Benha University
Faculty of Science
Botany Department

Algae and Algal physiology (638 B)
Date: 30/5/2016
Time: 3 Hours
Pre-master of Microbiology - Final Exam

1- Discuss the following topics:

a) Structure of plastid, arrangement of envelopes and thylakoids in algae.
b) Nitrogen as a nutrient for algal growth.

2- Write briefly about the following:

a) Lag or induction phase of algal growth in batch culture.
b) Pigments involved in algal photosynthesis.
c) Characteristic features of heterocyst for nitrogen fixation.

3- Give brief account on:

a) Addition of EDTA to algal growth media.
b) Protective mechanism of nitrogenase enzyme.
c) Microcystin toxin.

Best wishes

نموذج الأجابة

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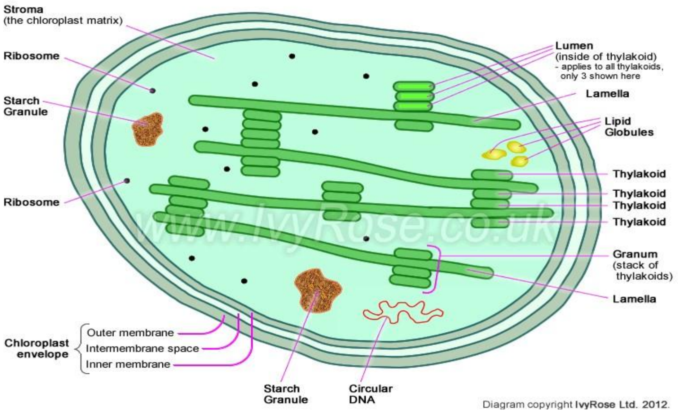


What is a chloroplast ? A chloroplast is a type of organelle. Chloroplasts are the site of photosynthesis in eukaryotic cells. Therefore they are only present in photosynthetic cells e.g. plant cells and algae.

Chloroplasts are bigger than mitochondria. That is why chloroplasts settle first when photosynthetic cells are homogenized and centrifuged. A typical chloroplast has a biconvex shape and a maximum dimension of about 5μm (i.e. 5 micrometers = 0.005 mm

•Although they exist within cells, chloroplasts are sometimes referred to as "**semi-autonomous organelles**" because they contain their own DNA and reproduce independently of the nucleus of the eukaryotic cell in which they are located.

•Each chloroplast is surrounded by a double-layered membrane i.e. it is enclosed by two membranes separated by an intermembrane space. In green plants both the inner membrane and the outer membrane surrounding chloroplasts are lipid-bilayer membranes.

Structure of (or within) **chloroplasts**:

1.	**Chloroplast Envelope**:	- Each chloroplast is enclosed (surrounded by) a chloroplast envelope consisting of three layers: 1. The **outer membrane** is a phospholipid membrane 2. The **intermembrane space** 3. The **inner membrane** is a phospholipid membrane Overall the chloroplast envelope is semi-permeable. It is permeable to glucose molecules and certain ions including Fe^{2+} and Mg^{2+}, and oxygen and carbon dioxide.
2.	**Stroma** (chloroplast matrix)	- The chloroplast matrix is called the stroma and contains enzymes that catalyze the **light-independent** reactions of photosynthesis.
3.	**Thylakoids** each thylakoid has a **lumen**	- Thylakoids are also referred to as **thylakoid membranes**. They are disc-shaped structures that are the sites of light absorption at which the **light-dependent** reactions of photosynthesis take place. The region within the membrane forming each thylakoid (by enclosing the contents of the thylakoid) is called the **lumen** of the thylakoid. Either on the surface of, or embedded within, thylakoids are: • **chlorophyll molecules** - on the surface of thylakoids. (Chlorophyll is **green** and plentiful in chloroplasts in plant cells, hence many plants are also green! Although chlorophyll is the main pigment in chloroplasts there are also other pigments - different pigments absorb different wavelengths of light.) • **accessory pigments** • **enzymes** • **electron transport systems** The light absorbing molecules within thylakoid membranes are arranged in **photosystems**. Thylakoids are also the sites at which ATP synthesis occurs within chloroplasts.
4.	**Grana** (plural), singular - **Granum**	- Thylakoids are arranged in stacks called grana (*plural*). A single **granum** is a stack of several thylakoids one on top of another. As shown in the diagram and model above, there are many such **grana** within each chloroplast.
5.	**Lamellae** (plural) singular - **Lamella**	- As shown above, stromal **lamellae** connect two or more **grana** to each other. In this way the lamellae act as a "skeleton" of the chloroplast, maintaining efficient distances between the **grana**, thereby maximizing the overall efficiency of the chloroplast.
6.	**Circular DNA**	- Each chloroplast contains one or more molecules of small circular DNA.
7.	**Starch granules**	- Starch exists in chloroplasts in the form of tiny lumps called "granules" or sometimes "grains". These are present because they are the (insoluble) storage carbohydrate product of photosynthesis.
8.	**Lipid globules**	- Lipid globules are also present in chloroplasts.
9.	**Ribosomes**	- Chloroplasts contain the smaller type of ribosomes (i.e. "70S ribosomes"), which is the same type as those freely distributed around the cytoplasm of prokaryotic cells

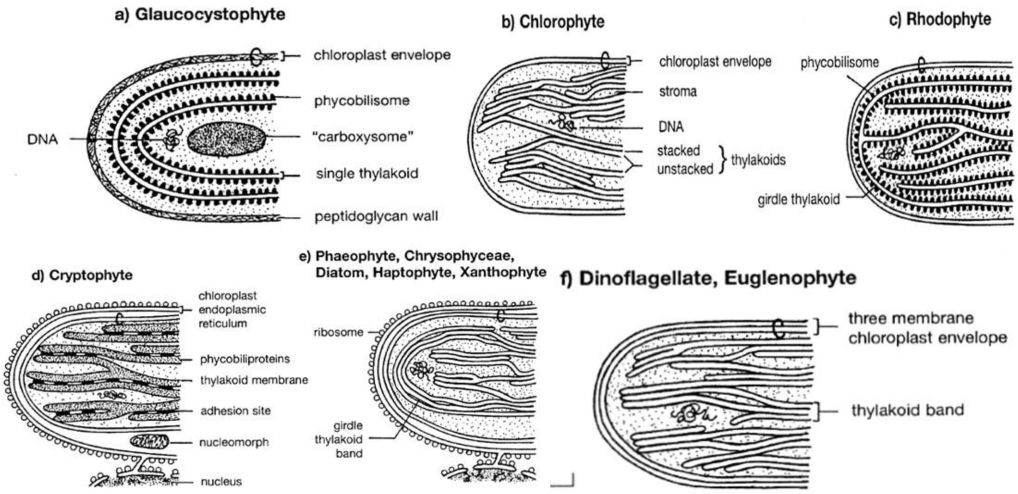


Fig. 1. Diagrams to show the typical arrangement of plastid envelopes and thylakoids in a) glaucocystophytes; b) chlorophytes; c) rhodophytes; d) cryptophytes; e) chrysophytes, phaeophytes, diatoms, xanthophytes, haptophytes, etc and f) dinoflagellates and euglenophytes.

b) Nitrogen as a nutrient for algal growth.

Nitrogen

After carbon, nitrogen is the most important nutrient contributing to the biomass produced.

Typical responses to nitrogen limitation is discoloration of the cells (decrease in chlorophylls and an increase in the carotenoids) and accumulation of organic carbon compounds such as polysaccharides and certain oils (PUFAs).

Nitrogen is mostly supplied as nitrate (NO_3), but often ammonia and urea are also used.

Some cyanobacteria are capable of utilizing elemental nitrogen by the reduction of N2 to NH4 , a process catalyzed by the enzyme nitrogenase.

	Organic	Inorganic
Particulate	Living biomass Detritus Feces, etc.	Ammonia adsorbed to suspended sediments
Soluble	Enzymes Amino acids Organic acids Waste products	Nitrate Nitrite Ammonia Dissolved N_2 gas

Table 2. Different forms of nitrogen (N) commonly found in ponds.

Nutrients are present in several forms in aquatic systems, including dissolved inorganic, dissolved organic, particulate organic, and biotic forms. Only dissolved forms are directly available for algal growth:

Element	Form Available to Plants	Major Functions
Macronutrients		
Carbon	CO_2	Major component of plant's organic compounds
Oxygen	CO_2	Major component of plant's organic compounds
Hydrogen	H_2O	Major component of plant's organic compounds
Nitrogen	NO_3^-, NH_4^+	Component of nucleic acids, proteins, hormones, and coenzymes
Sulfur	SO_4^{2-}	Component of proteins, coenzymes
Phosphorus	$H_2PO_4^-$, HPO_4^{2-}	Component of nucleic acids, phospholipids, ATP, several coenzymes
Potassium	K^+	Cofactor that functions in protein synthesis; major solute functioning in water balance; operation of stomata
Calcium	Ca^{2+}	Important in formation and stability of cell walls and in maintenance of membrane structure and permeability; activates some enzymes; regulates many responses of cells to stimuli
Magnesium	Mg^{2+}	Component of chlorophyll; activates many enzymes
Micronutrients		
Chlorine	Cl^-	Required for water-splitting step of photosynthesis; functions in water balance
Iron	Fe^{3+}, Fe^{2+}	Component of cytochromes; activates some enzymes
Boron	$H_2BO_3^-$	Cofactor in chlorophyll synthesis; may be involved in carbohydrate transport and nucleic acid synthesis
Manganese	Mn^{2+}	Active in formation of amino acids; activates some enzymes; required for water-splitting step of photosynthesis
Zinc	Zn^{2+}	Active in formation of chlorophyll; activates some enzymes
Copper	Cu^+, Cu^{2+}	Component of many redox and lignin-biosynthetic enzymes
Molybdenum	MoO_4^{2-}	Essential for nitrogen fixation; cofactor that functions in nitrate reduction
Nickel	Ni^{2+}	Cofactor for an enzyme functioning in nitrogen metabolism

2- Write briefly about the following:

a) Lag or induction phase of algal growth in batch culture.

Growth phases of microalgae cultures

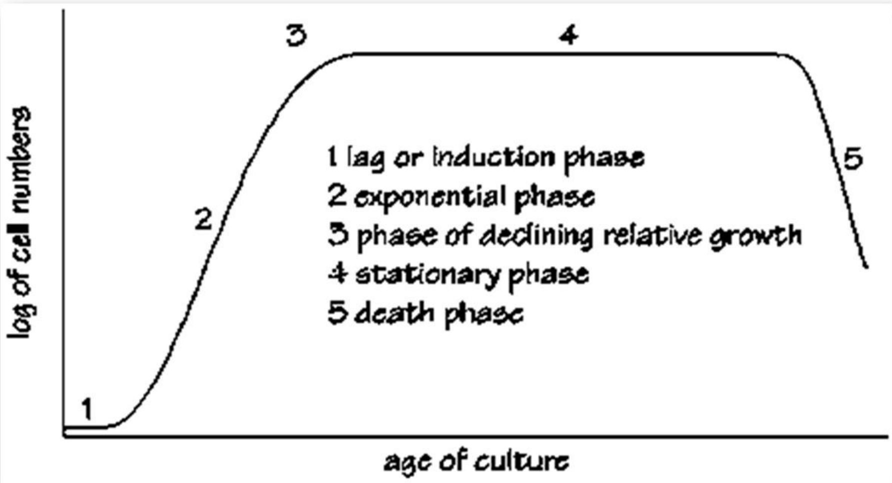
The growth of an axenic culture of micro-algae is characterized by five phases

1- lag or induction phase

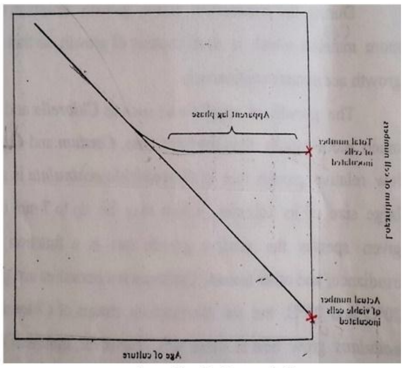
This phase, during which little increase in cell density occurs, is relatively long when an algal culture is transferred from a plate to liquid culture. Cultures inoculated with exponentially growing algae have short lag phases. The lag in growth is attributed to the physiological adaptation of the cell metabolism to growth, such as the increase of the levels of enzymes and metabolites involved in cell division and carbon fixation.

2- exponential phase

During the second phase, the cell density increases as a function of time t according to a logarithmic function: Ct = Cemt. ،
with Ct and C The .rate growth specific = m and ،respectively ،، and t time at concentrations cell the being ،
.temperature and intensity light ،species algal on dependent mainly is rate growth specific

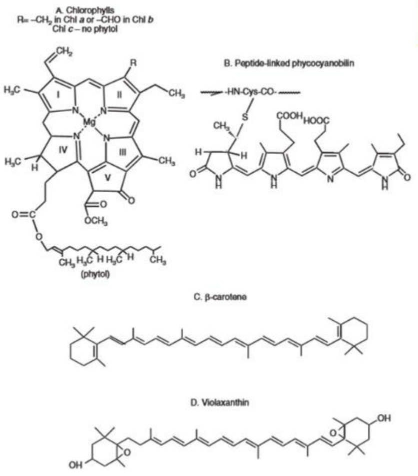


Five growth phases of micro-algae cultures



1- lag or induction phase

b) Pigments involved in algal photosynthesis.



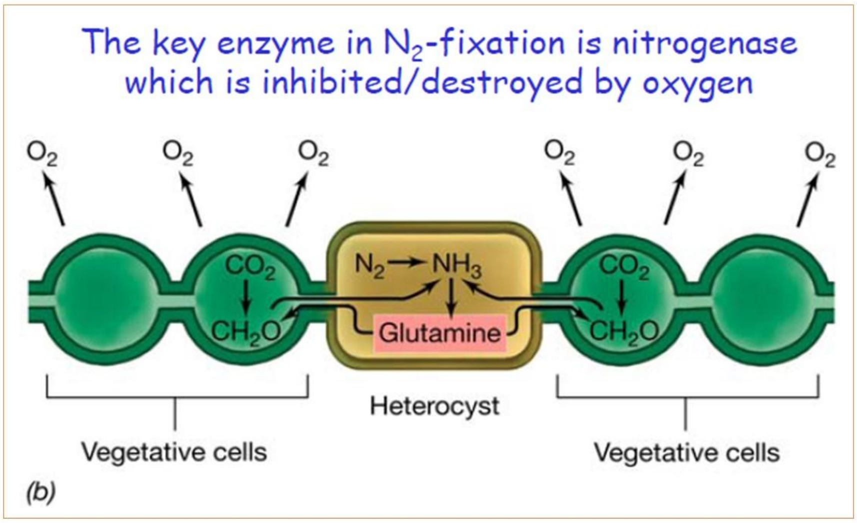
Photosynthetic pigments

Photosynthetic pigments are categorized into three classes. Chlorophylls, Caretenoids, Phycobilins

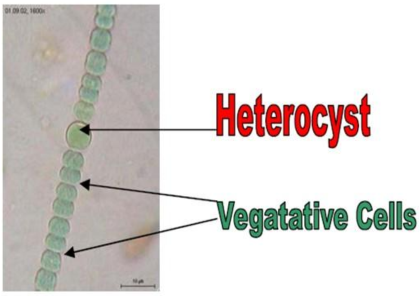
c) Characteristic features of heterocyst for nitrogen fixation.

Characteristic features of Heterocyst:

1. **The Heterocyst is the site for cyanobacterial nitrogen fixation which is an enlarged cell, and may be present terminally or intercalary in the filamentous cyanophycean algae.**
2. **In the process of cyanobacterial nitrogen fixation, hydrogen gas (H_2) is also evolved as a by product and 40% of it is recycled, whereas remaining 60% hydrogen gas can be used by as a source of future clean fuel.**
3. **The Heterocyst is made up of three (3) different cell wall layers- the outer fibrous and middle homogenous layers are made up of non-cellulose polysaccharide. Whereas, the inner laminated layer is made up of glycolipids.**
4. **These special cell wall layers permit the atmospheric N_2 (g) to diffuse inside, whereas on the other hand they stop the atmospheric O_2 (g) to come inside.**
5. **This is a damage-control mechanism for the enzyme nitrogenase, as the nitrogenase is sensitive to O_2 and cold, and cannot function in the presence of O_2 (g).**



In some cyanobacteria nitogen fixation occurs in heterocycts. These cells only have Photosystem I



The other cells have both photosystem I and photosystem II, which generates oxygen when light energy is used to split water to supply H_2 for synthesis of organic compounds.

3- Give brief account on:

a) Addition of EDTA to algal growth media.

<u>Phosphorus</u>

Phosphorus is essential for growth and many cellular processes such as energy transfer, biosynthesis of nucleic acids, DNA, etc.

The preferred form in which it is supplied to algae is as orthophosphate PO_2

the most important growth limiting factors. This is because it is easily bound to other ions (e.g. CO2 3 and iron) resulting in its precipitation (**problem)** and consequently rendering this essential nutrient unavailable for algal uptake

The addition of metal chelators such as EDTA dissolves in water has alleviated this problem of mineral precipitation due to pH difference .

It is thought chelators influence the availability of these elements. Chelators act as trace metal buffers, maintaining constant concentrations of free ionic metal. It is the free ionic metal, not the chelated metal, which influences microalgae, either as a nutrient or as a toxin. Without proper chelation some metals (such as Cu) are often present at toxic concentrations, and others (such as Fe) tend to precipitate and become unavailable to phytoplankton.

EDTA may have an additional benefit of reducing precipitation during autoclaving

b) Protective mechanism of nitrogenase enzyme.

- nitrogenase active in anaerobic condition
- nitrogenase enzyme is highly sensitive to oxygen
- nitrogenase is inactivated when oxygen reacts with the iron component of the proteins

Protective mechanism against Oxygen

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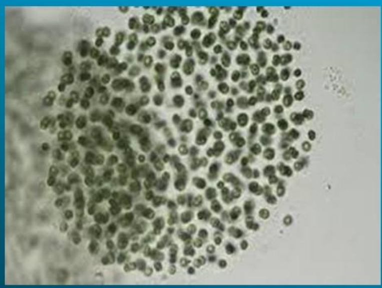
4. These special cell wall layers permit the atmospheric N_2 (g) to diffuse inside, whereas on the other hand they stop the atmospheric O_2 (g) to come inside.

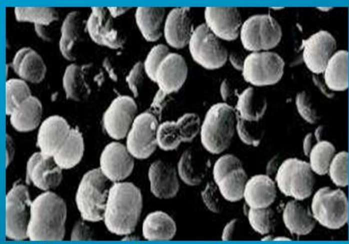
5. This is a damage-control mechanism for the enzyme nitrogenase, as the nitrogenase is sensitive to O_2 and cold, and cannot function in the presence of O_2 (g). Degredation of photosysten II involved in O2 production

c) Microcystin toxin.

Mirocystin:

Globally, microcystins are the most commmonly detected cyanobacterial toxin
. The liver is the ultimate target organ for toxic effects.
Microcystins are highly toxic with low doses required for lethal effects.
There is some evidence that microcystins can act as tumor promoters.



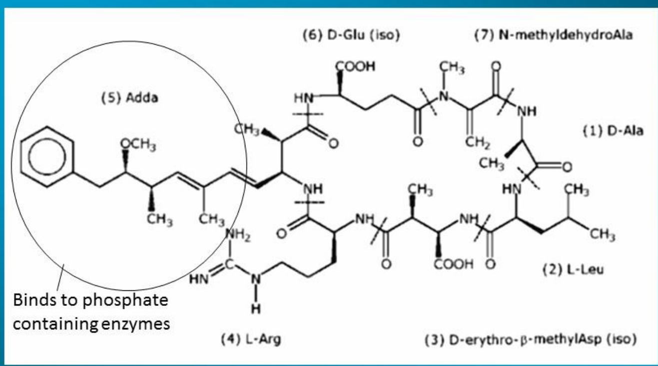


(Microcystis aeruginosa)

Microcystin toxin:

-large, cyclic peptide with several structural variants
-the linear form (break-down or precursor products) is 100x less toxic compared with the cyclic toxin

Microcystins: polypeptide that has >70 structural variants that alter potential toxicity by 20-fold. Principal damage to liver with inhibition of protein phosphatase 2a enzyme.



Microcystin can kill fish at 60-70 ng/mL

Fate and Breakdown of Microcystins

- In dark waters, microcystins can persist for months or even years.

- Microcystins remain potent after boiling and have a high degree of chemical stability. Sunlight will slowly breakdown microcystins.

- Despite their chemical stability, microcystins are susceptible to bacterial breakdown. These types of bacteria are found in lake water and sediment, river water and sewage effluent.