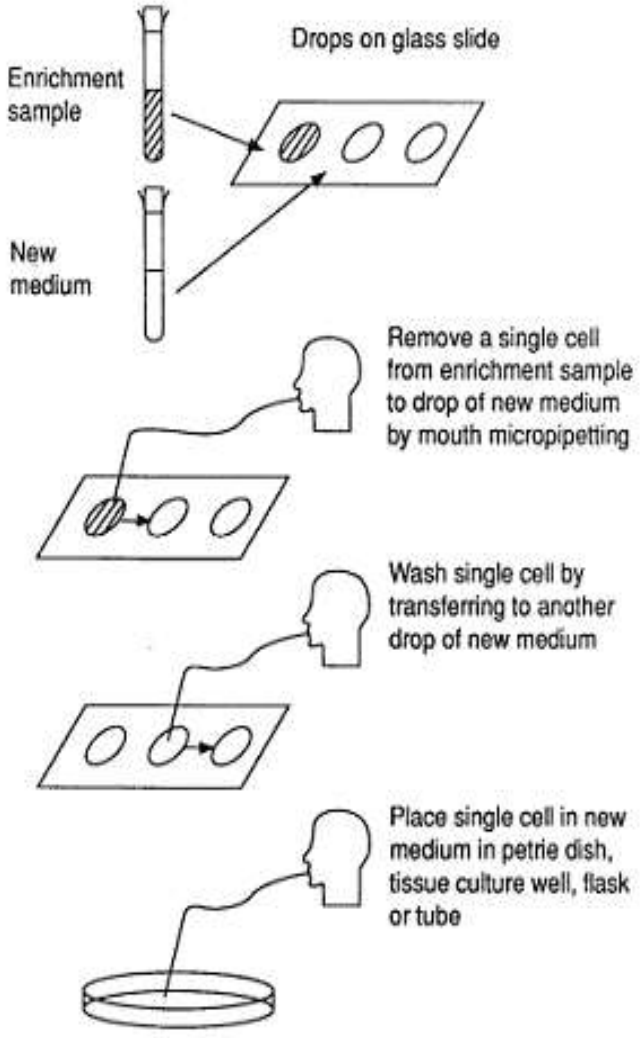
Air is also pumped into the culture vessel. This air passes down a long glass tube to the bottom of the culture and

bubbles up. This serves to keep the culture well suspended as well as high in oxygen and CO2. The air flowing into the culture vessel flows out through an outflow tube. As fresh medium is added to the culture vessel the level of the liquid in the culture vessel rises. When that level reached the bottom of the outflow tube old medium and cells flow out of the culture vessel into a waste flask. There is one other glass tube in the culture vessel, the sample port. When you need a sample of cells from the culture vessel you open up the clamp on the sample port and medium and cells flow out. When you have enough you reclamp the sample port.

When choosing a culture medium the nature habitat of the species should be considered in order to determine its environmental requirements. Algae media refers to the solution or culture in which algae grow, and there are two major types of algae media, enrichment and artificial media. An enrichment medium is generally made by adding soil extracts to distilled or natural water or by simply adding chemical nutrients to seawater or lake/dam water. The artificial medium uses "pure" water and "pure" chemicals and doesn’t include additions of soil extracts or natural lake or sea water. This artificial medium is mostly used under laboratory conditions to exacting standards, although unknown impurities can still be present in even the most carefully prepared artificial medium.

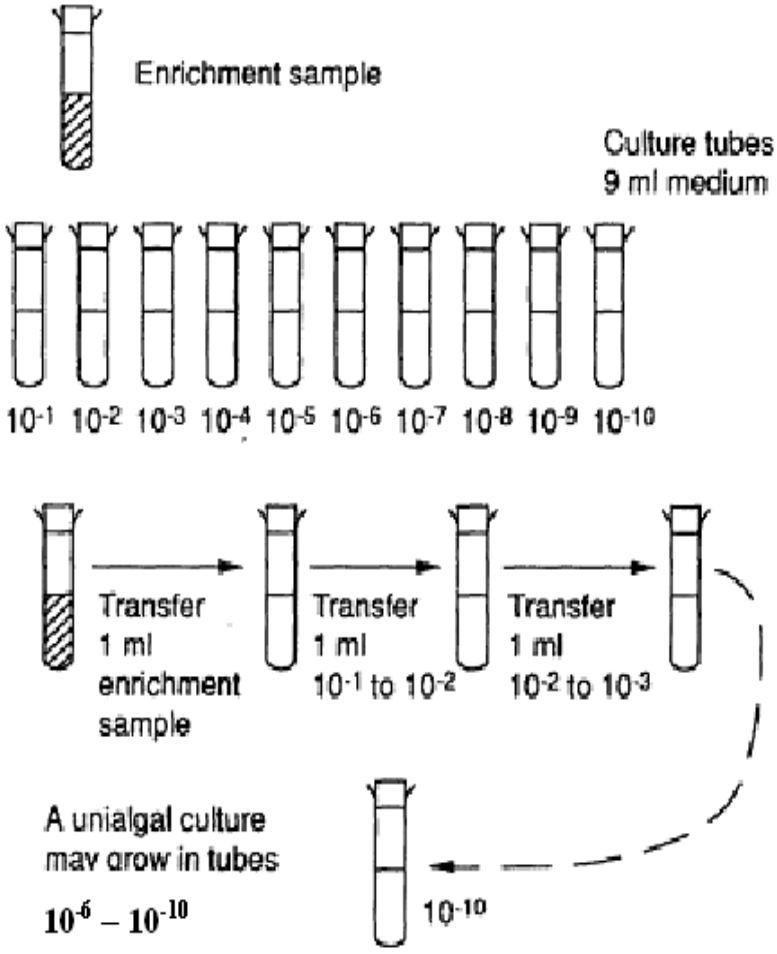
MICROALGAL ISOLATION TECHNIQUES

(a) Micromanipulation:



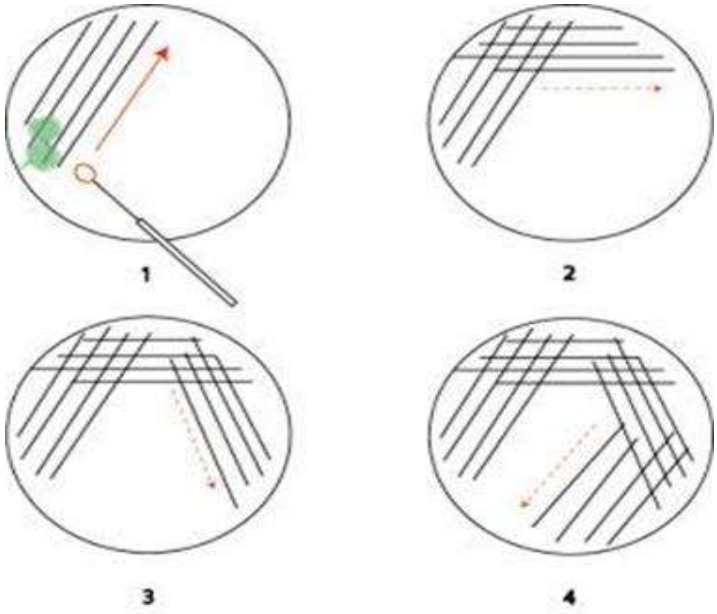
(b) Serial dilution:

dispense 9 ml of media into each of ten test tubes with sterile automatic dispenser. Label tubes 10^{-1} to 10^{-10} indicating dilution factor .Aseptically add 1 ml of enrichment sample to the first tube (10-1) and mix gently.Take 1 ml of this dilution and add to the next tube (10-2), mix gently. Incubate test-tubes under controlled temperature and light conditions



(c) Streak plating:

Prepare petri dishes containing growth medium solidified with 1-1.5% agar medium.Place 1—2 drops of mixed phytoplankton sample near the periphery of the agar. use the sterile loop to make parallel streaks of the suspension on the agar ·Remove a sample using a sterilized wire loop and place in a drop of sterile culture medium on a glass slide. Check microscopically that the desired species has been isolated and is unialgal .Repeat the streaking procedure. This second streaking reduces the possibility of bacterial contamination and of colonies containing more than one algal species.Transfer selected colonies to liquid or agar medium.



INDICES OF GROWTH OF ALGAE

In growing algae culture yield, dry weight, optical density of a suspension of algal cells and increase in cell number are used as a characteristic of increase of growth. Other indices of growth, such as accumulation of carbon, nitrogen, protein, or some products of cell metabolism (starch, acids) are used in growth measurement.

1) Yield as a growth indicator:

yield as an expression of organic production, is usually given in terms of fresh or dry weight of the organic mass produce over the period of the time per unite of volume or unit of area occupied by organism.

2) Determination of yield: Y=X1-X0/A(or V)

Where X1& X0 are quantitative expressions of the mass of cells at the beginning and at the end of the growth period and A (or V) the area or the volume occupied by population of microbial growth.

ALGAL NUTRITION

(1) PHOTOTROPHIC: using light to produce carbohydrate from H2O and Co_2

(2) CHEMTROPHIC: employing inorganic substance

(3) HETEROTROPHIC: employing organic substance

(4) MIXOTROPHIC: autotrophic and heterotrophic

(5) Phagotrophic: which ingest organic and inorganic substance.

(6) Auxotrophic: is the inability of an organism to synthesize a particular organic compound required for its growth

ALGAL NUTRIENTS

Sixteen chemical elements are known to be important to alga's growth and survival. The sixteen chemical elements are divided into two main groups: non-mineral and mineral.

3) Non-Mineral Nutrients: The Non-Mineral Nutrients are hydrogen (H), oxygen (O), & carbon (C).These nutrients are found in the air and water .Algae use energy from the sun to change carbon dioxide (CO2 - carbon and oxygen) and water (H2O- hydrogen and oxygen) into starches and sugars. These starches and sugars are the alga's food.

4) The mineral nutrients: are divided into two groups : macronutrients and micronutrients.Macronutrients can be broken into two more groups :

(1) The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K). These major nutrients usually are lacking because algae use large amounts for their growth and survival .

(2) The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S).

(A) Macronutrients element:

(1) Phosphorus: is an essential part of the process of photosynthesis .Helps with the transformation of solar energy into chemical energy; proper plant maturation; withstanding stress.Effects rapid growth

(2) Potassium: algae require potassium ion as activator of enzymes helps in the building of protein, photosynthesis.

(3) Nitrogen: Nitrogen is a major component of proteins and amino acids.

(3) Calcium: required by most of algae for growth an essential part of plant cell wall structure

(4) Magnesium: is part of the chlorophyll in all green plants and essential for photosynthesis. It also helps activate many plant enzymes needed for growth

(5) Sulfur: Essential plant food for production of protein.

(B) Micronutrients element: Micronutrients are those elements essential for plant growth which are needed in only very small (micro) quantities. These elements are sometimes called minor elements or trace elements. The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Providing micronutrients (as well as macronutrients) to growing plants.

Micronutrient element consider essential to all algae: An essential nutrient is a nutrient that the cell cannot synthesize on its own -- or not to an adequate amount

(1) Iron (Fe): iron required in biological oxidation and reduction reaction Essential for formation of chlorophyll.

(2) Manganese (Mn): Functions with enzyme systems involved in breakdown of carbohydrates, and nitrogen metabolism.

(3) Chloride (Cl): Aids plant metabolism.

(4) Molybdenum (Mo) Helps in the use of nitrogen

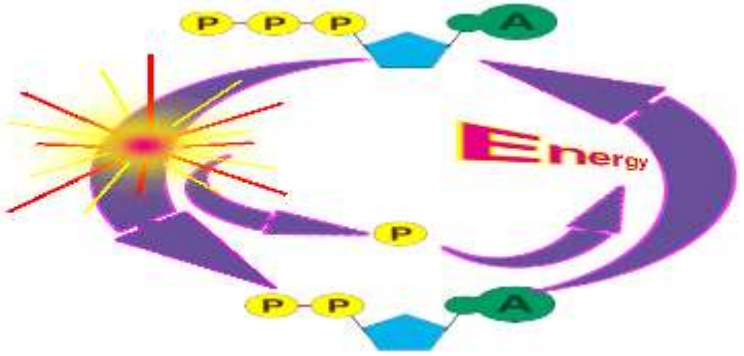
(5) Zinc (Zn) Essential for the transformation of carbohydrates.

(6) Boron (B): Helps in the use of nutrients and regulates other nutrients . Aids production of sugar and carbohydrates .

(7) Copper (Cu): Important for reproductive growth.

The vital role of ATP

This is the energy-rich compound that is the source of energy for all living things. It is a nucleotide, comprising a 5C sugar (ribose); an organic base (adenosine); and 3 phosphate groups (2 in ADP). This is continually recycled thus:



ATP + H2O □ ADP + Pi.

The amount of energy released from the hydrolysis of one molecule of ATP is small and can thus be used to energize a single reaction, whilst, being a single reaction, it is also very fast. These are significant advantages over using glucose directly.

Photosynthesis

($CO_2 + H_2O \rightarrow C_6H_{12}O_6 + O_2$)

Nearly all life on Earth depends on this process, yet it is very inefficient. Half of all energy from the Sun is used to evaporate water; 15% is reflected and 32% passes through the leaf untouched. That leaves (sorry!) only 2% of the incident radiation to be trapped and used in photosynthesis.

There are two parts to this process:

1- The light-dependent reactions (which take place in the thylakoid membranes, or grana, of the chloroplasts), in which light energy, trapped by chlorophyll, is used:

•to split water into O_2 and H^+ ions, stored as reduced NADPH and

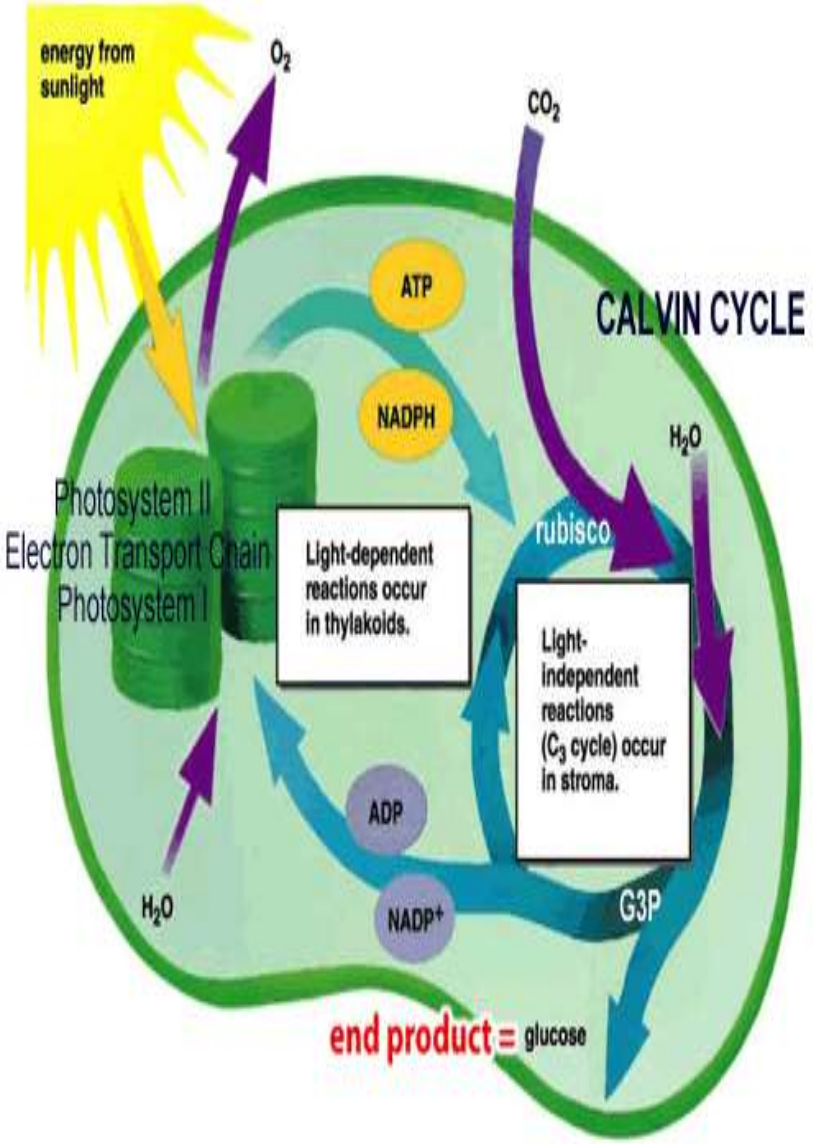
•to be stored as chemical energy by converting ADP + Pi to ATP.

2- The light-independent reactions, (take place in the stroma of the chloroplasts) in which:

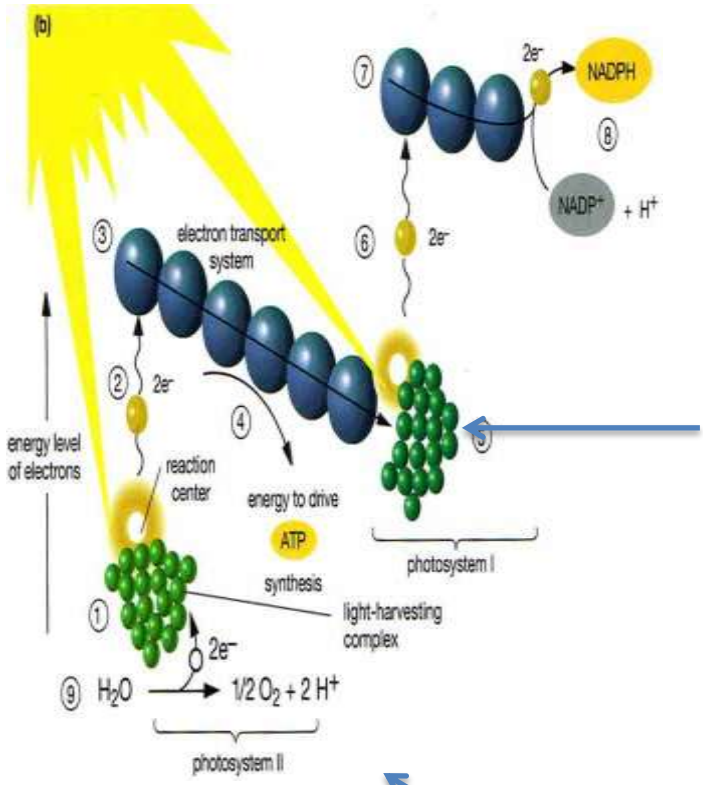
•the ATP and reduced NADP (from the light-dependent reactions) are used to reduce CO_2 to glucose.

The thylakoid membranes provide a large surface area (to trap light), and contain molecules of chlorophyll arranged in clusters called photosystems. Each photosystem

contains several hundred molecules of chlorophyll and acts as a single light-harvesting system.



1. The light-dependent reactions



This (confusingly!) begins with **Photosystem II**, where **trapped light energy is used to split water**, a process known as **photolysis:**

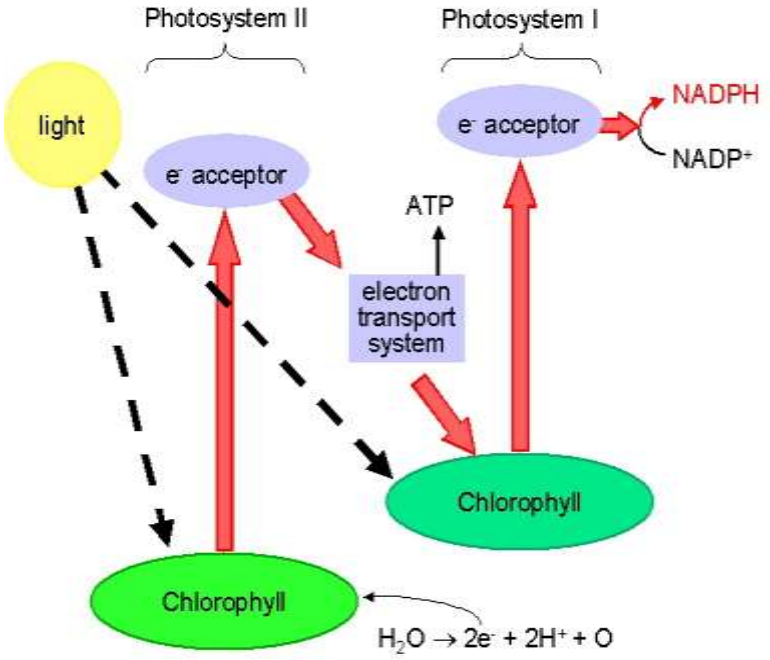
$$H_2O \rightarrow 2H^+ + 2e^- + \tfrac{1}{2}O_2$$

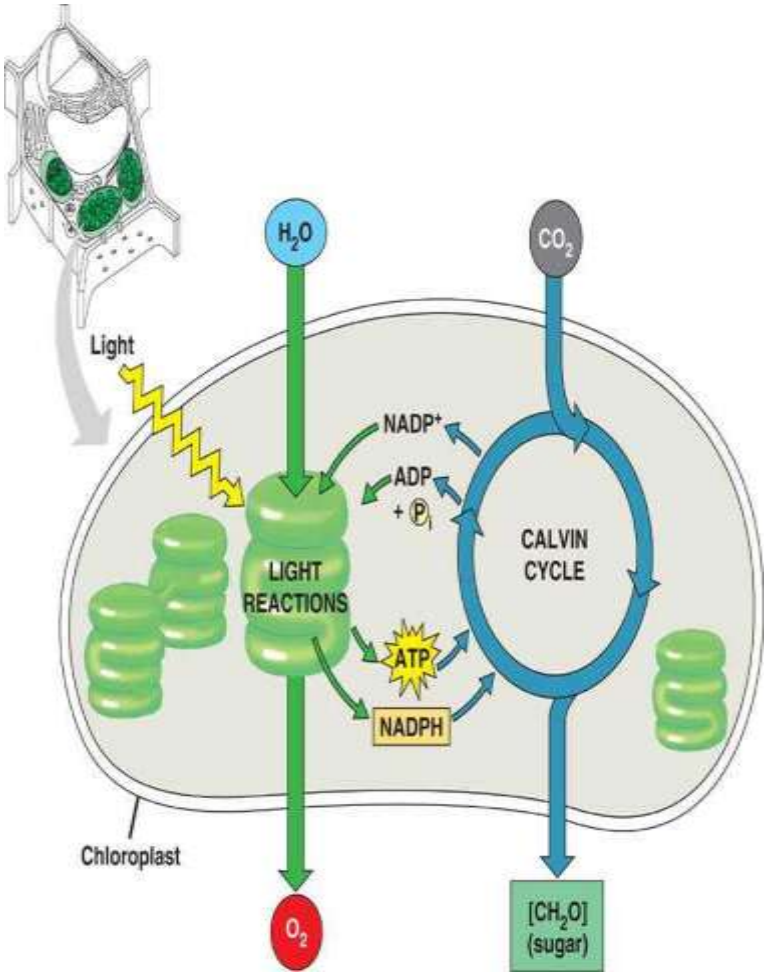
The electrons are used to generate ATP, by passing them **along a series of electron carriers**, losing energy as they do so, **before they join Photosystem I**,

Replacing electrons lost there. **Photosystem I** also traps light energy, and uses it to excite electrons along a series of carrier molecules.

Combined with the H^+ ions formed in Photosystem I, they react with NADP to produce reduced NADP (also known as $NADPH_2$):

$$NADP + 2H^+ + 2e^- \rightarrow \text{reduced NADP}$$





The end-products of the light reaction are thus **ATP** and **reduced NADP**, (also called **NADPH**) which move into the **stroma** of the chloroplast ready to act as the raw materials for the **light-independent reactions** (see right).

2. The light-independent reactions

The diagram (below) shows very clearly all that!

The cycle involves:

A 5-carbon sugar (**ribulose bisphosphate – RuBP**) **combines with CO_2**, forming two **molecules of a 3-carbon compound**, **glycerate 3-phosphate**, **GP** (2 x 3C = 6C!).

The GP is then reduced to triose phosphate,

TP, which **requires both ATP and reduced NADP** (which were made in the light-dependent reactions).

Some of the TP is used to make glucose, amino-acids and lipids, whilst the **rest is converted back into RuBP** – which **requires another molecule of ATP**.

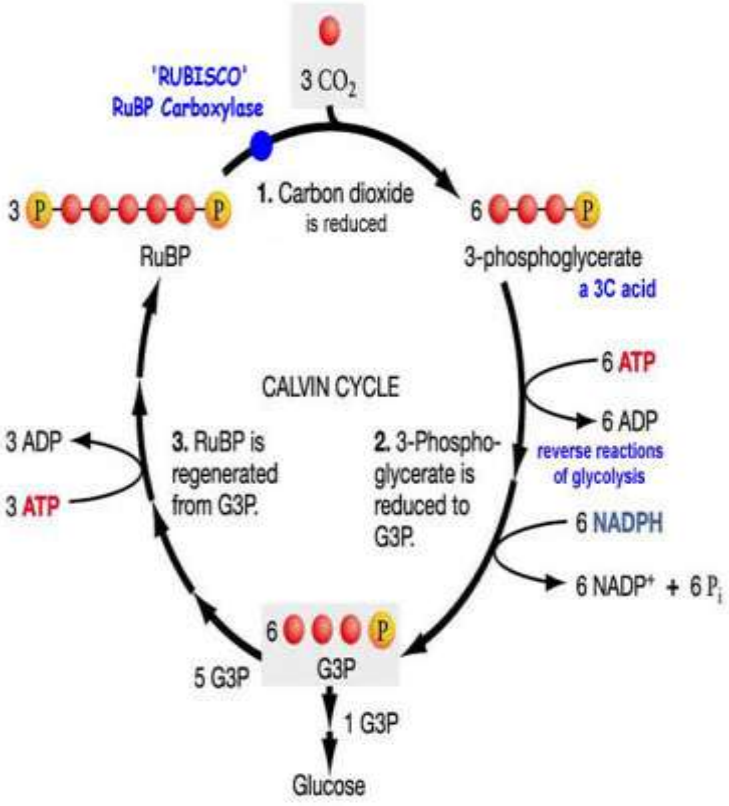
The sequence is thus:

$RuBP + CO_2 \rightarrow GP + ATP + NADPH_2 \rightarrow TP \rightarrow$ glucose etc. <u>or</u>

$RuBP + CO_2 \rightarrow GP + ATP + NADPH_2 \rightarrow TP + ATP \rightarrow$ RuBp

(Thus completing the cycle)

+



N.B. When answering a question on this cycle, remember that **an increase in the level of a substance means that the next step is blocked.** So, for example, if there is an increase in RuBP levels, it would suggest that there is a lack of CO_2**.** The same applies for the other stages – a build-up of GP suggests a lack of ATP (i.e the light reaction is not working).

NOTE THAT it is NADP that is used in

Photosynthesis, and **NOT** NAD!!!

Respiration

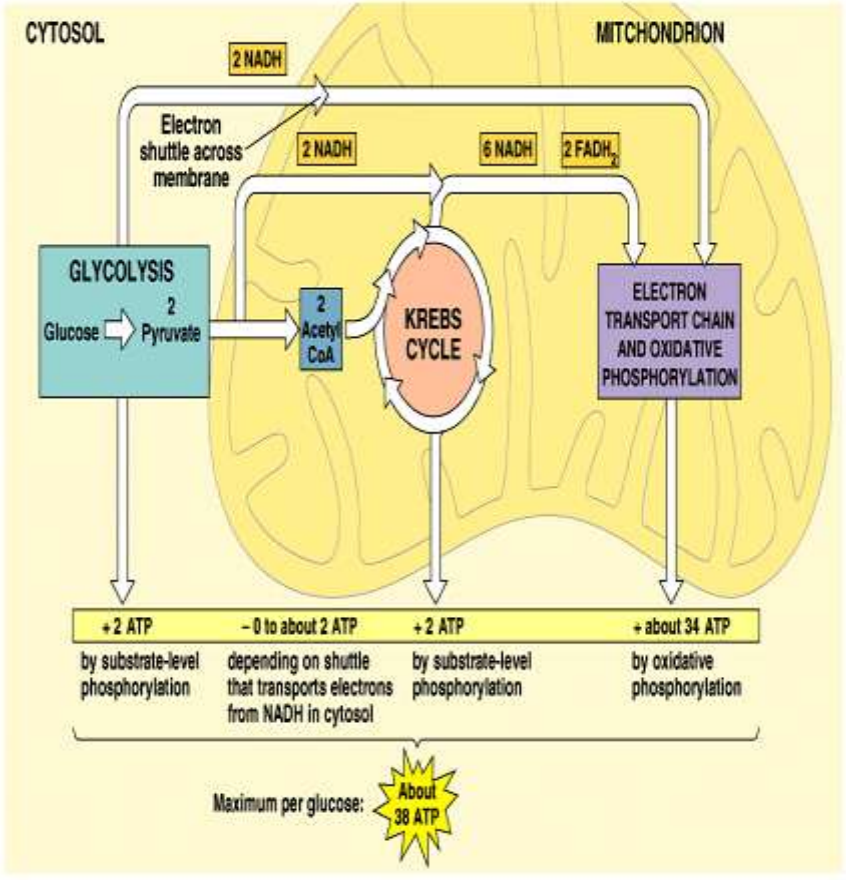
($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ (forming 38 ATP)

This is the process by which all living things obtain energy. We are only interested in the version of the process that takes place inside Eukaryotic cells. The many steps of the whole process allow small amounts of energy to be released at each step and also allows for substances other than glucose (fatty-acids, glycerol, amino-acids) to be used for respiration when needed.

There are 4 main stages:

1. **Glycolysis: Glucose → 2 x pyruvate (in the cytoplasm, no oxygen required). A net gain of 2 molecules of ATP and two molecules of reduced NAD (NADH).**
2. **The Link Reaction: Pyruvate → Acetyl CoA + NADH + CO_2 the 'link' to mitochondria**
3. **The Krebs (or TCA) Cycle: In the mitochondrial matrix, this uses oxygen to breakdown the Acetyl CoA through a complex cycle to release CO_2 and 'store' hydrogen as reduced NAD (NADH) and reduced FAD (FADH). Two molecules of ATP are also made per glucose molecule.**

4. **The Electron Transport Chain (E.T.C.): On the mitochondrial cristae, this uses oxygen to oxidise all the NADH molecules and FADH molecules made in all the earlier stages to water and release a great deal of energy – stored as ATP. By far the main site of ATP synthesis (34 molecules of ATP).**

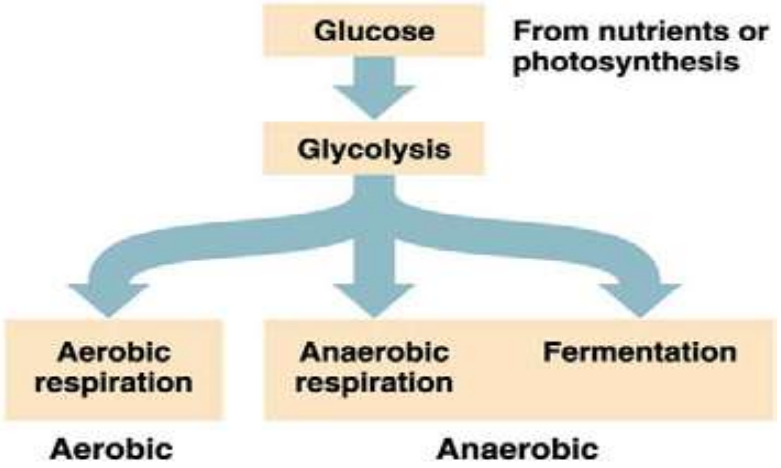


Note that respiration can also be **anaerobic**, i.e. **take place without oxygen.** This is much **less efficient, forming only 2**

molecules of ATP per glucose. There are two different types of anaerobic respiration, or **fermentation**:

Animals (only!): Glucose → 2 x lactic acid + 2ATP

Plants and Fungi: Glucose → 2 x ethanol + $2CO_2$ + 2ATP



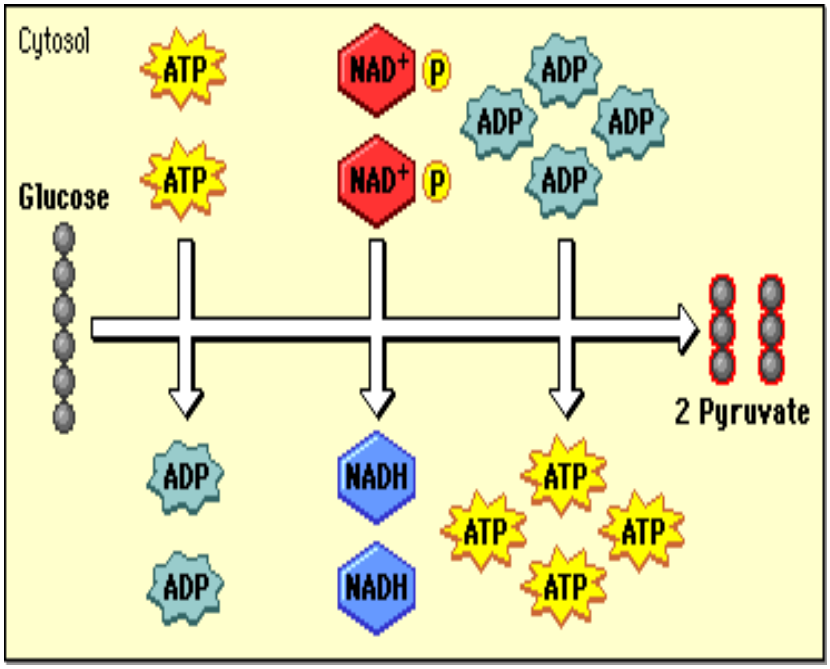
BOTH forms of anaerobic respiration **recycle the reduced NAD (NADH) back to NAD, thus allowing glycolysis to continue**.

The **lactic acid** animals produce **is broken down in the liver** (aerobically), causing the **oxygen debt** we experience at the end of violent (anaerobic) exercise.

Glycolysis is the **first stage of respiration**. It **breaks glucose down into two 3-C molecules (called pyruvate)** and at the same time makes it much more reactive. **Two molecules of ATP are used up** in this process, but **4 ATP molecules are formed** (**so net +2 ATP overall**). At the same time, **2 molecules of NAD are reduced** (to **NADH**) - see left. Since our cells have only a little NAD (it's a B-group vitamin), this **must** be recycled if glycolysis is

to continue. This takes place in the ETC in the mitochondria –see below.

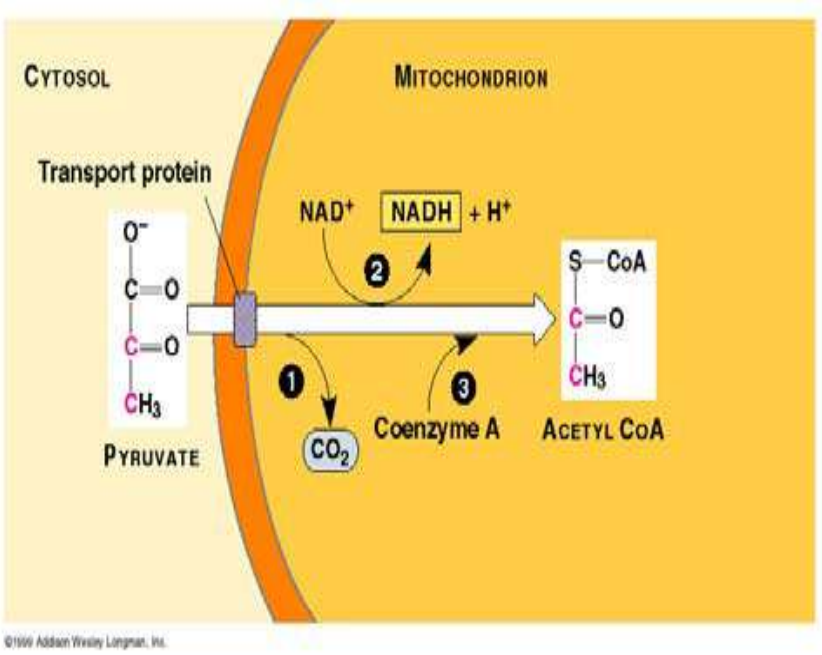
GLYCOLYSIS



This is the 'link' between the cytoplasm and the mitochondrion. The diagram (left) shows that, as the 3C pyruvate is converted to the 2C Acetyl CoA, **as well as a molecule of CO_2, a further molecule of NAD is reduced** (to **NADH**).

This is the first time that oxygen is required (for anaerobic respiration, see later)

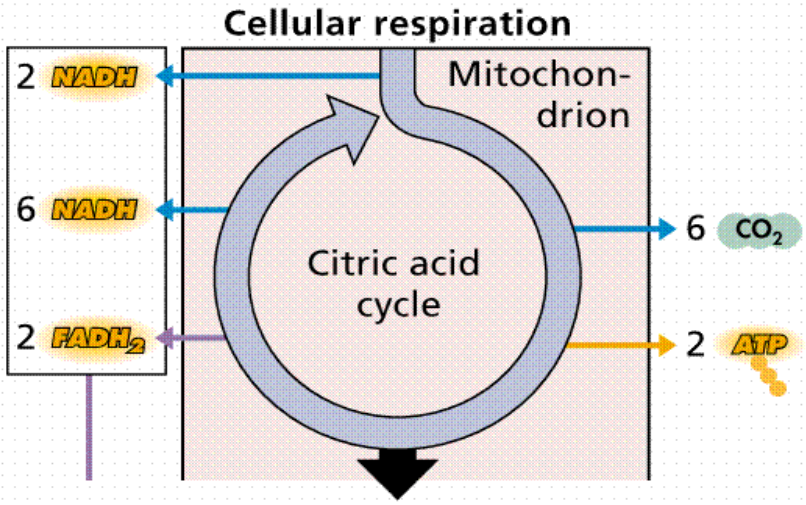
The link reaction



This takes place in the **matrix of the mitochondria** and questions on Kreb's Cycle take the form of 'count the carbons'! The **2C acetyl** group (the CoA returns to the cytoplasm to repeat the link reaction) **combines with the 4C oxaloacetate** to form the **6C citrate** molecule. This then gets converted **back** into the **4C oxaloacetate** in the rest of the cycle, so releasing **2 molecules of CO_2**.

Three molecules of reduced NAD (NADH) are also formed, together with **one molecule of reduced FAD (FADH), and one molecule of ATP.**

The Krebs cycle (or TCA cycle)



Now, since **two** molecules of acetyl CoA were formed from **each** molecule of glucose, it follows that **there are two turns of Kreb's cycle for each glucose molecule**. That means that **all the above numbers need to be doubled** to get the overall totals:

- **6 molecules of NADH and**
- **2 molecules of FADH and**
- **2 molecules of ATP**

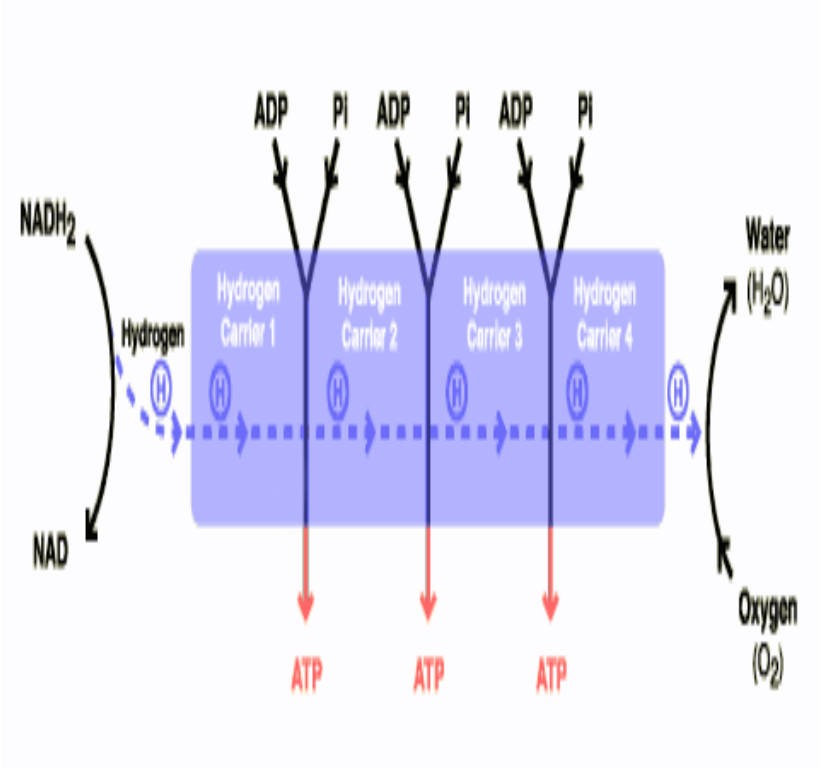
All **from one molecule of glucose**!

This series of reactions **takes place on the cristae,** the inner membrane of the mitochondria. A series of reactions **combines the H^+ ions and electrons released from the reduced NAD** (**NADH**) **with oxygen**. This **forms water** and the **energy released is trapped as ATP**.

From each molecule of reduced NAD (NADH), 3 molecules of ATP are formed

From each molecule of reduced FAD (FADH), 2 molecules of ATP are formed.

The Electron Transport Chain (ETC)



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Respiratory Quotient

This is used to calculate what substance an organism is respiring. It is calculated from the formula: **RQ = CO2 produced**

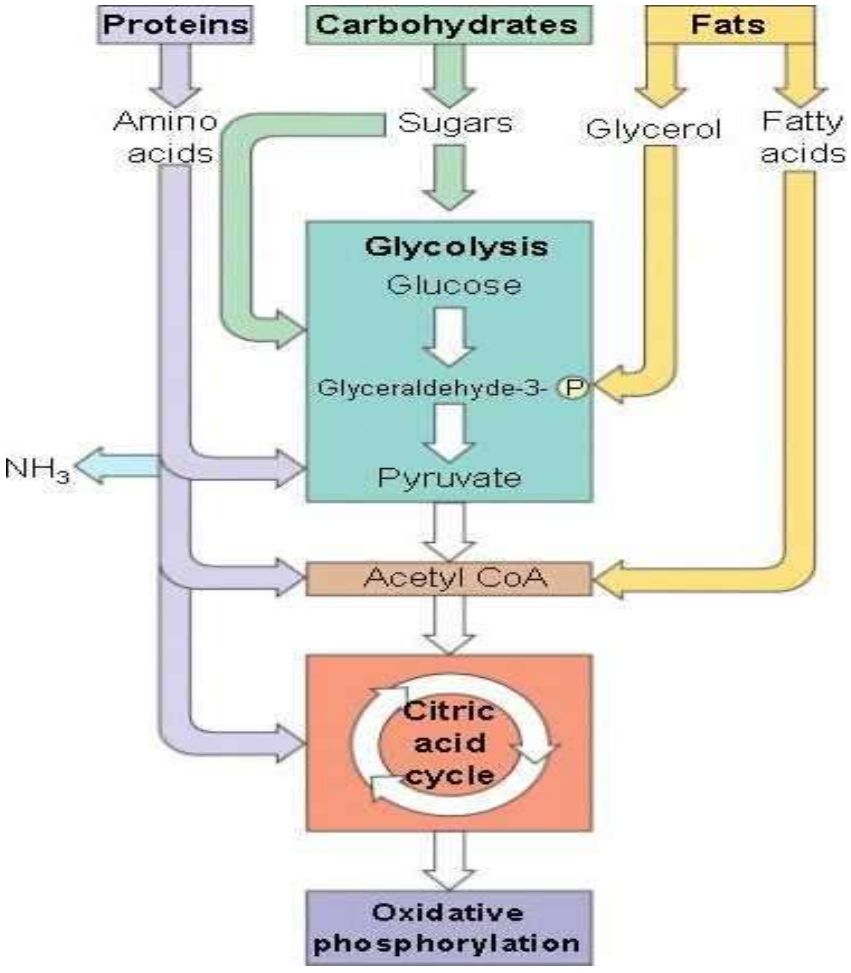
O_2 consumed

If the RQ is **0.7, then lipids are being respired**

If the RQ is **0.9, then amino-acids are being respired**

If the RQ is **1.0 then carbohydrates (any) are being respired**

If the RQ is **above 1.0, then respiration is anaerobic**.

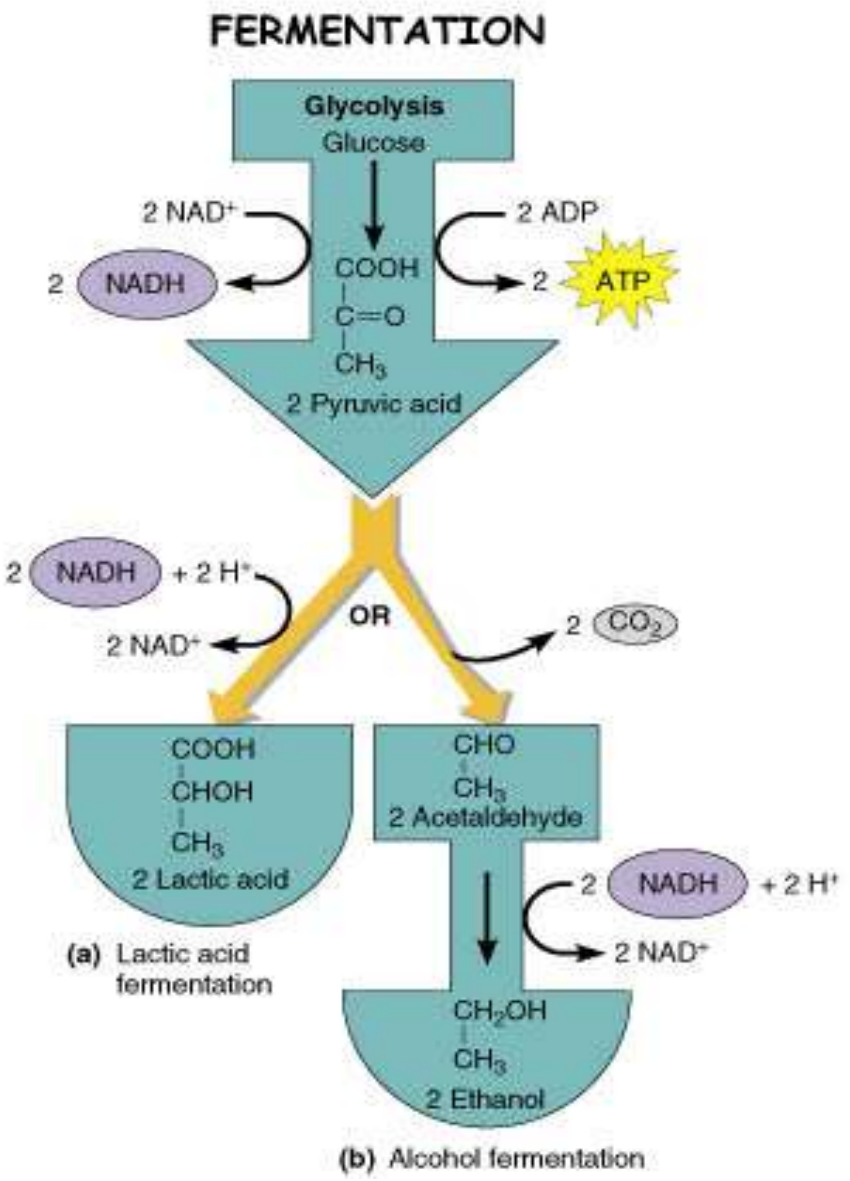


Fermentation

The **two** forms of anaerobic respiration are both shown in the diagram (right).

Note that **neither method gains any more ATP from the pyruvate**, but, by **recycling the reduced NAD (NADH), they both allow glycolysis to continue**, even in the absence of oxygen.

A side-effect is that the chemicals formed (**ethanol** and **lactic acid**) are **less toxic** than pyruvic acid (**pyruvate**).


FERMENTATION
Glycolysis
Glucose
2 NAD+
2 NADH
2 ADP
2 ATP
COOH
C=O
CH3
2 Pyruvic acid
2 NADH + 2 H+
2 NAD+
OR
2 CO2
COOH
CHOH
CH3
2 Lactic acid
(a) Lactic acid fermentation
CHO
CH3
2 Acetaldehyde
2 NADH + 2 H+
2 NAD+
CH2OH
CH3
2 Ethanol
(b) Alcohol fermentation

NITROGEN FIXATION

⇒ ***Our goal is to learn how N_2 an inert gas, becomes part of the structure of organic molecules***

⇒ ***Secondly, to study the function of nitrogen compounds in plants and bacteria***

⇒ ***To study the nitrogenase complex and learn its secrets for fixing nitrogen***

5) Role of nitrogen in the biosphere

- **The growth of all organisms depends on the availability of mineral nutrients, and ammonia is more important than nitrogen, which is required in large amounts as an essential component of proteins, nucleic acids and other cellular constituents.**
- **There is an abundant supply of nitrogen in the earth's atmosphere - nearly 78% in the form of N_2 gas.**

- **However, N_2 is unavailable for use by most organisms because there is a triple bond between the two nitrogen atoms, making the molecule almost inert. In order for nitrogen to be used for growth it must be "fixed" (combined) in the form of ammonium (NH_4) or nitrate (NO_3) ions.**

Microorganisms have a central role in almost all aspects of nitrogen availability and thus for life support on earth:

some bacteria can convert N_2 into ammonia by the process termed nitrogen fixation; these bacteria are either free-living or form symbiotic associations with plants or other organisms (e.g. termites, protozoa) other bacteria bring about transformations of ammonia to nitrate, and of nitrate to N2 or other nitrogen gases many bacteria and fungi

degrade organic matter, releasing fixed nitrogen for reuse by other organisms.

All these processes contribute to the nitrogen cycle.

We shall deal first with the process of nitrogen fixation and the nitrogen-fixing organisms, then consider the microbial processes involved in the cycling of nitrogen in the biosphere.

Nitrogen fixation:

- **A relatively small amount of ammonia is produced by lightning.**
- **Some ammonia also is produced industrially by the Haber-Bosch process, using an iron-based catalyst, very high pressures and fairly high temperature. But the major conversion of N_2 into ammonia, and thence into proteins, is achieved by microorganisms**

in the process called nitrogen fixation (or dinitrogen fixation).

How powerful is Nitrogen Gas?

❖ Haber Process:

$$N_2\,(g) + 3H_2\,(g) \overset{300\ atm.}{\rightleftharpoons} 2NH_3\,(g) + 92\ kJ$$

❖ Biological nitrogen fixation:

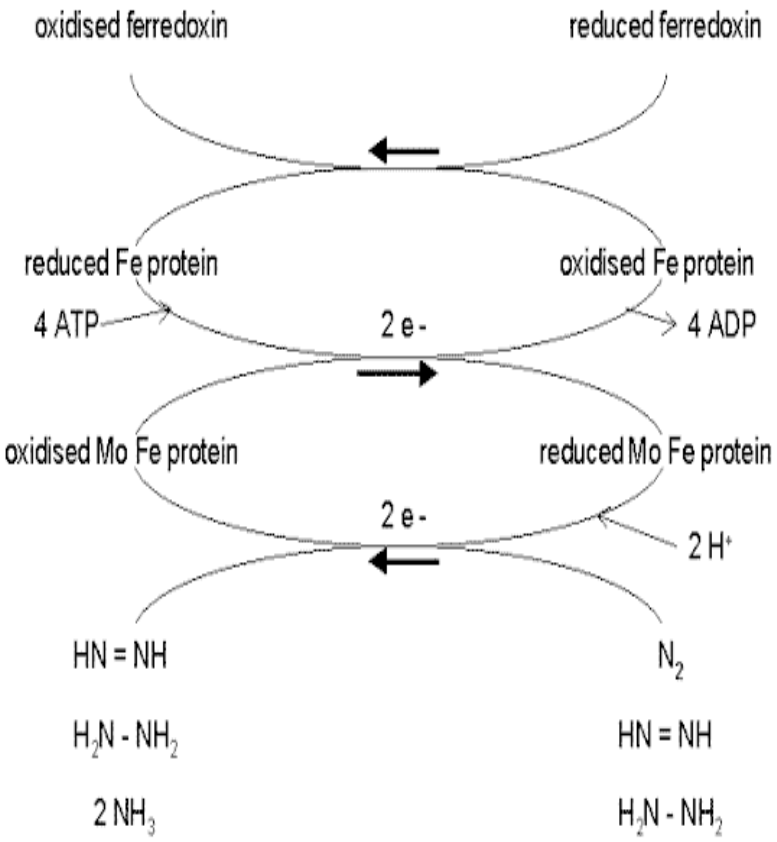
Biological nitrogen fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions):

$$N_2 + 8H^+ + 8e^- + 16ATP + 16H_2O \xrightarrow{\text{nitrogenase}} 2NH_3 + 16ADP + 16Pi + H_2 + 16H^+$$

This reaction is performed exclusively by prokaryotes (the bacteria and related organisms), using an enzyme complex termed nitrogenase. This enzyme consists of two proteins - an iron protein and a molybdenum-iron protein, as shown below.

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2, producing HN=NH. HN=NH is reduced to $H_2N\text{-}NH_2$, and this in turn is reduced to $2NH_3$.

Depending on the type of microorganism, the reduced ferredoxin which supplies electrons for this process is generated by photosynthesis, respiration or fermentation.



Factors affecting N_2 fixation

1. Presence of nitrate or ammonium: More N2, No, N2 fixation

2. Presence of certain inorganic substances Ca, Co, Mo – influence N2 fixation

3. Availability of energy source – addn. of C source increase N2 fixation

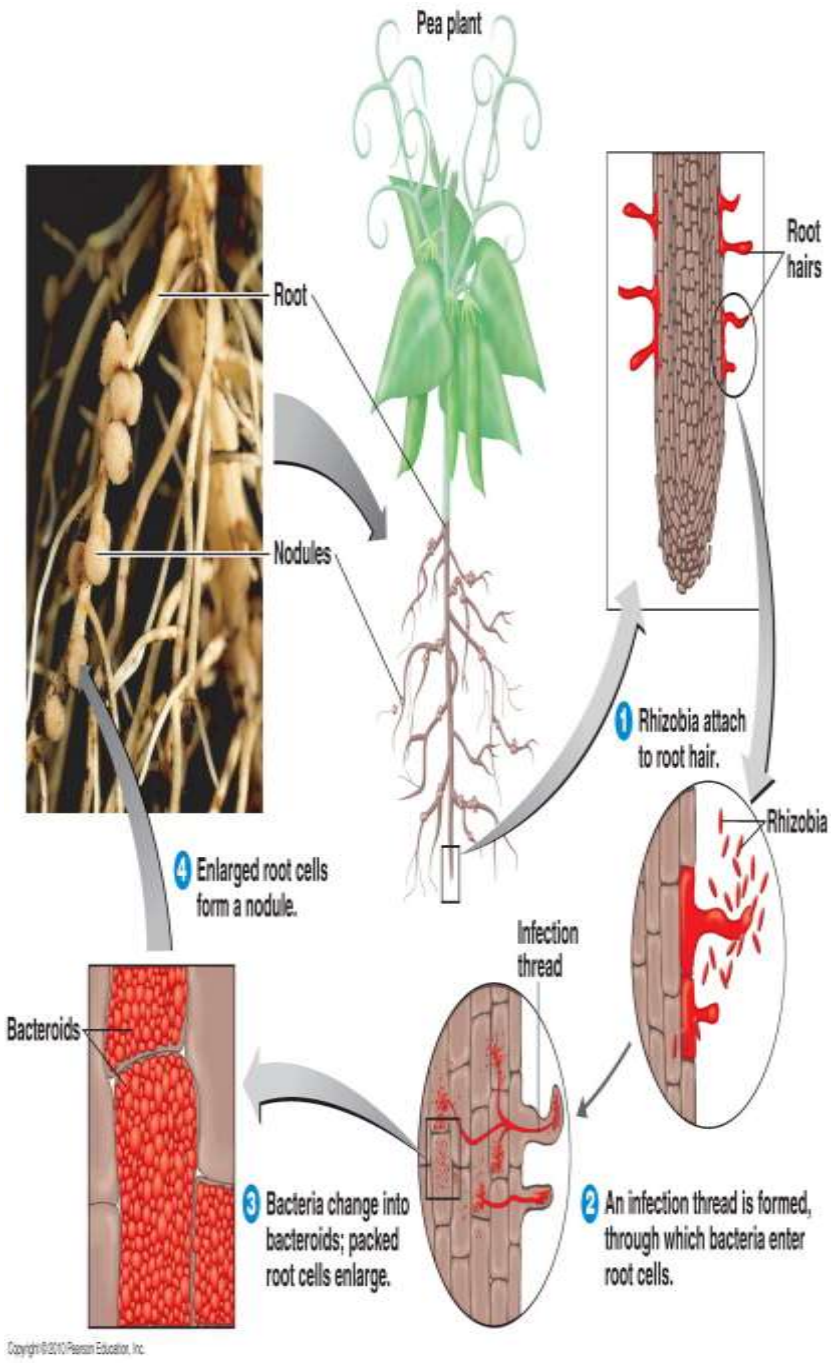
4. PH: Neutral – favours *Azotobacter*

5. Soil moisture: Adequate is good for fixation

6. Temperature: Mesophilic – 30°C.

Ammonia assimilation N2 fixation results in NH4 formation which reacts with organic acids and form amino acids which is mediated by ammonia assimilating enzyme.

Formation of a Root Nodule:


Pea plant
Root
Nodules
Root hairs
1 Rhizobia attach to root hair.
Rhizobia
Infection thread
2 An infection thread is formed, through which bacteria enter root cells.
3 Bacteria change into bacteroids; packed root cells enlarge.
Bacteroids
4 Enlarged root cells form a nodule.
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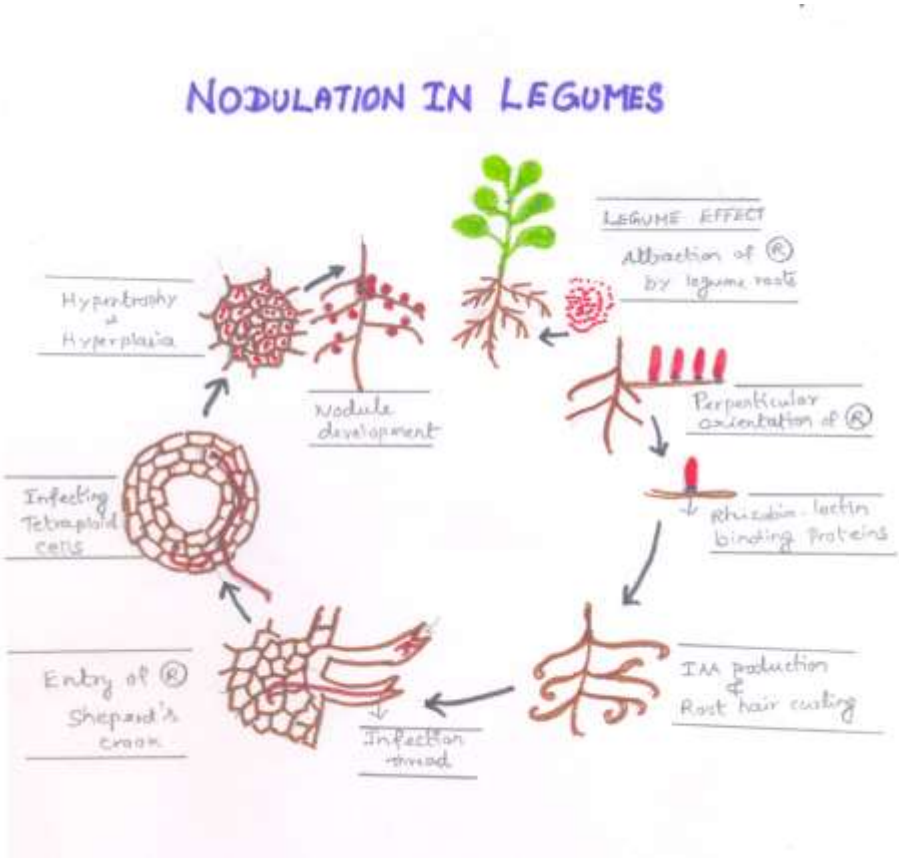

Factors affecting nodulation

- **Temperature and light**
- **Combined Nitrogen**
- **Hydrogen iron concentration**
- **Mineral nutrition-Co, Mo, P, Ca**
- **Ecological factors**
- **Salinity and alkalinity**

ECONMIC IMPORTANTS OF ALGAE

1- Algae as food

2- Algae as fodder for cattle

3- Algae as fertilizers

4- Algae in pisi culture

5- Algae in industry

6- Algae in reclamation of alkaline or use soils

7- Antibiotics

8- Role of algae in disposal sewage

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Good Luck