Microalgae

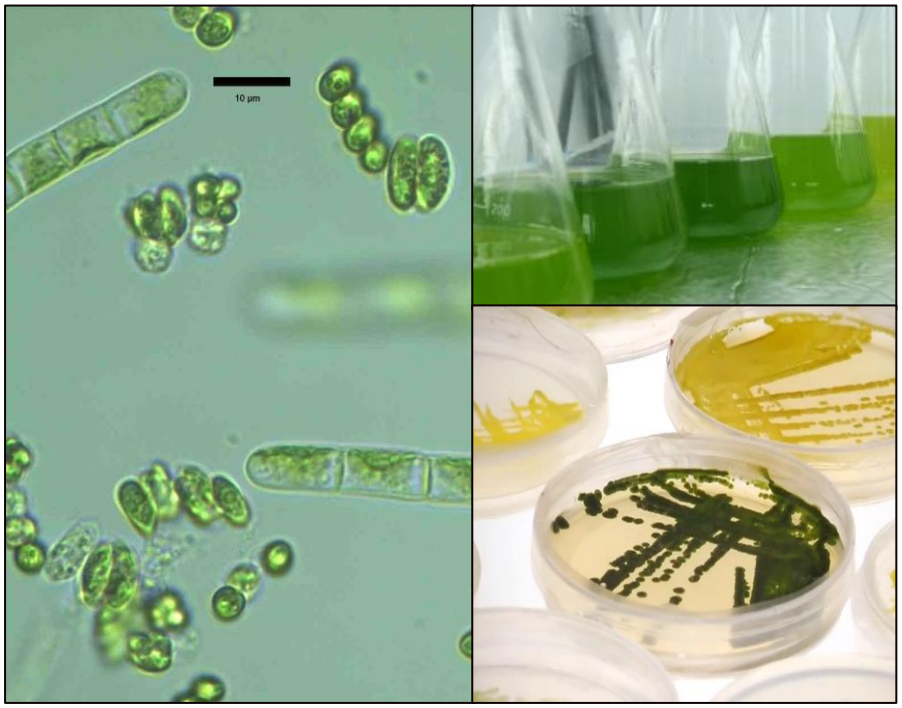
SAPIENZA
UNIVERSITÀ DI ROMA

Algae

Is generally considered an algae any organism with chlorophyll *a* and with a thallus not differentiated in roots, stem and leaves.

Macroalgae

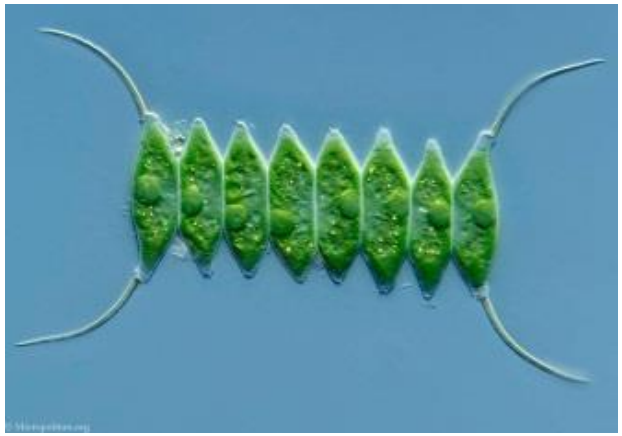
Microalgae

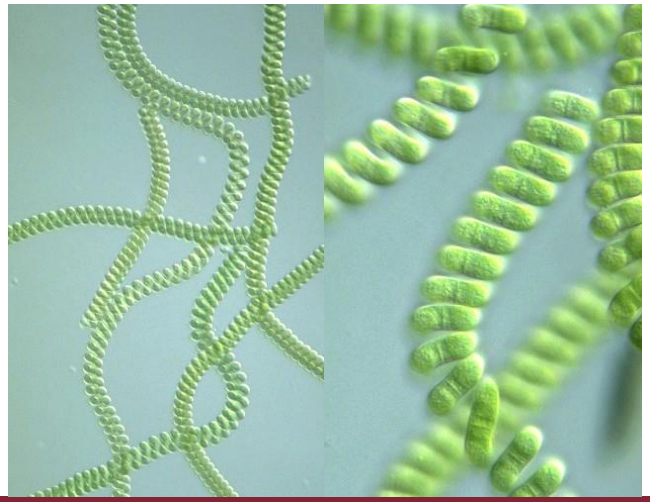


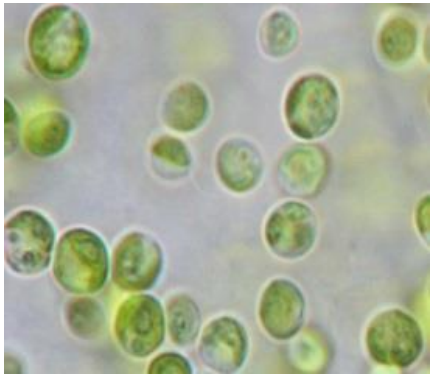
Microalgae

Microalgae are photosynthetic microorganisms

- It is estimated that more than 50,000 species exist
- Some species are found in saltwater and other in freshwater
- Cell size is generally included between 2 and 40 µm
- Microalgae can be divided in eukaryotic and prokaryotic (i.e. cyanobacteria)

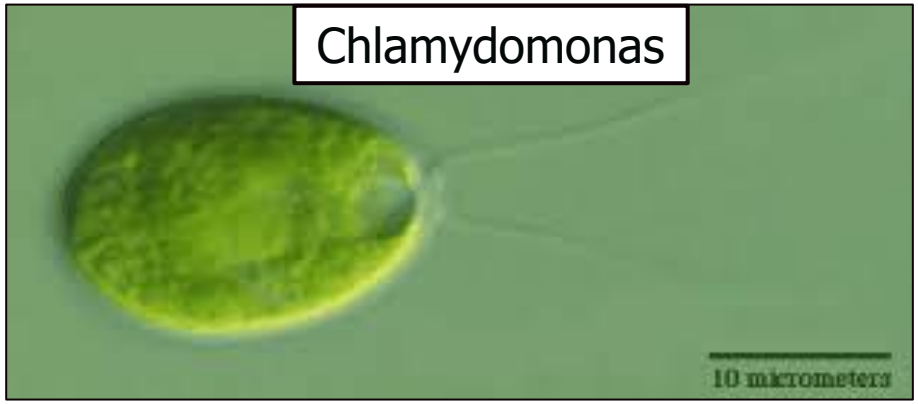


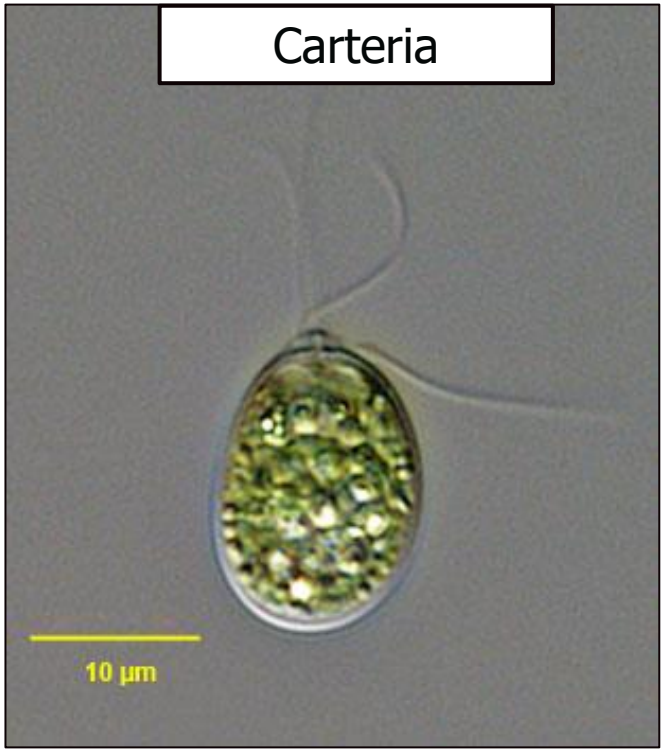




Morphology

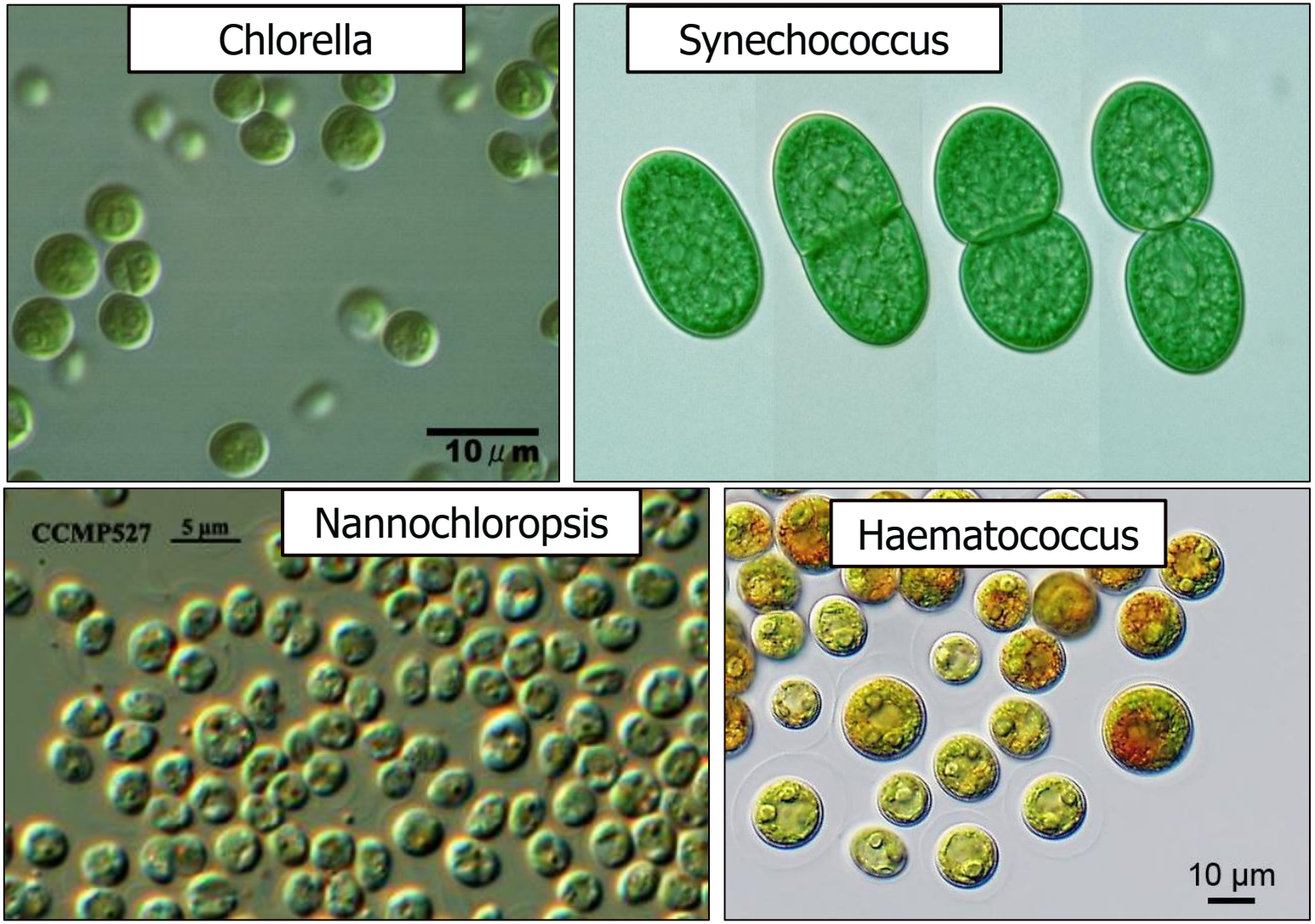
Unicellular flagellate





Microalgae can have one, two or three flagella

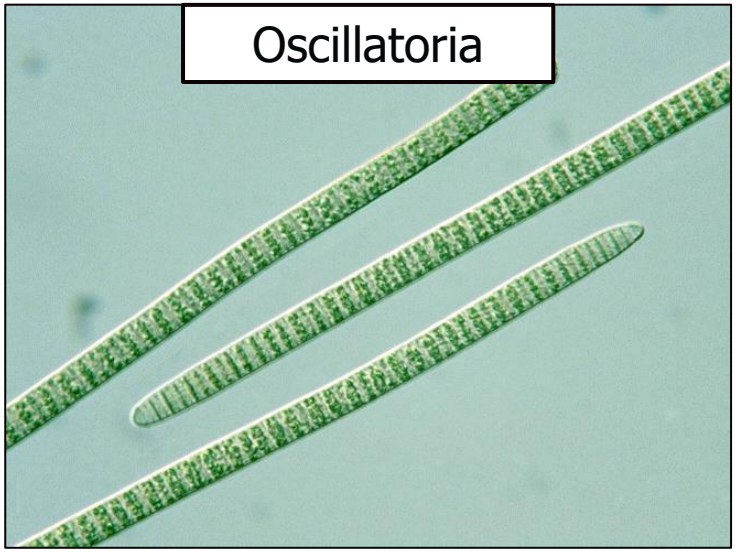
Unicellular non-flagellate



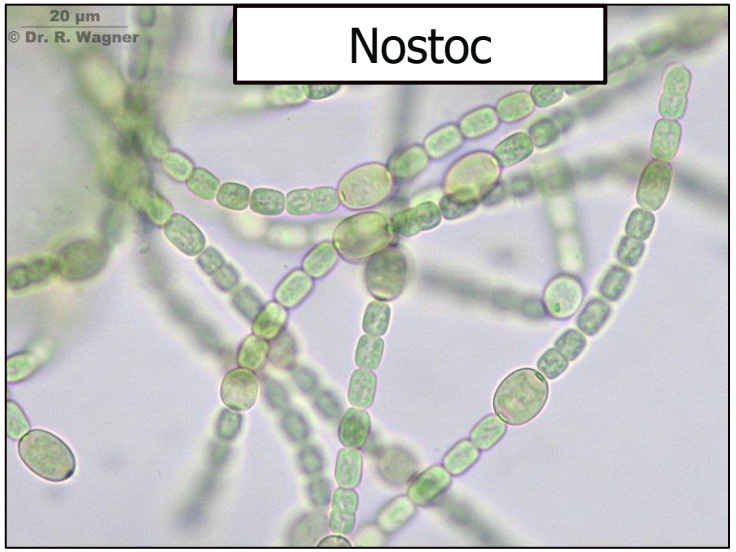
Colonial non-flagellate

Microalgae are bind together by a mucilaginous sheath. The cells are physiologically independent, however they can be influenced by the colony life (light, gas, nutrients gradients).

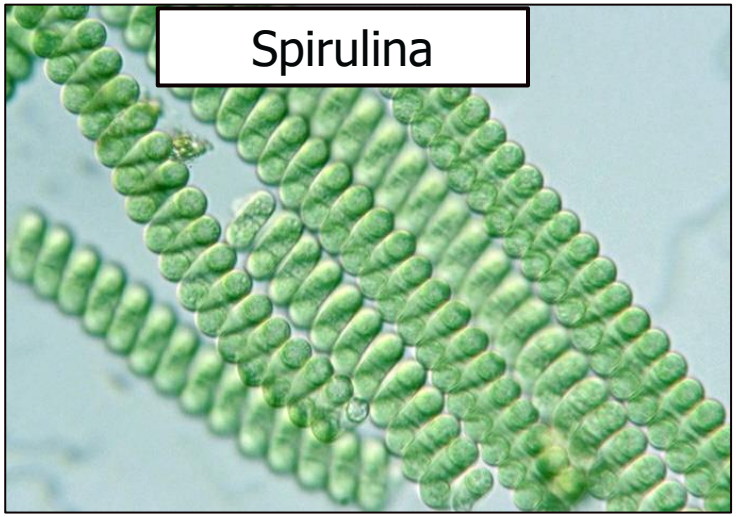
Some cyanobacteria species live in filamentous colony. Filaments are build up by single cells bind together in long chains.



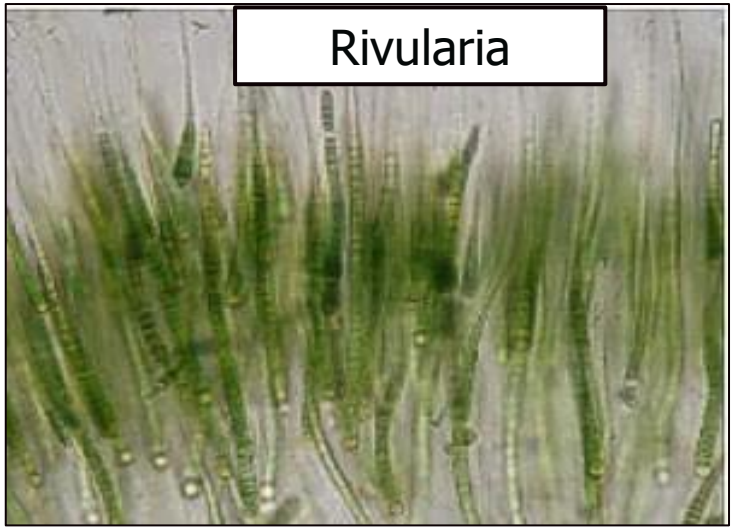
Simple filaments



Twisted filaments

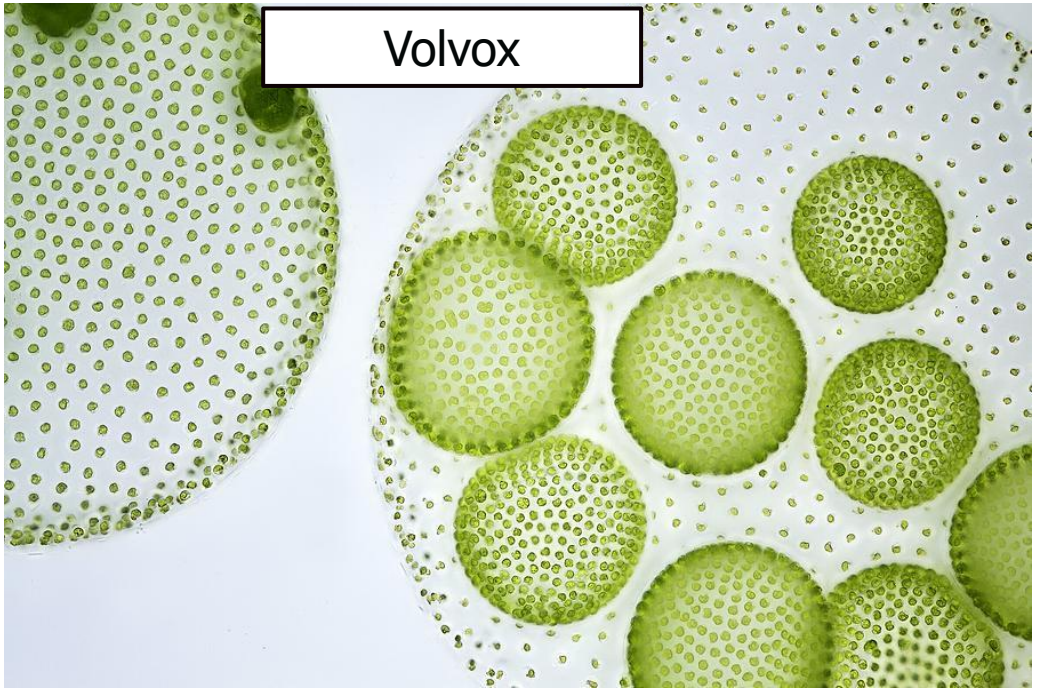


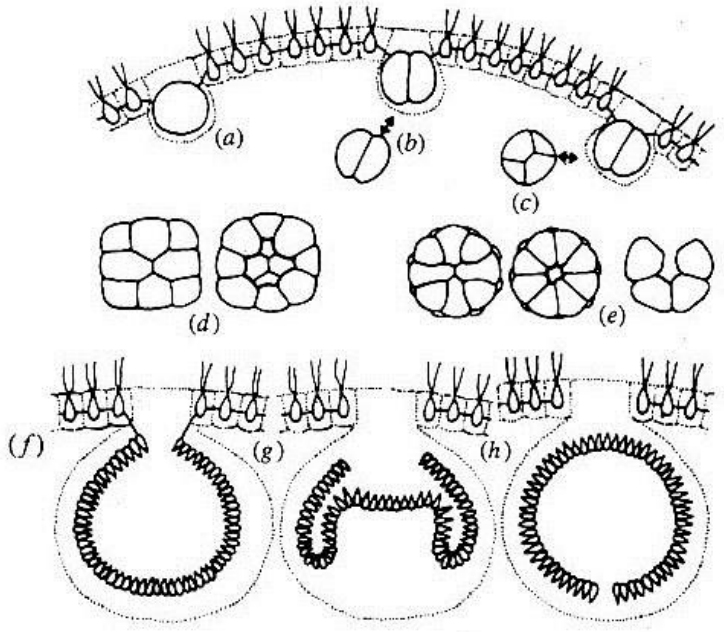
Helical filaments



Branched filaments

Colonial flagellate





Volvox are biflagellate microalgae able to generate spherical mobile colonies. Daughter colony are generated by the reproduction of some reproductive cells. Initially they are inside the parents, later the parents disintegrate.

Cell organization

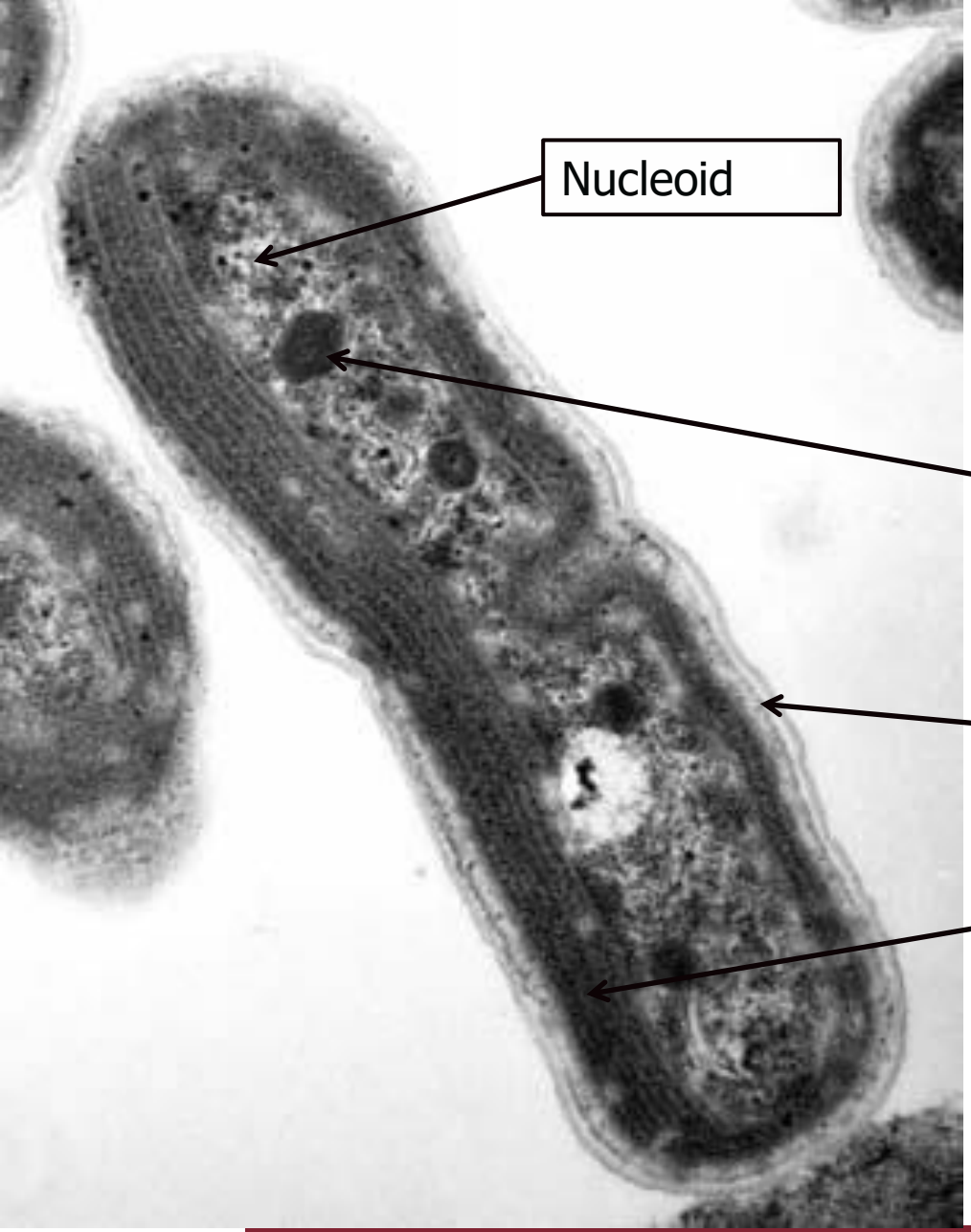
Prokariotic cell

- There is not nucleus, DNA is free in cytoplasm (nucleoid) and not organized in chromosomes.
- No organelles.
- Chlorophyll is in the cytoplasm.
- Cell wall: lipopolysaccharides, peptidoglycan.
- Stored material: glycogen, polyhydroxyalkanoates, hydrocarbons, lipids, cyanophycin.

Eukariotic cell

- DNA is organized in chromosomes in the nucleus.
- There are many specialized organelles (Golgi body, vacuoles, mitochondria, endoplasmic reticulum, plastides).
- Chlorophyll is in the plastides.
- Cell wall: cellulose, hemicellulose, pectin, agar, carrageenan.
- Stored material: starch, lipids.

Prokaryotic cell

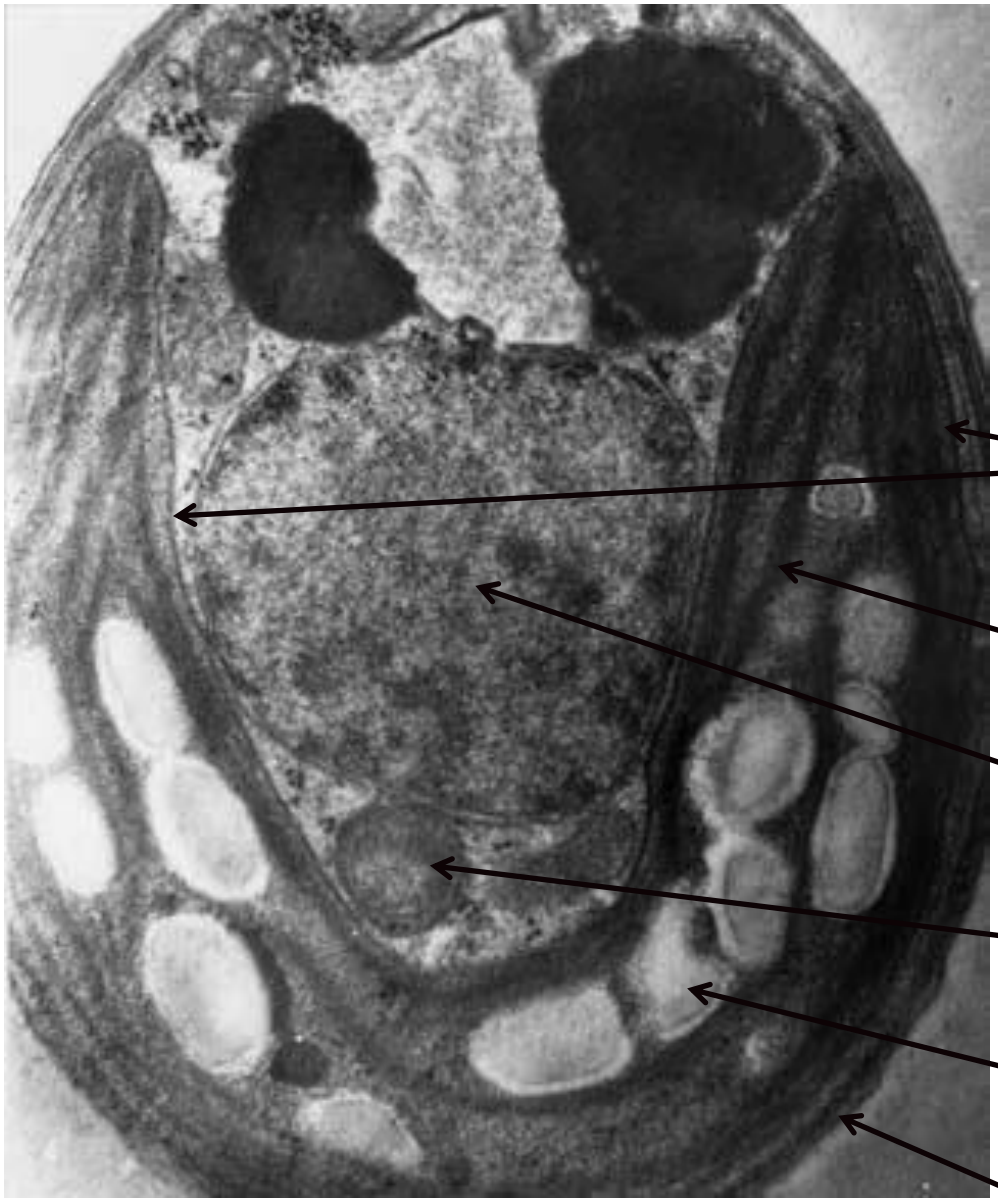


Electron micrograph of a cell of *Synechococcus sp*. during duplication

Thylakoids are membrane system containing chlorophyll. In prokaryotic cell they are in the cytoplasm.

Eukaryotic cell

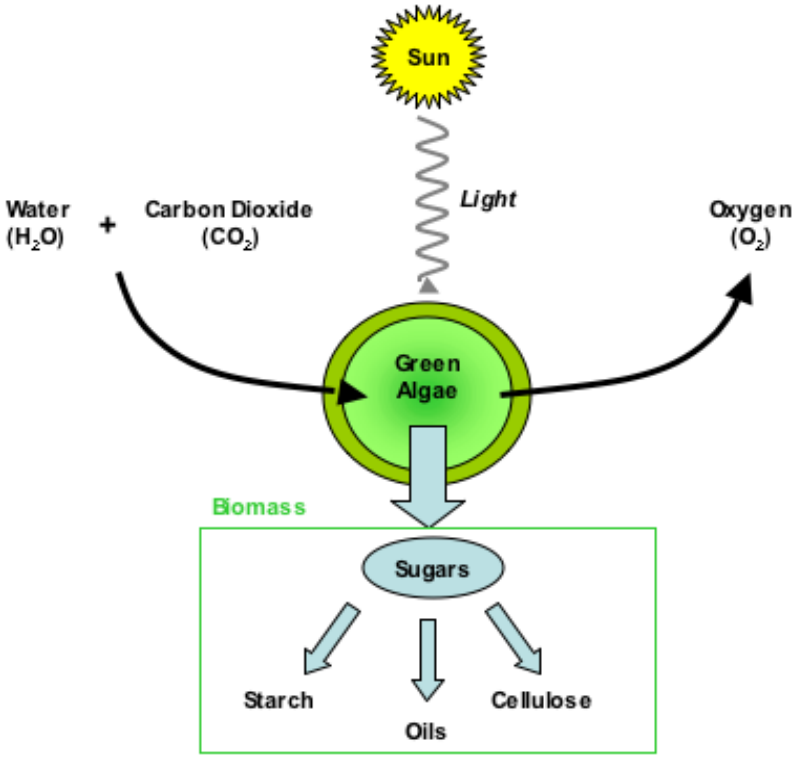
Electron micrograph of a cell of *Chlorella vulgaris*



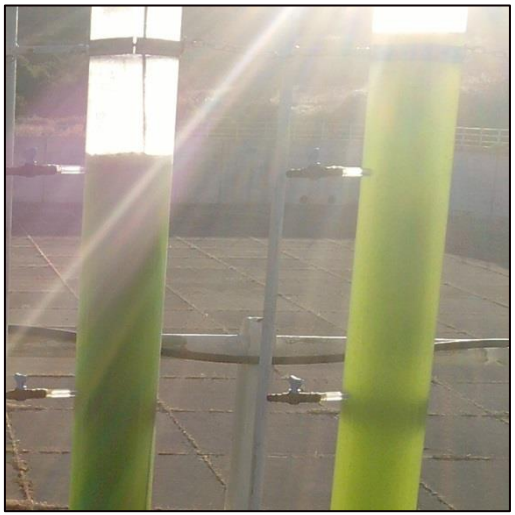
Photoautotrophic metabolism

When microalgae grow in autotrophic condition they utilize light as source of energy and CO_2 as source of carbon.

Photosynthesis is the biochemical process at the base of autotrophic metabolism.



Graphic©SustainableGreenTechnologies-2008



Microalgae by utilizing light energy and CO_2 maintain their internal homeostasis and synthesize new biomass.

Through photosynthesis microalgae use light energy to split water in O_2, protons and electrons. While O_2 is released outside the cell, protons and electrons are utilized to reduce CO_2 to carbohydrates.

Photosynthesis is a complex process which can be divided in two phases: light phase and dark phase.

$12\ H_2O + light \rightarrow 24\ H^+ + 24\ e^- + 6\ O_2$ Light phase

$6\ CO_2 + 24\ H^+ + 24\ e^- \rightarrow C_6H_{12}O_6 + 6\ H_2O$ Dark phase

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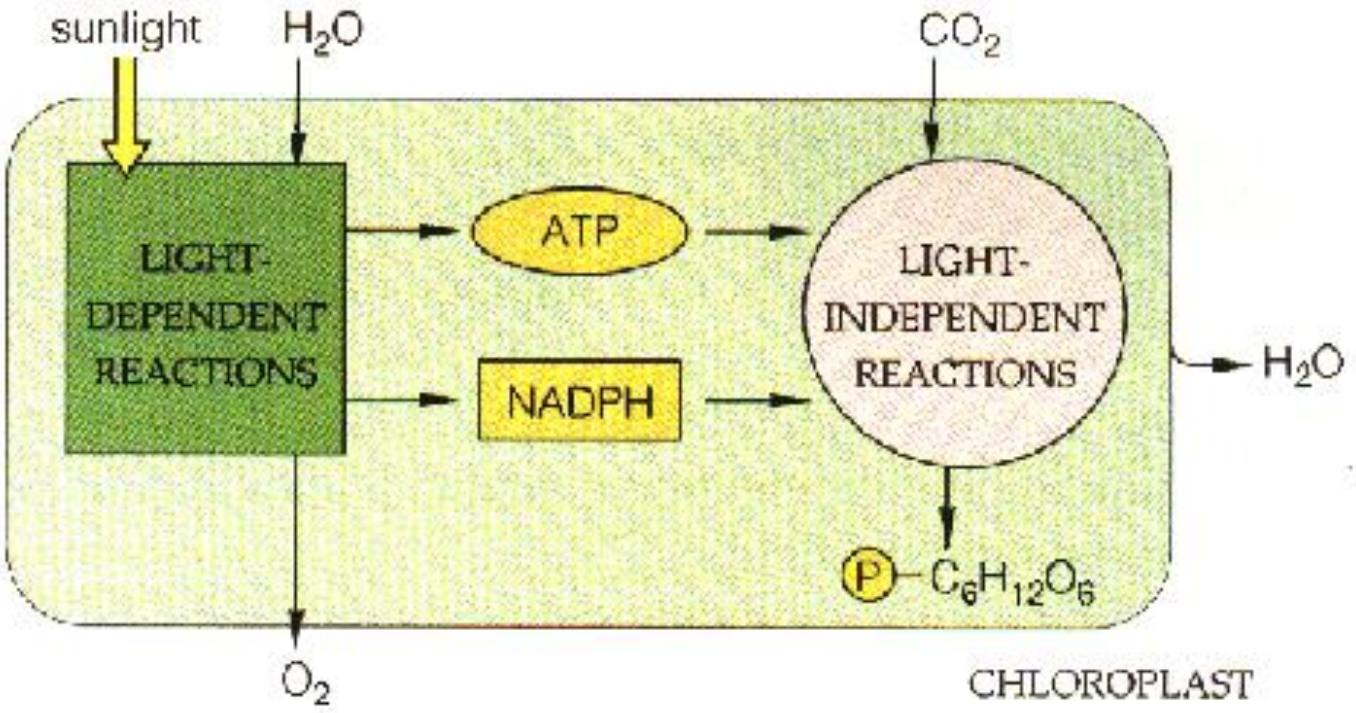
$6\ H_2O + 6\ CO_2 + light \rightarrow C_6H_{12}O_6 + 6\ O_2$ General photosynthesis reaction

Light phase is characterized by light-dependent reactions, they can be carried out only under illumination

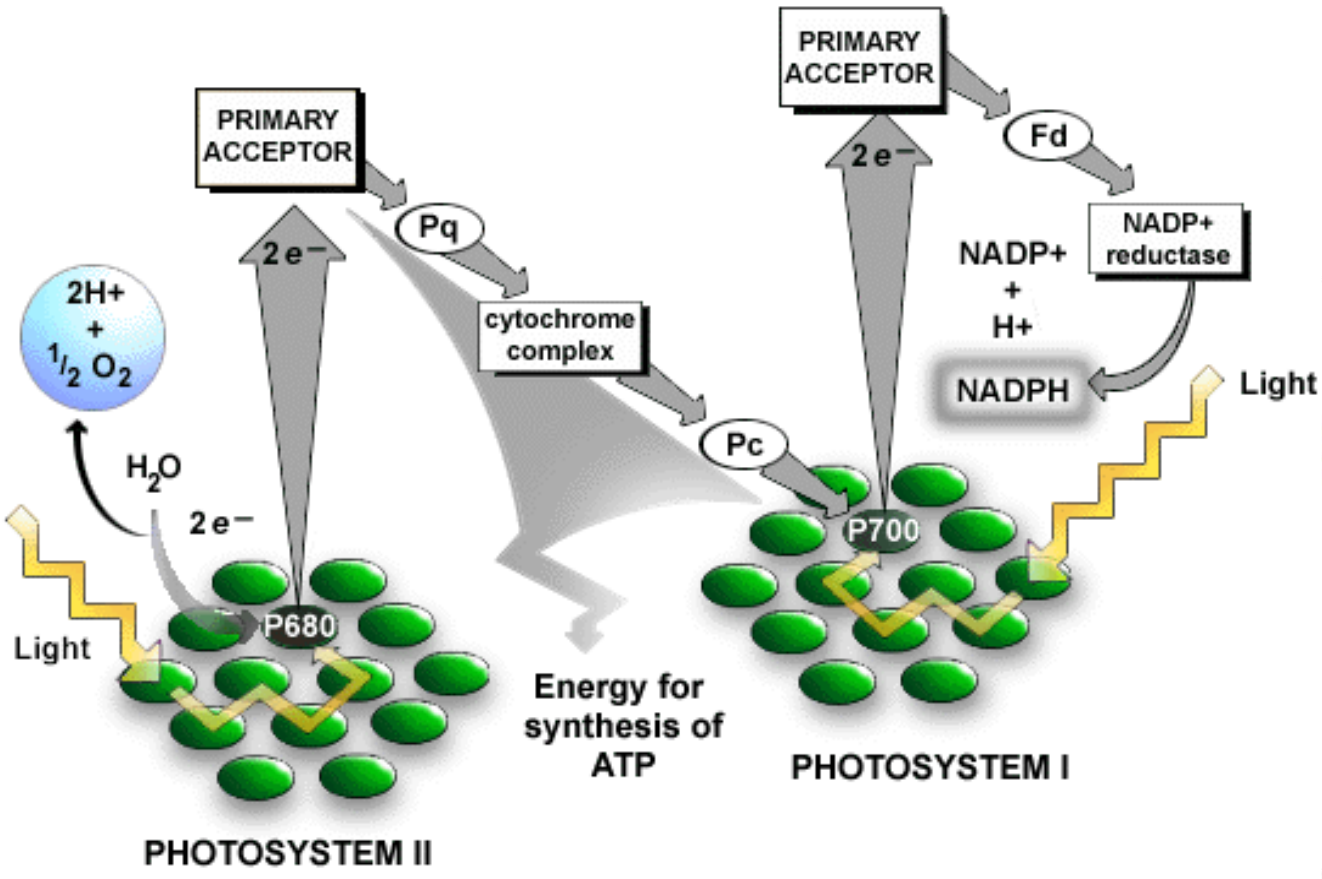
Dark phase is characterized by light-independent reactions, they can be carried out under darkness as well as under illumination.



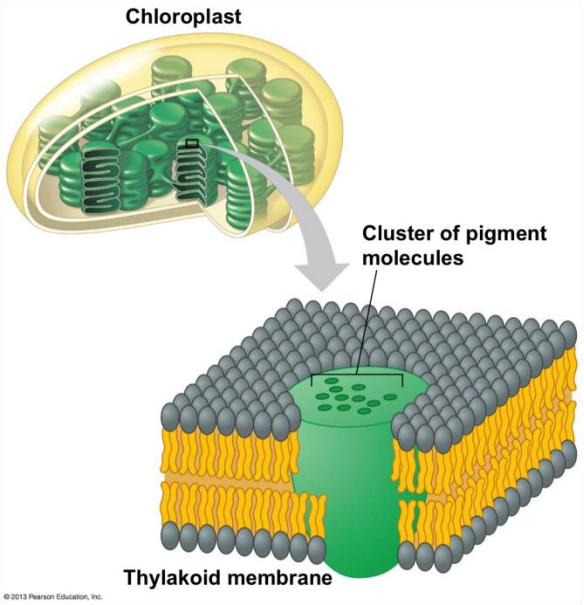
NADPH is the electron carrier of the cell, used in redox reaction.

ATP is the energy coin of the cell, consumed in endergonic reaction (biomass synthesis).

Light phase



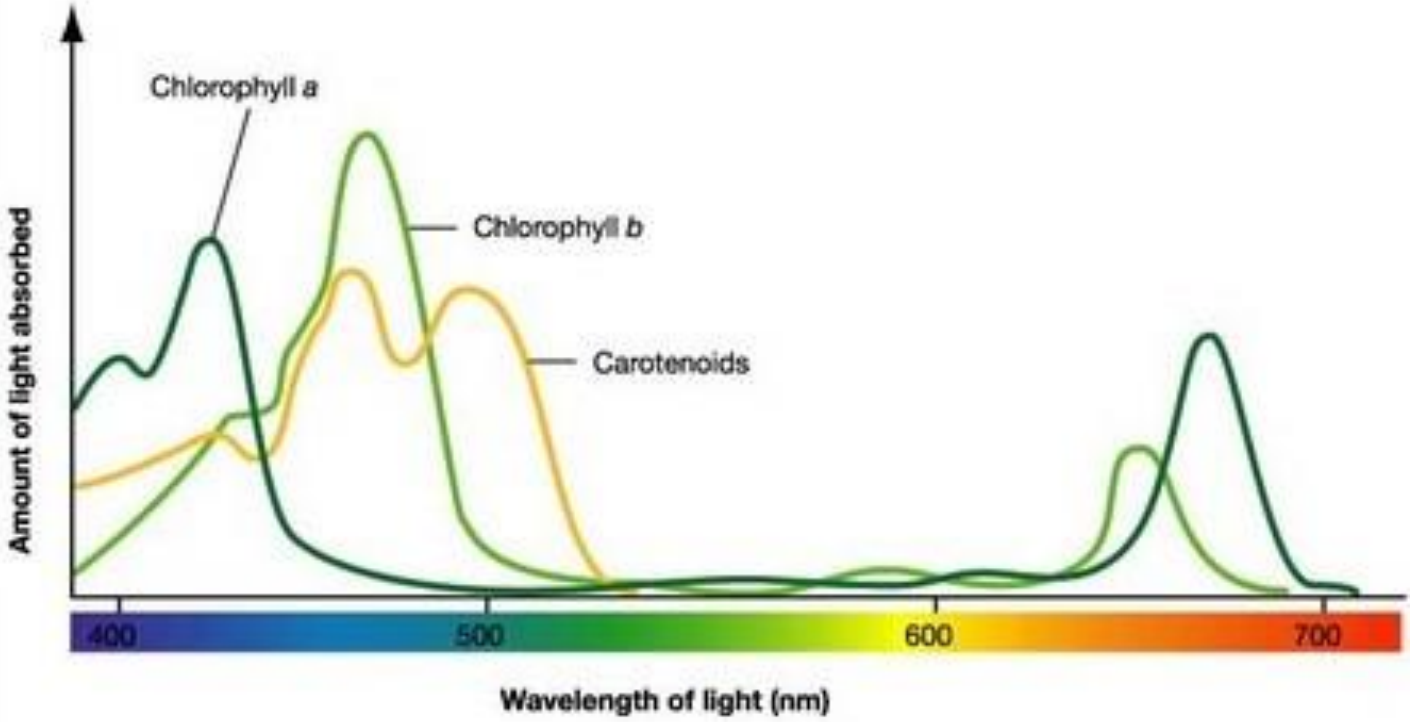
This phase is carried out on the membrane of the thylakoids



Chlorophyll is the green pigment present in photosystems and responsible of light energy harvesting. This energy is used to split water and reduce the primary acceptor. In this phase light energy is converted to chemical energy (ATP and NADPH).

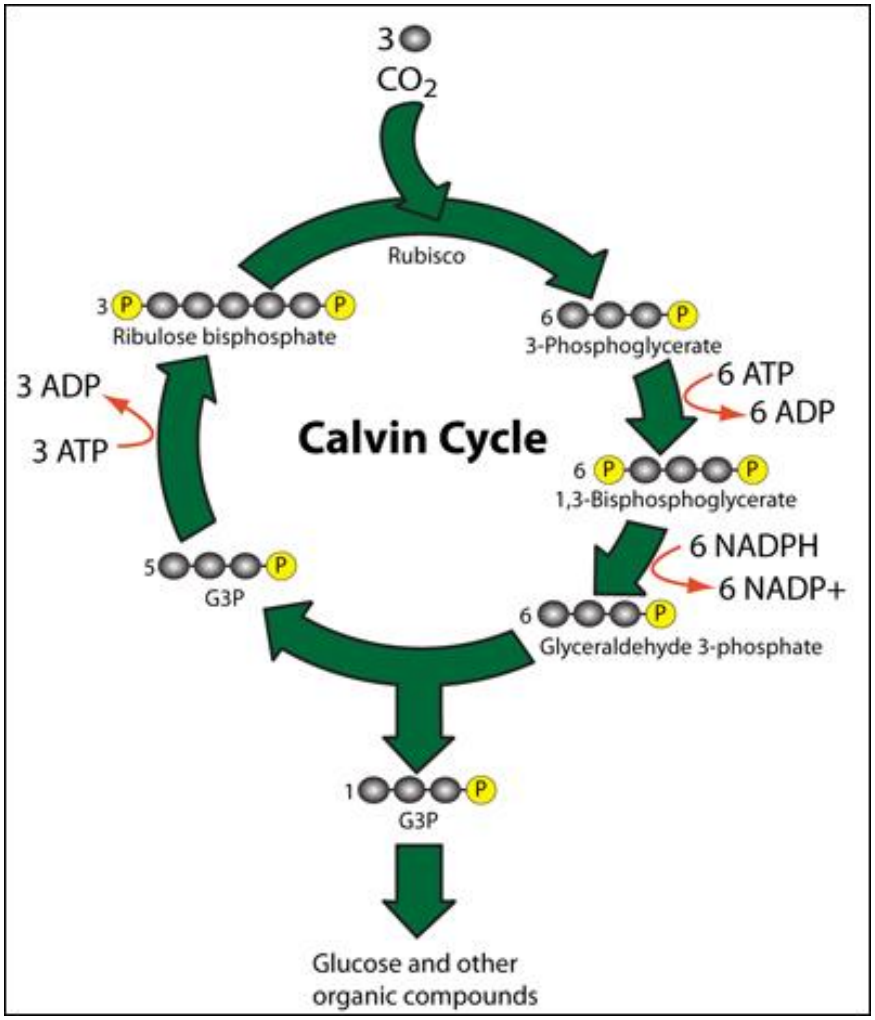
Photosynthetically active radiation (PAR)

Solar energy is not all available for photosynthetic activity.
Photosynthetically active radiation is the amount of light effectively available for photosynthesis. It depends by adsorption spectra of photosynthetic pigments and from light nature.
For any light source it is possible quantify photons effectively available for microalgae. These are generally expressed as μmol/m^2/s or μE/m^2/s. For sunlight each W/m^2 is equivalent to 0.219 μmol/m^2/s.



Green light is not adsorbed by chlorophyll. This is why microalgae and superior plants are green.

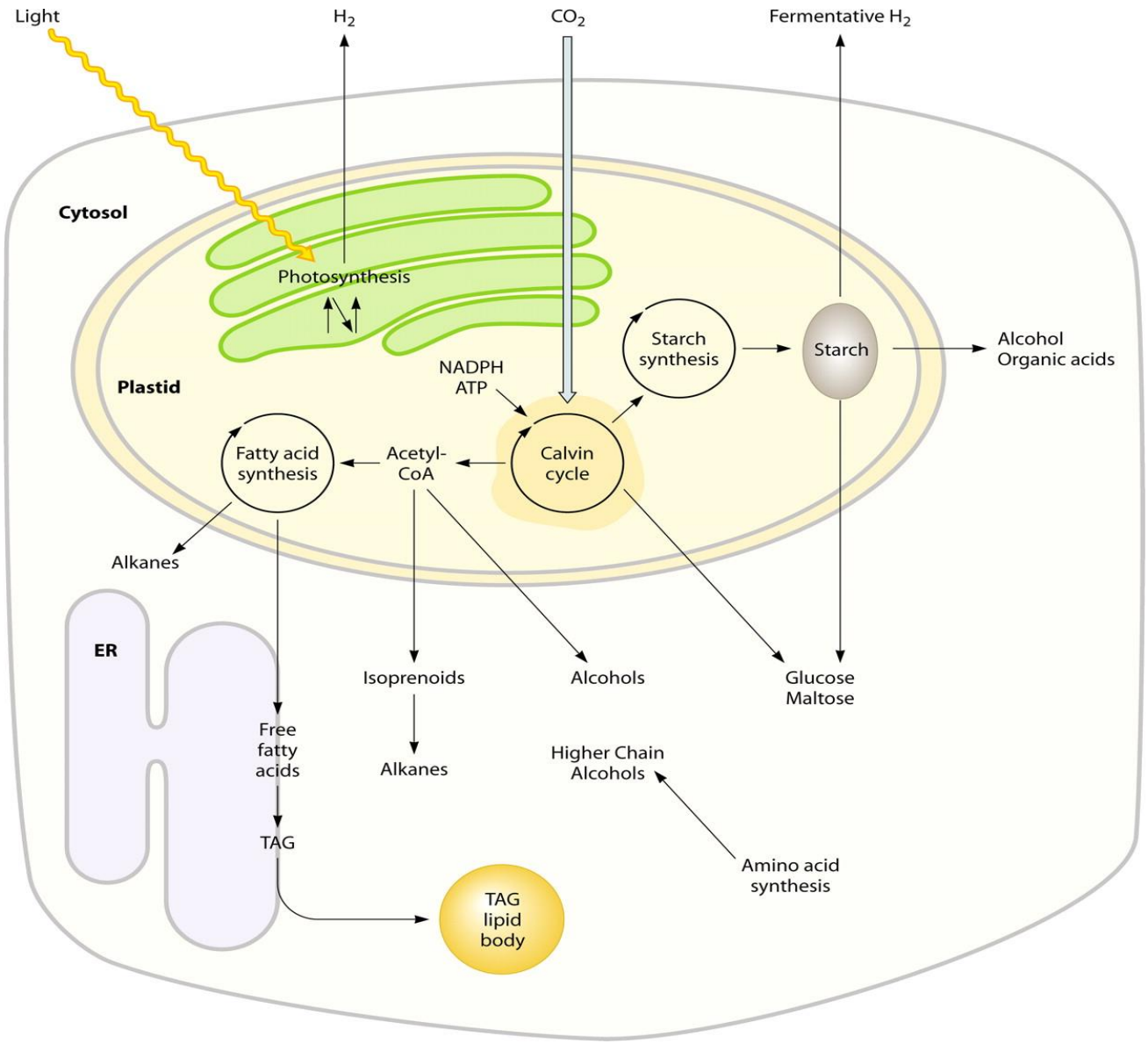
Dark phase (Calvin Cycle)



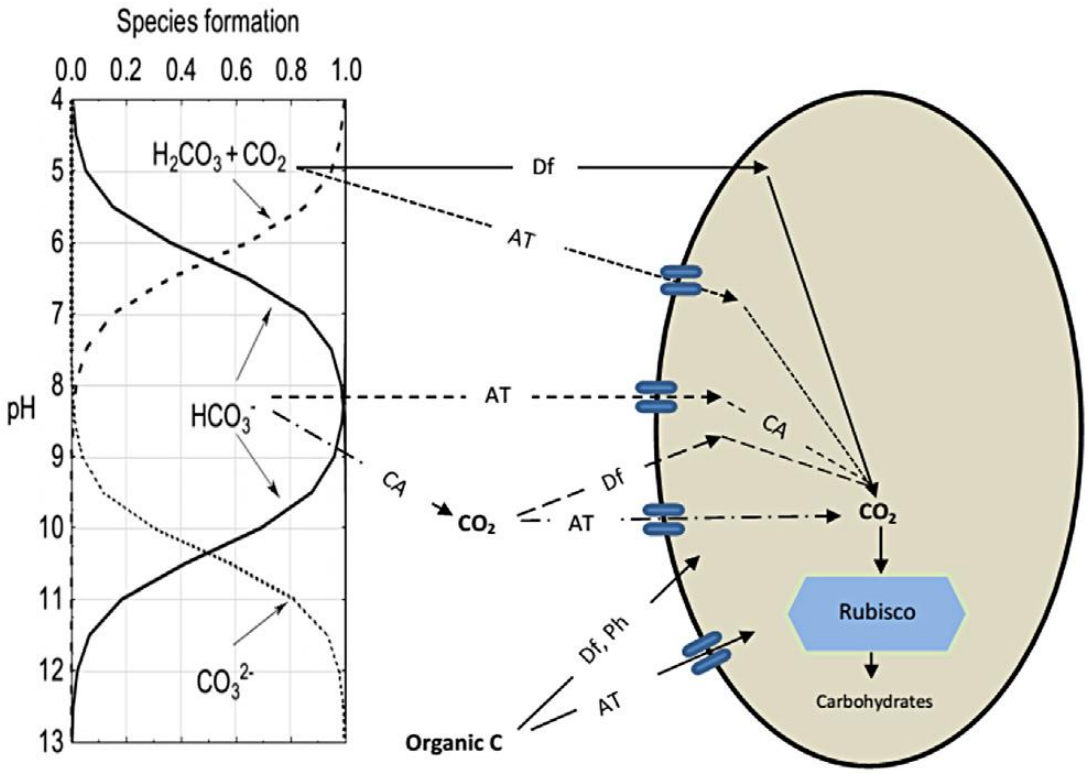
ATP and NADPH produced in light phase are utilized to reduce CO_2 in carbohydrates

RUBISCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is fundamental enzyme responsible of CO_2 fixation in organic molecules. It is present in all photosynthetic organisms.

In eurkaryotic algae dark phase is carried out in the stroma of plastids. In prokaryotic algae it is carried out in cytoplasm.


Light
H_2
CO_2
Fermentative H_2
Cytosol
Photosynthesis
Plastid
NADPH
ATP
Starch synthesis
Starch
Alcohol
Organic acids
Fatty acid synthesis
Acetyl-CoA
Calvin cycle
Alkanes
ER
Isoprenoids
Alcohols
Glucose
Maltose
Free fatty acids
Alkanes
Higher Chain Alcohols
TAG
Amino acid synthesis
TAG lipid body

Inorganic carbon assimilation



CO_2 is not the only inorganic carbon form that can be assimilated by microalgae.

CO_2, HCO_3^- and CO_3^{2-} concentration are function of pH.

All the forms should be converted in CO_2 into the cell because this is the only form usable by Rubisco.

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$
$$H_2CO_3 \leftrightarrow HCO_3^- + H^+$$
$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+$$

Salts requirement

Besides C, H and O, fixed trough photosynthetic activity, there are other essential elements which should be assimilate for microalgal growth.

Main are N, P, K, S, Si, Na, Ca, Mg, Fe, Cl, B, Mn, Cu, Zn, Mo.

Nitrogen

Nitrogen is the third most abundant element in microalgal biomass after carbon and oxygen. Its content ranges from 1% up to 14%. Nitrogen represent about 16% of protein dry weight. It is essential for the synthesis of proteins, nucleic acids (DNA, RNA) and pigments (as chlorophyll).

Microalgae generally take nitrogen from inorganic ions as nitrate and ammonium. Some algae (e.g. Nostoc) are able to fix atmospheric nitrogen (N_2). Also organic molecules as urea and amino acids can be used as nitrogen sources.

Phosphorous

Phosphorus is an important constituent in nucleic acids (DNA, RNA), phospholipids (which make up the membranes), proteins and other important molecules as ATP. Its biomass content varies from 0.05% up to 3.3%.

Generally phosphorous is present in the medium as phosphate salts. In natural environment is often a limiting element for microalgal growth.

Potassium

Along with nitrogen and phosphorus is one of the primary macronutrients for biomass production. Potassium content in some microalgae ranges from 1.2% to 1.5%. It is an activator for a number of enzymes involved in photosynthesis and respiration, it affects protein and carbohydrate synthesis and regulates the osmotic potential of cells. It is present in the medium as K^+ form as phosphate salts, nitrate salts or other salts.

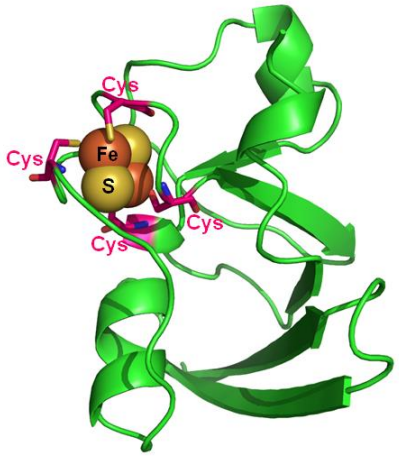
Other nutrients

Sulfur is a constituent of some essential amino acids, vitamins, regulatory compounds and secondary metabolites. It is present in water mainly as sulfate ions, which can be actively taken by microalgae.

Magnesium is a fundamental constituent of chlorophyll. Moreover it is essential for many enzymatic reactions, as, for example, reactions that involved ATP and carbon dioxide fixation.

Calcium is a significant element for microalgal growth, since it is an important constituent of cell walls. It affects also cell division and morphogenesis. It can reach 8% of microalgal dry weight.

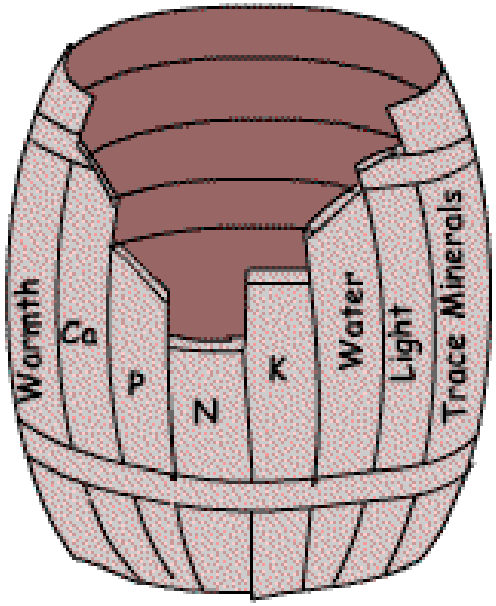
Iron is involved as cofactor in many enzymatic reactions, mainly in redox reaction (e.g. in respiration and photosynthesis).



Ferredoxin

Concept of the limiting factor

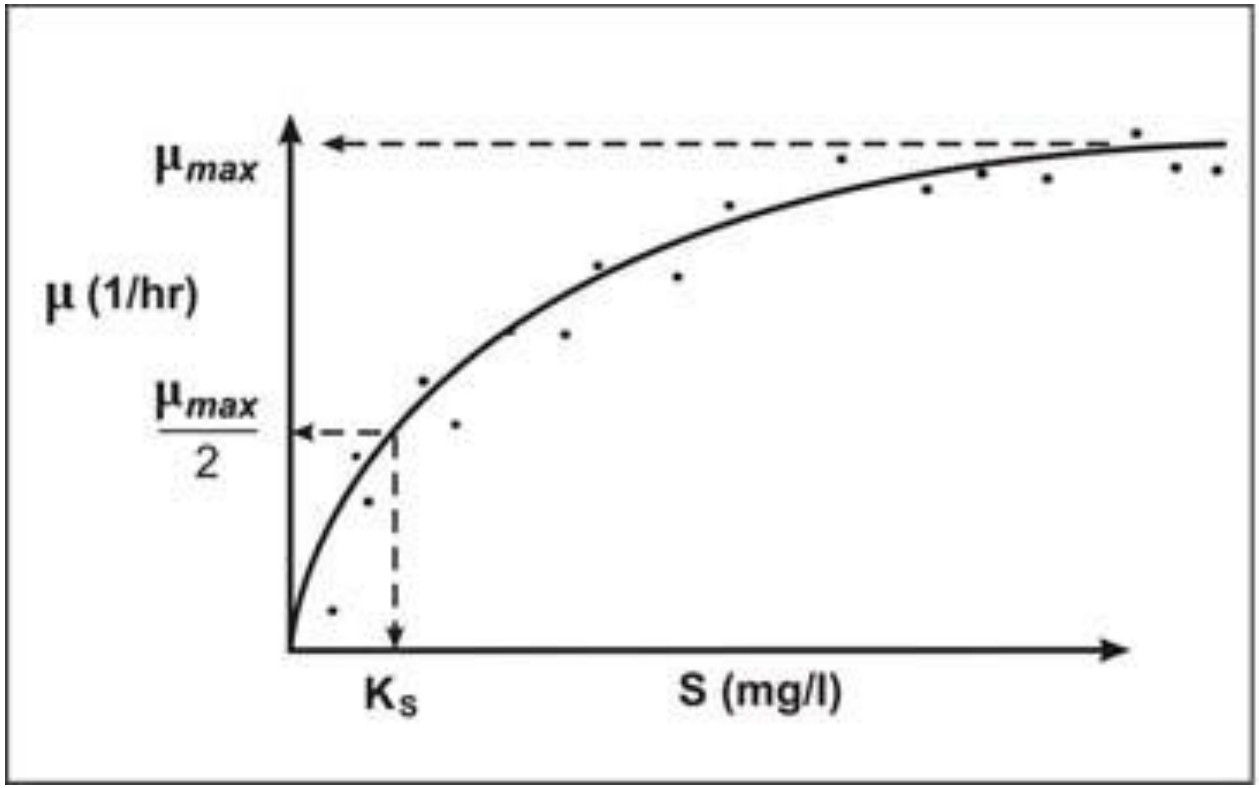
Liebig's law of the minimum: the rate of growth of an organism doesn't depend on the overall nutrient concentration but on the concentration of limiting factor.



Liebig's Barrel Analogy

Limiting factor is not the nutrient at the lesser concentration but the nutrient that determines rate of growth and that a change in its level will result in a change in the growth rate.

Monod Equation



$$\mu = \mu_{max}\left(\frac{S}{S+K_S}\right) \quad (5)$$

$$\frac{dx}{dt} = \mu x$$

S is the limiting substrate

K_s is the half-saturation constant

μ_{max} is the maximum rate of growth

Monod equation is a classic model to describe dependence of μ from [S]. It is generally used to describe light dependence.

Cultivation in photoautotrophic conditions

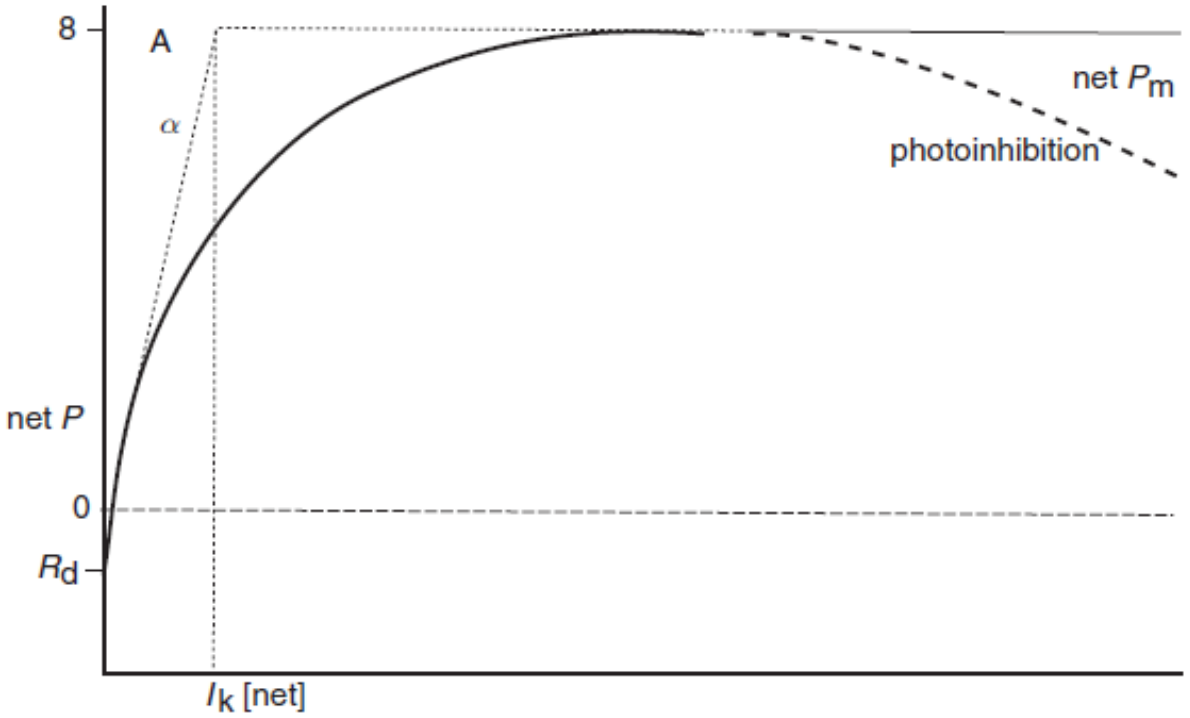
When microalgae are cultivated in photoautotrophic condition the main issue is light supply.

- Light-limited region
- Light-saturated region
- Photoinhibited region

Photosynthesis is measured as CO_2 assimilation or O_2 evolution.

Light absorbed is a small part of incident light.

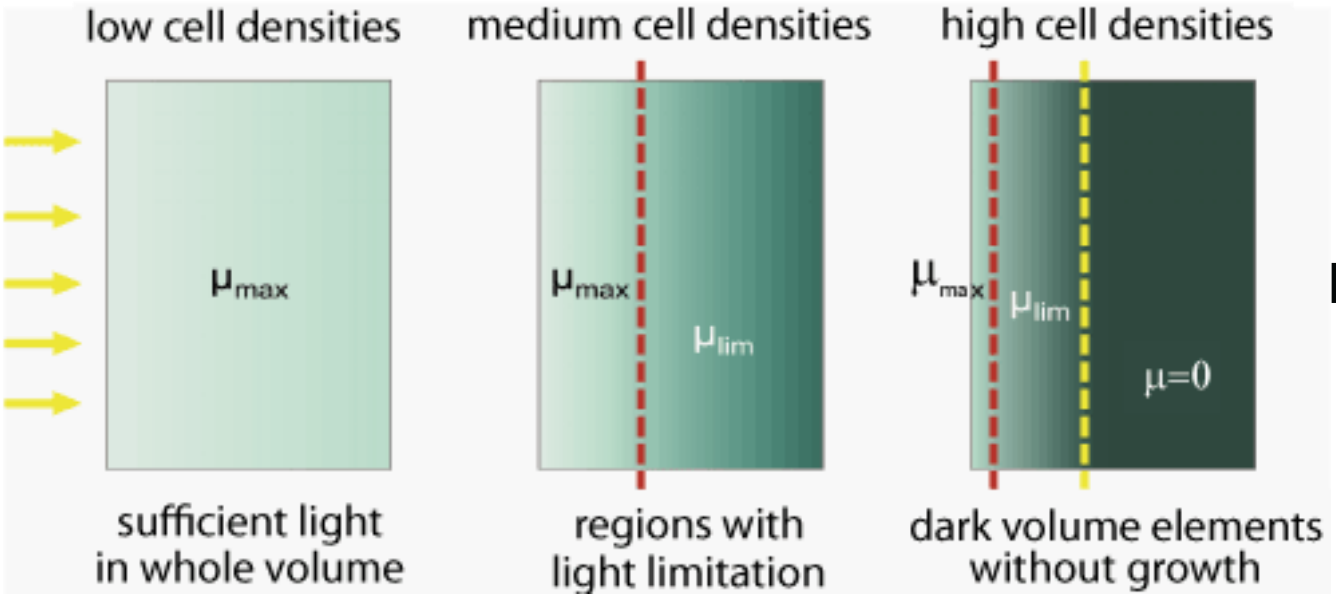
Photosynthesis versus Irradiance



P_{max}: light-saturated rate
α: ratio between photosynthesis and irradiance
I_k: saturation irradiance
R_d: dark respiration

Issues in reactor design

Enhancement of S/V ratio: light is an essential nutrient in photoautotrophic conditions which should be supplied through external surface of the reactors.



$$\mu = \frac{\mu_{max}\, I}{K_I + I}$$

Reactors with high S/V ratio are preferred for cultivation in autotrophic condition. However excessive illumination should be avoided to prevent photoinhibition.

Material selection: plastic materials with **high transparency** are generally chosen to build reactors. Common employed materials are PVC, polycarbonate, polyethylene, polymethyl methacrylate. A good resistance to external agents (as UV irradiation, biodegradation and high salts concentration) is essential for plant **durability**.

Easiness to **cleaning**: reactor surfaces can be dirtied by microbial biofilm formation which reduce light penetration.

Prevention of **contamination** and of **water and nutrient loss**. Water can be lost by evaporation. Nitrogen can be converted in ammonia and volatilize in alkaline pH.

The possibility to **control operative condition** (pH, temperature, turbidity, gas concentration) is a key factor to maintain high productivity performance.

Energy requirements.

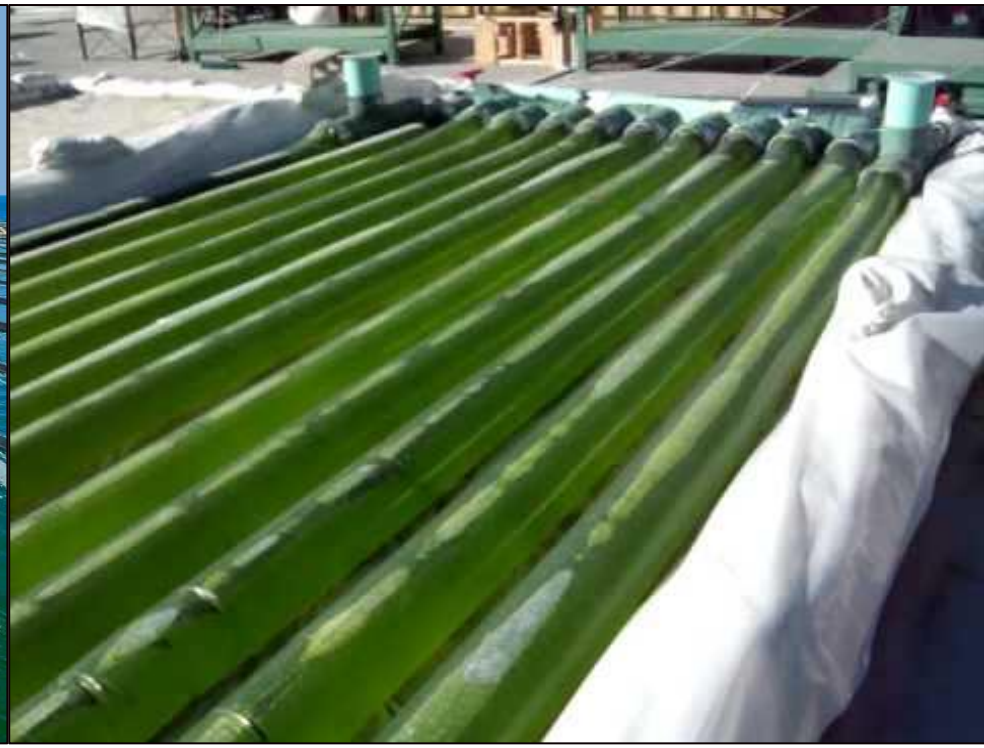
Operative and installation **costs**.

Photoautotrophic cultivation

Mainly two typologies of reactors may be employed for photoautotrophic microalgae cultivation: open ponds and closed photobioreactors.

Open ponds

Closed photobioreactors



Open ponds

Open ponds include natural ponds, as lakes, and artificial ponds.
Raceway ponds are the most commonly used systems. They are generally made of oval shaped channel where growth medium is recirculated. This system is widespread because it is durable, easy and economic to build up and to operate.

Cyanotech, Kona, Hawaii. Production of astaxanthin and Spirulina.

Deep: 0.2-0.3 m.

Water is recirculated by paddlewheel.

Also if surface can exchange gas with atmosphere, additionally submerged aerators may be used.

Walls and bottom can be made of sand, clay, cement or plastic.

Advantages and limitations of open ponds

Advantages

- Relatively cheap.
- Easy to clean.
- Non-agricultural land required.
- Low energy inputs.
- Low S/V ratio.

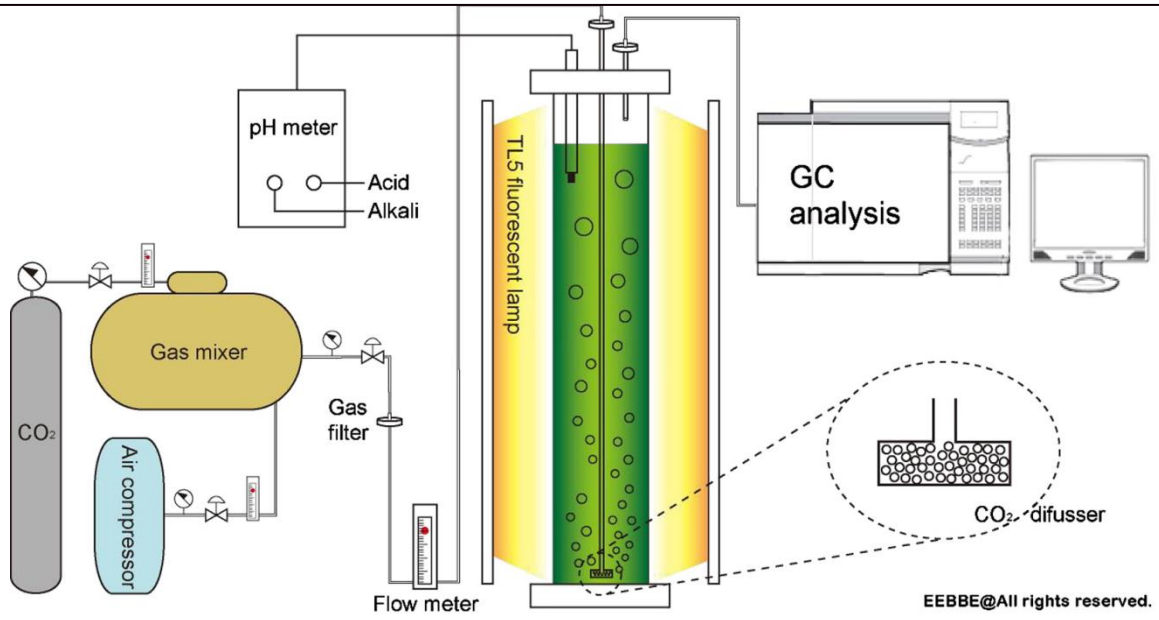
Limitations

- Low volumetric and per unit area productivity.
- Very susceptible to external contamination.
- Poor mixing, low light penetration and low CO_2 concentration.
- Water loss by evaporation.
- Low biomass concentration reached

In order to limit contamination is necessary to operate in very selective conditions. For example high pH, high temperature, high salt concentration.

Closed photobioreactors

Photobioreactors (PBR) can be defined as culture systems for photoautotrophs in which a great proportion of the light (>90%) does not impinge directly on the culture surface, but has to pass through the transparent reactor's walls to reach the cultivated cells. Consequently, PBR do not allow, or strongly limit, direct exchange of gases and contaminants (dust, microorganisms, etc.) between the culture and the atmosphere.



According to morphology PBR can be divided in: flat panel and tubular reactor; manifold or serpentine; horizontal, vertical, inclined or in spiral arrangement.

Tubular serpentine photobioreactors

Serpentine PBR are systems in which several straight transparent tubes are connected in series by U-bends to form a flat loop.

They can be arranged vertically or horizontally.

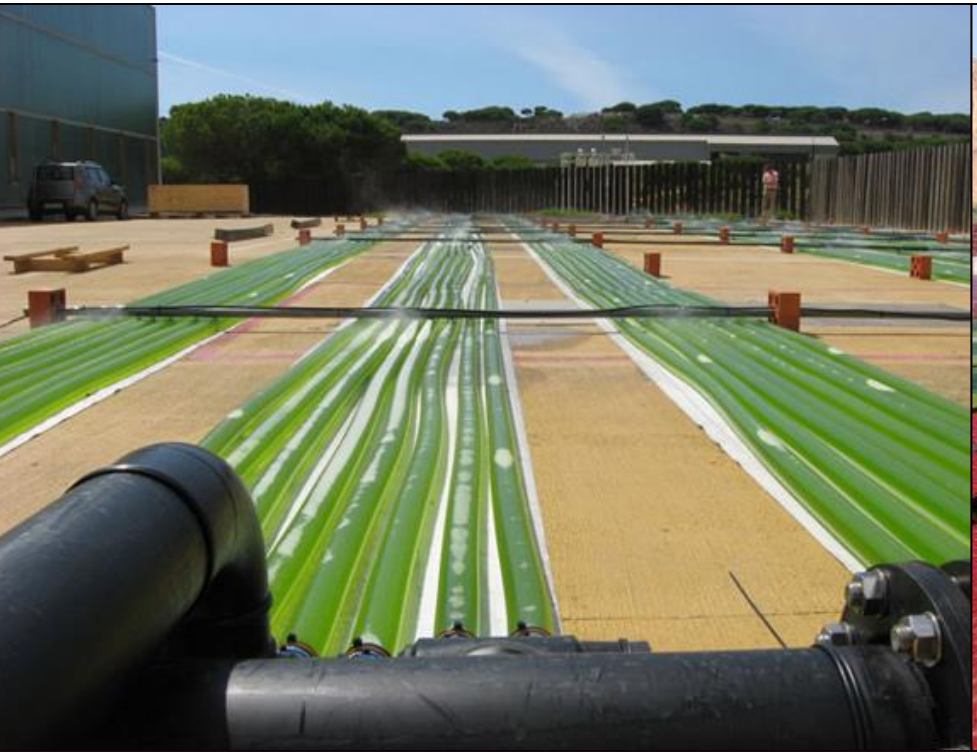
Gas exchange, temperature control, nutrient addition and harvesting are carried out in a separate section.

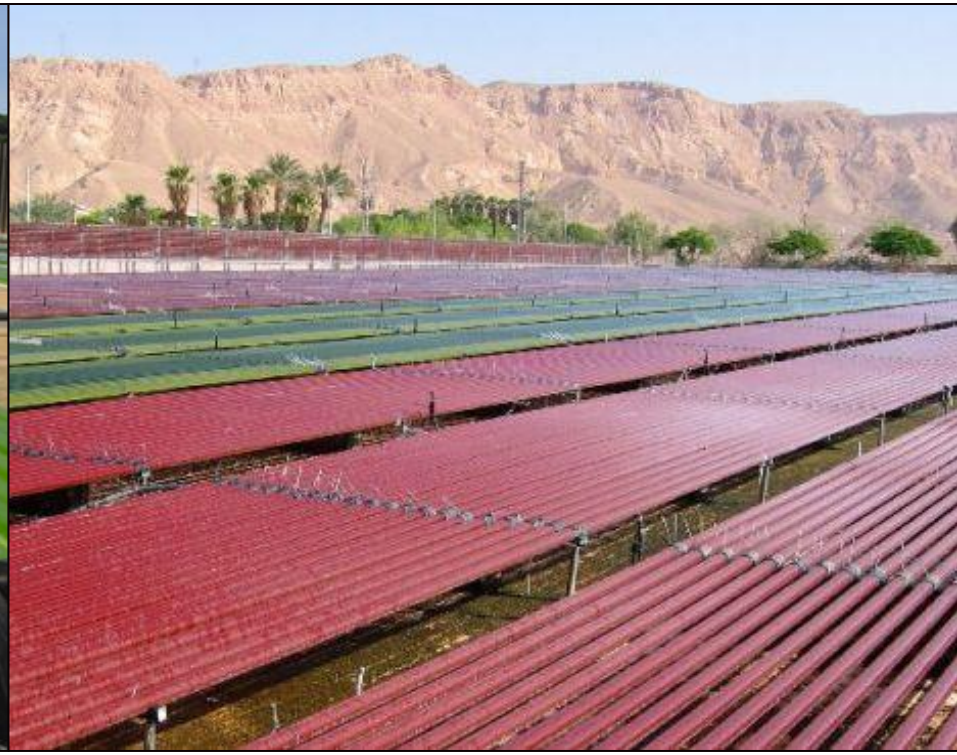
DURAN® glass tubing for the construction of photo-bioreactors (PBR).

Tubular manifold photobioreactors

In manifold PBR, a series of parallel tubes are connected at the ends by two manifolds, one for distribution and one for collection of the culture suspension.

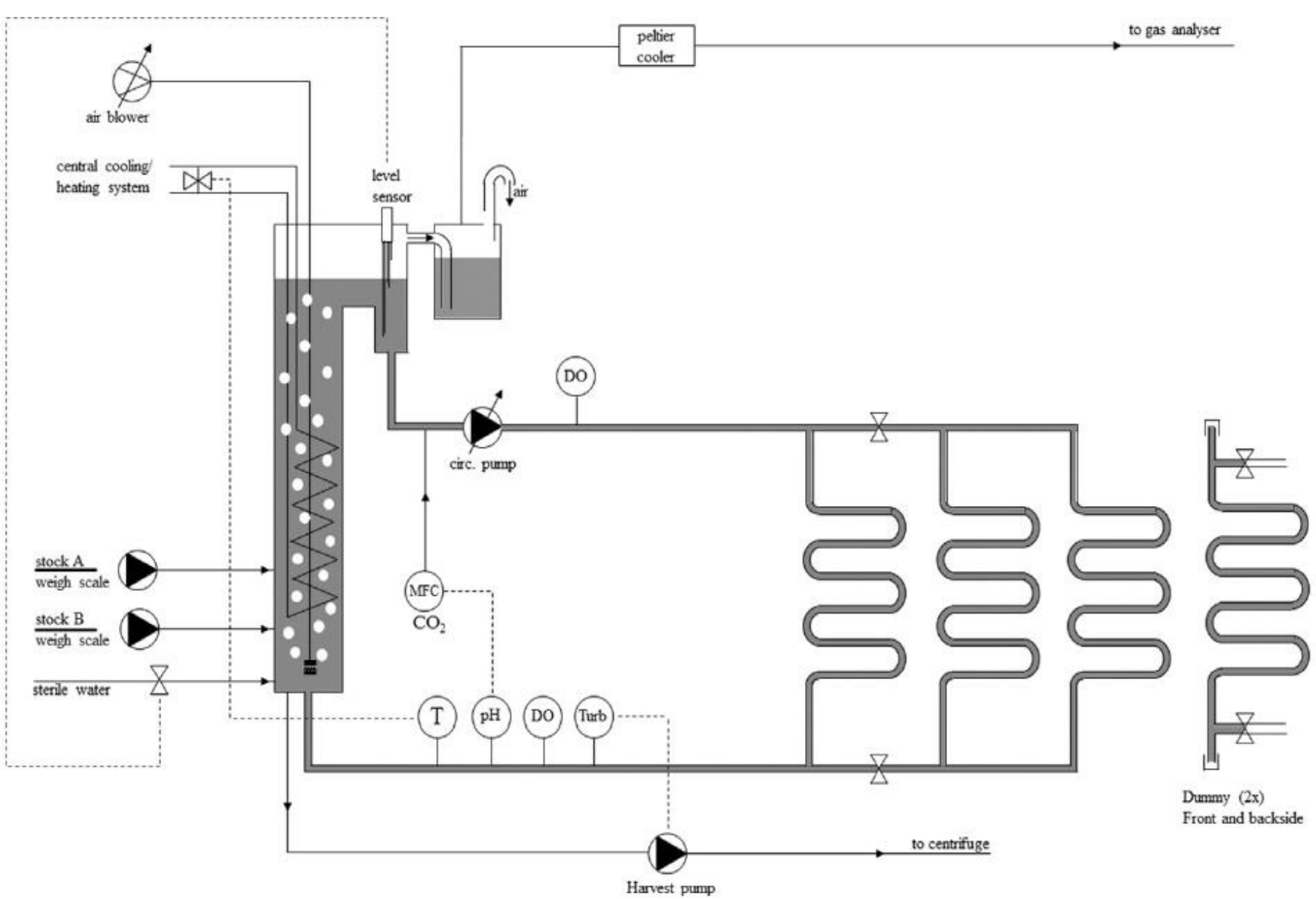
Absence of bends allows to reduce energy consumption to move the culture medium.





Tubular elical photobioreactors (bio-coil PBR)

Helical PBR consist of small-diameter, generally flexible tubes wound around an upright structure.


peltier cooler
to gas analyser
air blower
central cooling/ heating system
level sensor
air
DO
circ. pump
stock A weigh scale
stock B weigh scale
sterile water
MFC
CO_2
T
pH
DO
Turb
Dummy (2x) Front and backside
to centrifuge
Harvest pump

Oxygen accumulation

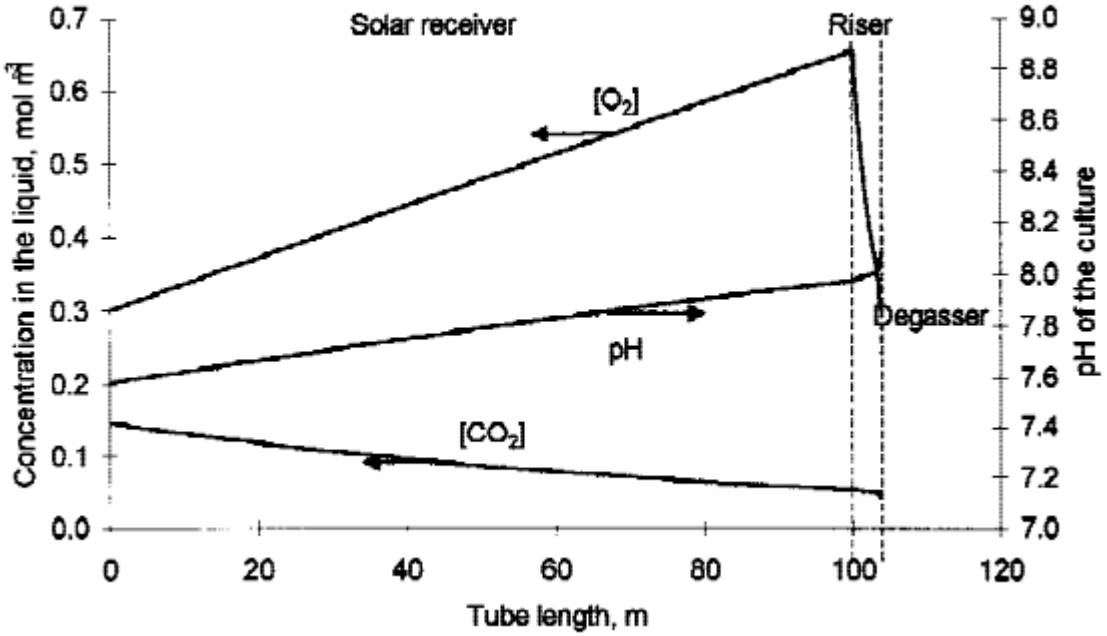
Closed tubular reactors are characterized by long residence time of the gas. This leads to an oxygen accumulation. Increment in oxygen concentration reduces photosynthesis efficiency because O_2 is a competitive inhibitor for RUBISCO.

When RUBISCO binds O_2 instead CO_2 it works as oxygenase catalyzing the reaction of O_2 with ribulose bisphosphate to form phosphoglycolate. This process is named **photorespiration**. Rate of photorespiration depend by O_2/CO_2 ratio.

Typical K_m value:

K_O = 200-600 µM
K_C = 1-20 µM

For this reason oxygen should be stripped at the end of the reactor.



Advantages and limitations of tubular photobioreactors

Advantages

- Large illumination surface area.
- Suitable for outdoor cultures.
- Good biomass productivity.
- Good parameters control.

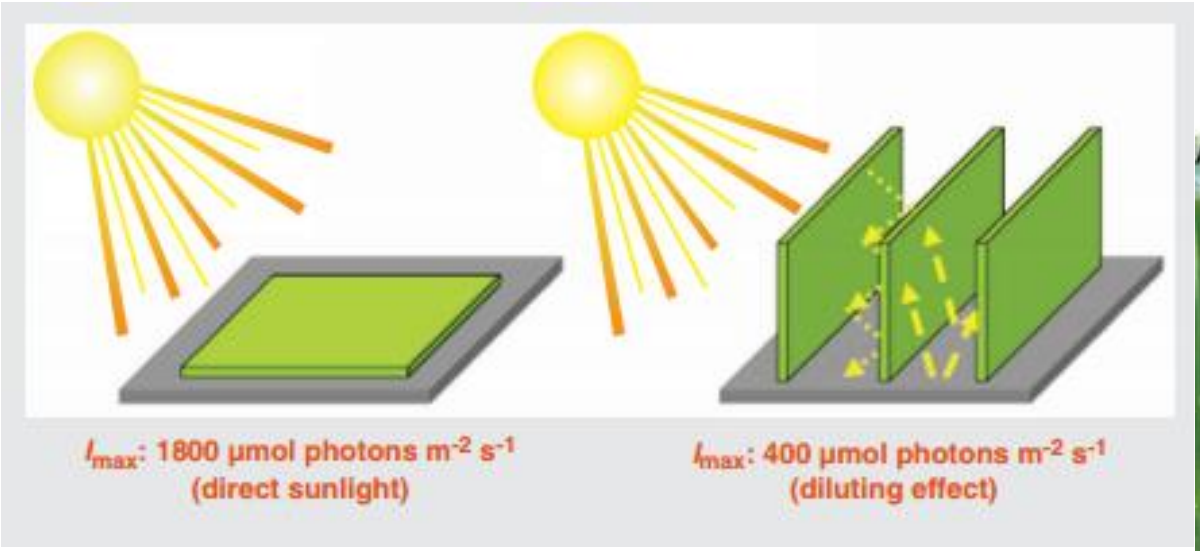
Limitations

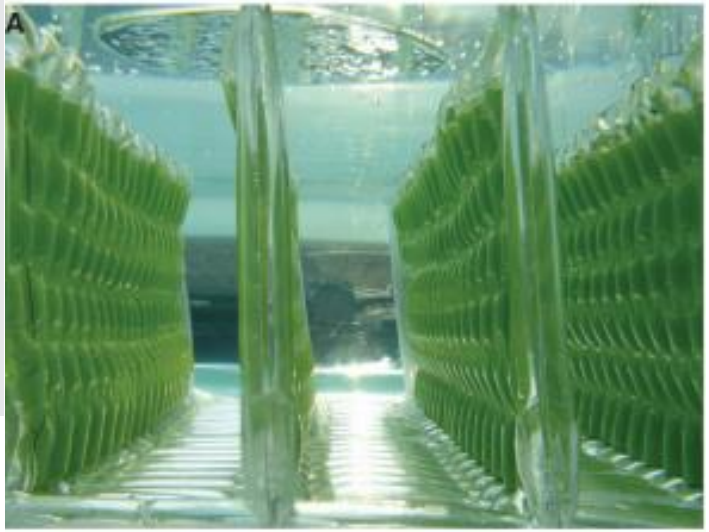
- High costs.
- High energy consumption.
- Fouling and wall growth.
- Large space required.
- Gradient of pH, dissolved oxygen and CO_2 along the tubes.

Flat panel photobioreactors

The small thickness of these reactors allows to reach high productivity and biomass concentration. High photosynthetic efficiencies can be achieved.

Oxygen accumulation is strongly reduced.

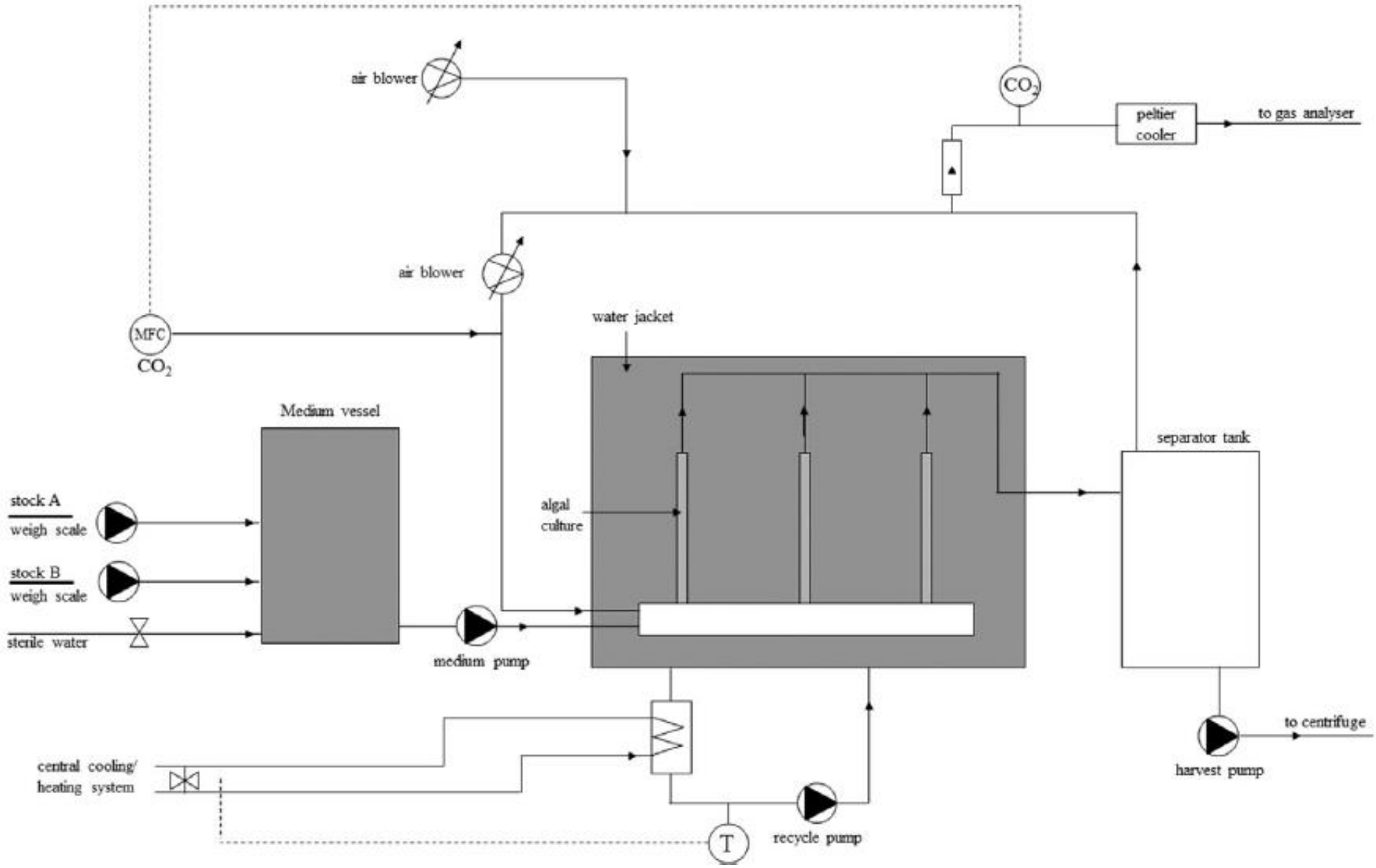




Light dilution is helpful to reduce photoinibition. It is a low cost system to obtain high photosynthetic efficiencies under bright sunlight.

Reactors submerged in large water volumes allow temperature control by low energy requirements.

Plastic bags can be used to build up low cost reactors, however polymers employed, as polyethylene, generally have short lifetime (1 year).


air blower
CO_2
peltier cooler
to gas analyser
air blower
water jacket
MFC
CO_2
Medium vessel
separator tank
stock A
weigh scale
algal culture
stock B
weigh scale
sterile water
medium pump
to centrifuge
central cooling/ heating system
harvest pump
T
recycle pump

Advantages and limitations of flat panel photobioreactors

Advantages

- High biomass productivity.
- Easy to sterilize.
- Low oxygen accumulation.
- Good light path.
- Large illumination surface area.
- Suitable for outdoors cultures.

Limitations

- Difficult scale up.
- Difficult temperature control.
- Fouling and wall growth.
- Cost and energy consumption.

Bubble column photobioreactors

Bubble column photobioreactors are built by vertical columns fed at the bottom with inlet gas. In some cases they are also internal illuminated through lamps or optical fibers.

Regulation of metabolism

The metabolism is the combination of all chemical cellular reactions. These reactions are essential to satisfy energy and material requirements of the cell.

In their natural environment microalgae, as any living organism, are exposed to continuous changes (e.g. change in pH, temperature, nutrient concentration, etc...). Consequently metabolism should be regulate in response to these changes to allow microalgal life and growth.

Regulation systems of the microalgae are the result of million of years of evolution.

For any microalgal strain optimal growth conditions are generally close to average environmental conditions of relative natural environment.

There are many ways by which cellular metabolism is regulated.

These can be summarized in control of gene expression and in control of enzymes activity.

Stress condition

Microalgae enter in a stress condition when changes in environmental conditions result in a metabolic imbalance that require biochemical and metabolic adjustments before a new steady state of growth can be established.

Environmental change ➡ Sensing mechanism ➡

Response mechanism adaptation ➡ New steady state

Environmental changes can take place at different timescale, for example:

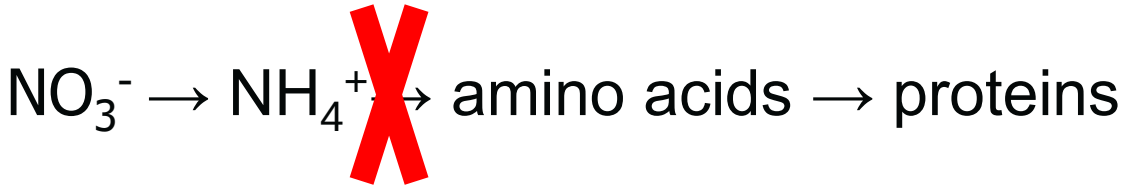
Circadian cycle: which include variation of light and temperature in 24h.

Seasonal cycle: which include variation in months.

Light-dark cycle: which include light variation in terms of fraction of seconds. It is common in reactors with a dense microalgal concentration and depends by culture mixing.

Nitrogen starvation

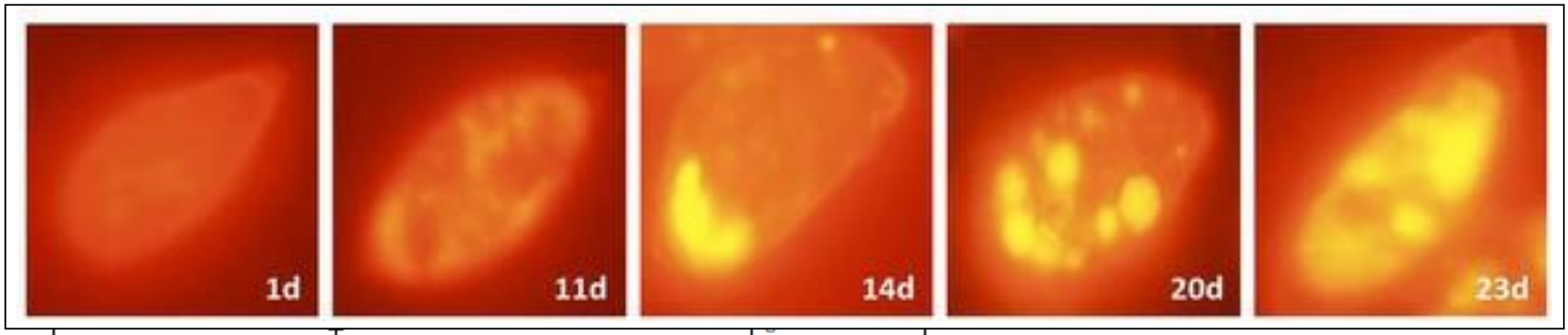
When nitrogen sources in the medium are depleted microalgae get in nitrogen starvation conditions. In this condition protein synthesis is inhibited because nitrogen for amino acids and DNA synthesis is missing.

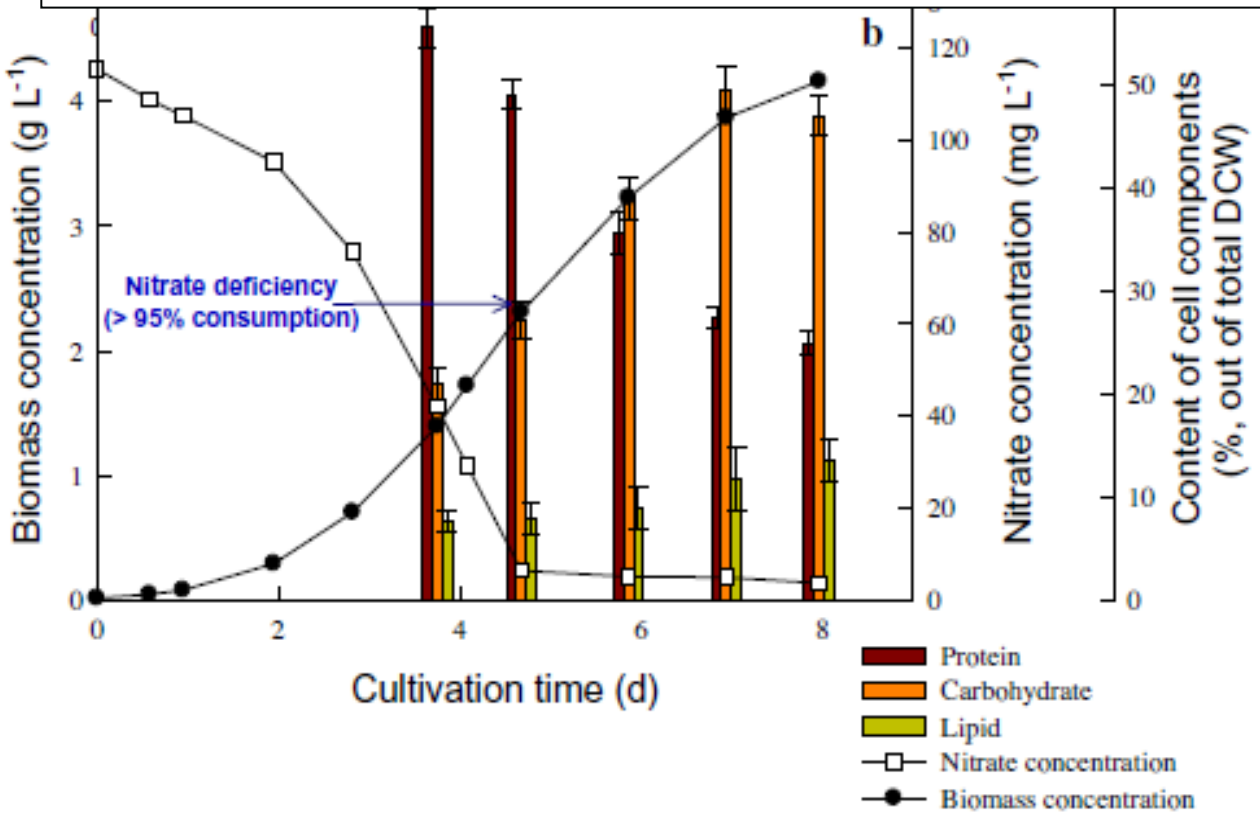


In these conditions microalgae can not use energy to replicate themselves and produce new cells, so energy is stored as reserve materials. Mainly reserve materials in microalgae are lipids (triglycerides) and starch. Some cyanobacteria store polyhydroxyalkanoates (PHA).

Under nitrogen starvation lipids can reach 85% of dry weight while starch can reach 60% of dry weight.

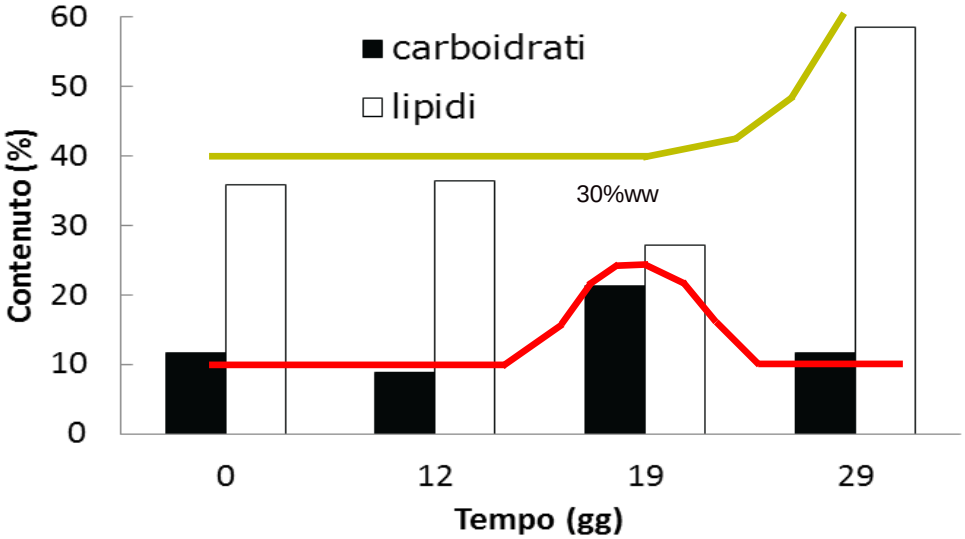
Phosphorous starvation leads to similar effects.

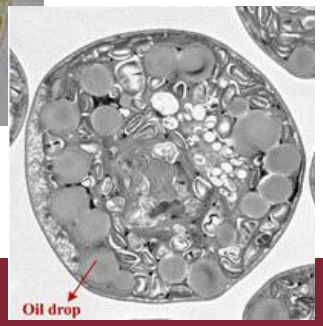


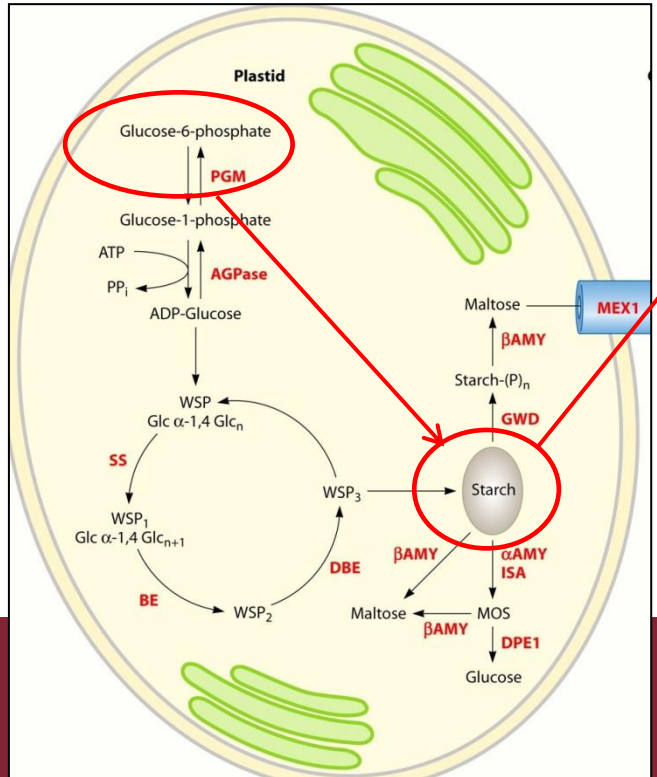


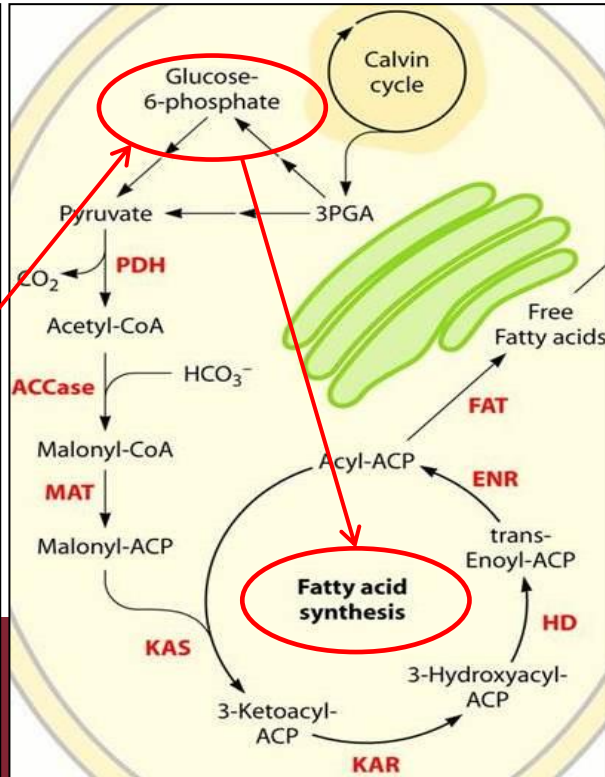
Increase in lipids stored in a strain of *Scenedesmus sp*. during different times of nitrogen starvation. Lipids are stained by adding Nile Red, a lipophilic stain visible through fluorescent microscopy.

Produzione di amido in coltivazione fototrofa outdoor





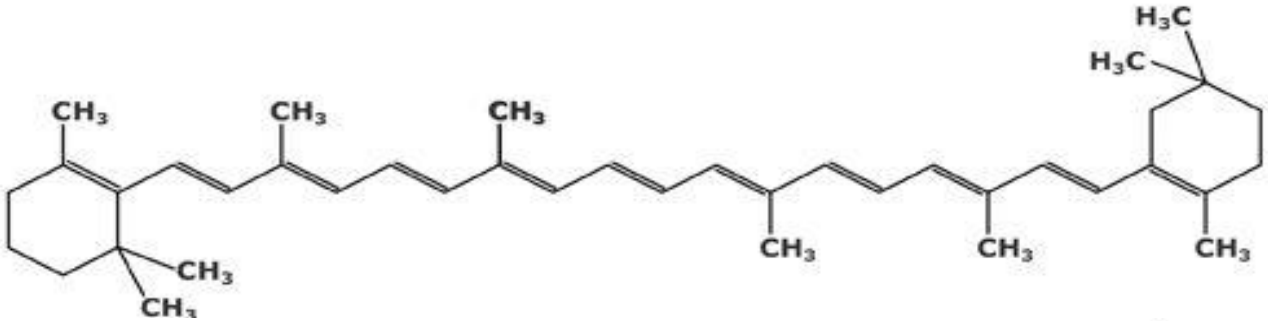




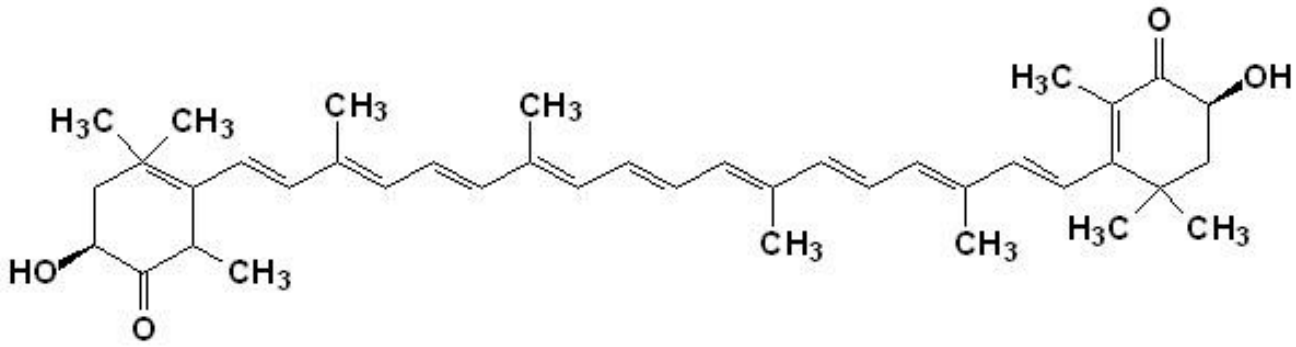
Carotenoid production

Carotenoids are yellow, orange or red lipophilic pigments with 40 carbon structures. All algae contain carotenoids with a greater variety then higher plants. Some carotenoids occur in most algal classes while other are restricted to only few classes. Average carotenoids concentration can change from very small content as 0.1% to high amount ad, for example, 14% in *Dunaniella* (β-carotene).

They are divided into two main groups, the carotenes (non-oxygenated molecules) and the xanthophylls (oxygenated molecules).



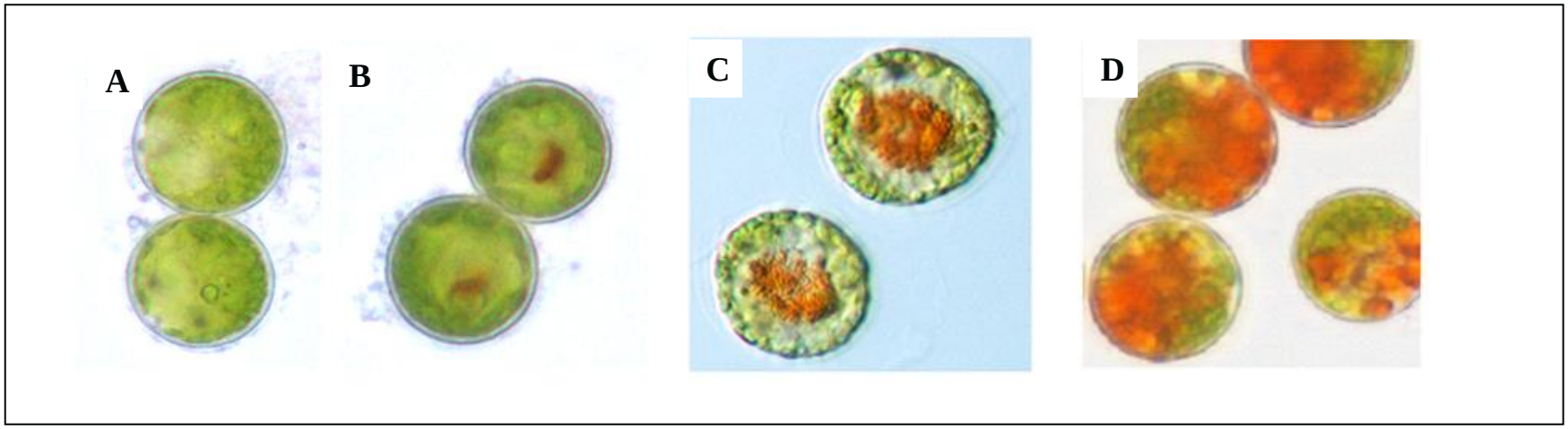
β-carotene



Astaxanthin

The main role of carotenoids are **photoprotection**, light harvesting, excess energy dissipation and structure stabilization.
When microalgae are subjected to high irradiance generally show relatively high carotenoid content. Carotenoids do not transfer excitation energy to reaction center and act as a screen to protect reaction center from excessive excitation.
They have strong **antioxidant** properties.

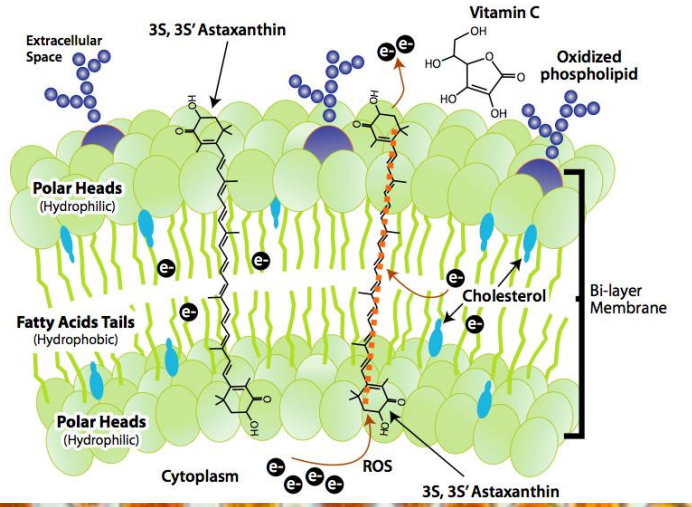
Dunaniella salina can store until 14% of β-carotene dry weight. *Haematococcus pluvialis* until 4% of Astaxanthin.



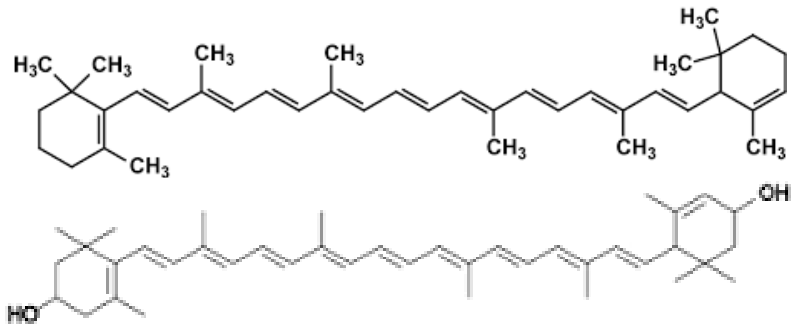
Increase in astaxanthin content in *Haematococcus pluvialis* under increasing jasmonic acid content expositions.

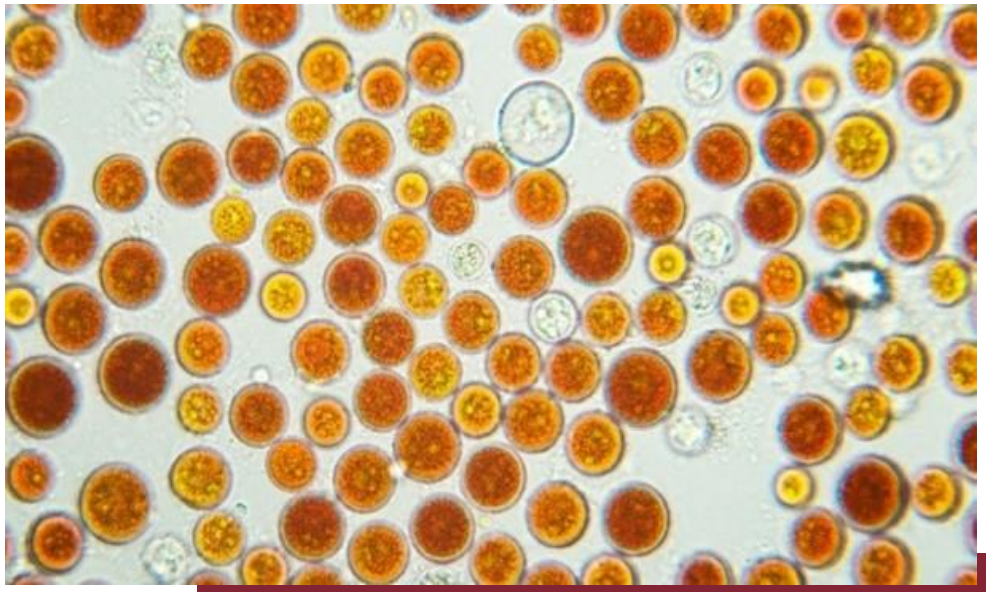
Microalgae: aspetti metabolici

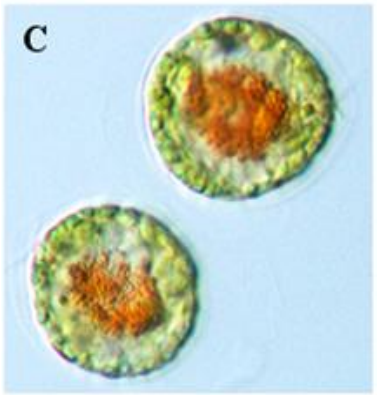
Stress ossidativi

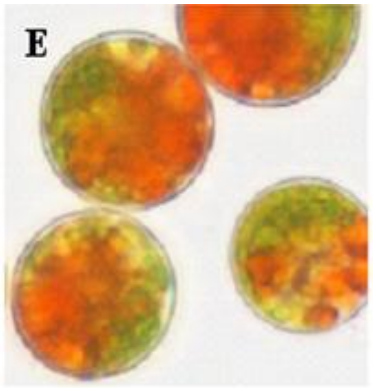


carotenes (non-oxygenated molecules)









Increase in astaxanthin content in *Haematococcus pluvialis* under increasing jasmonic acid content expositions.

Mixotrophic and heterotrophic metabolism

Not all microalgae are obligate autotrophs, many species are able to use also organic compounds as energy source and carbon source. These species can growth in mixotrophic and heterotrophic condition.

	Autotroph	Mixotrophic	Heterotrophic
Carbon source	Inorganic	Inorganic + organic	Organic
Energy source	Light	Light + $C_{organic}$	$C_{organic}$

The catabolism of organic carbon allow to produce energy by fermentation or respiration metabolism.

Organic substrates

Main organic substrates usable for mixotrophic and heterotrophic metabolism are: **glucose**, **glycerol**, **acetic acid, lactic acid, glutamate, alanine, asparagine**.

These molecules are readily available because they take part in important biochemical pathways. When organic substrates are in the medium metabolic changes occur in the cell allowing them usage.
For example when glucose is in the medium it induces the synthesis of glucose transport protein. The process is energy dependent.

Organic acid are more available at acid pH because the protonated form is more soluble in the cell membrane.

Amino acids can be used both as carbon and nitrogen source.

Some microalgae species are able to synthetize extracellular enzymes able to breakdown polysaccharides and proteins in simple available monomers as sugars and amino acids.

Heterotrophic growth

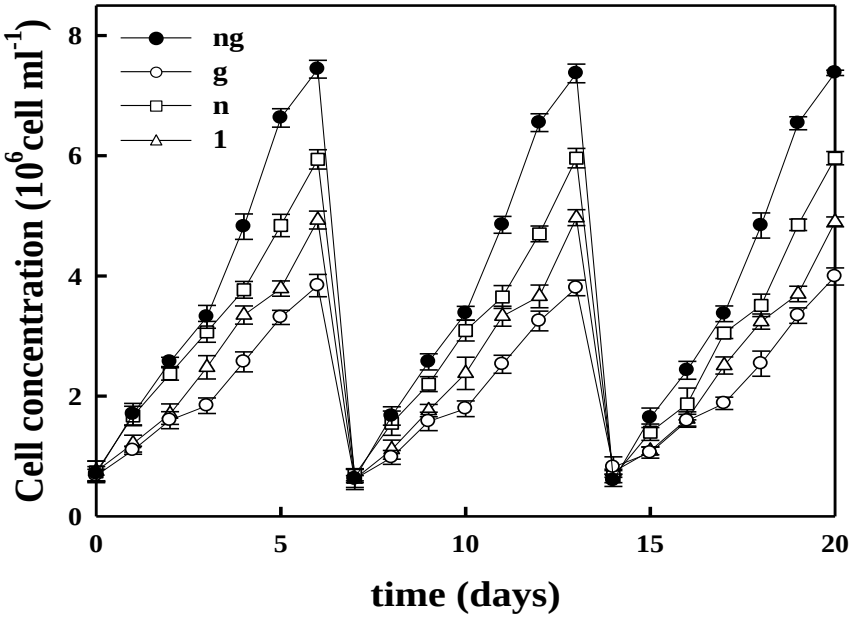
In heterotrophic conditions microalgae can grow also without light exposition and can reach higher biomass concentration than light-dependent growth. In these conditions microalgae can reach a biomass concentration of 45 g/L and a biomass productivity of 20 g/L/d. In photoautotrophic conditions maximum biomass concentration and biomass productivity which may be achieved are generally 3-4 g/L and 0.5-1 g/L/d respectively.

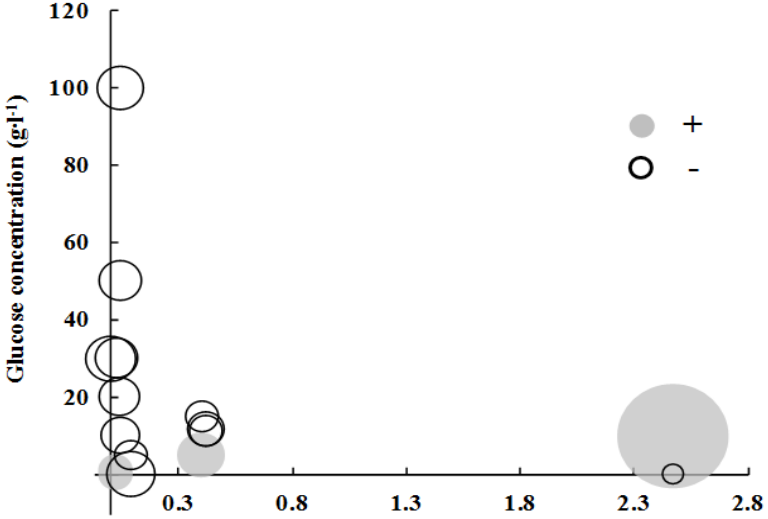
Some cyanobacteria are not able to assimilate organic carbon substrates.
Some microalgae have low affinity to organic substrates and as result they have low growth rate in heterotrophic culture.
Other microalgae species, as *Chlorella vulgaris*, have heterotrophic growth rate values comparable to values achieved in phototrophic condition.

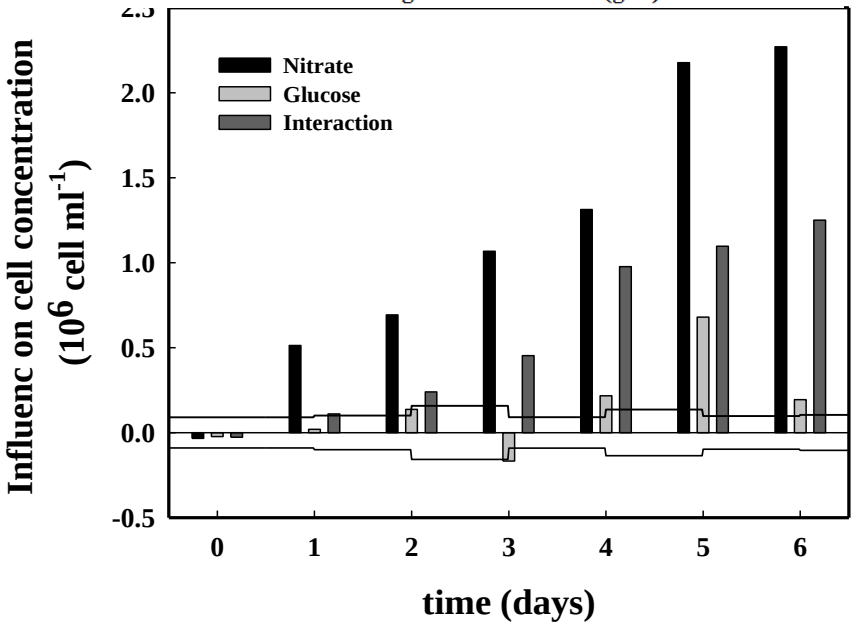
Organic substrates in high concentrations may inhibit microalgae growth. For example *Chlamydomanas reinhardtii* is inhibited by acetate concentration higher than 0.4 g/L.

Crescita eterotrofa

Inibizione da glucosio







Pagnanelli F., Altimari P., Trabucco F., Toro L. (2014) J. Chem. Technol. Biotechnol. 89(5), 652–661,

Mixotrophic growth

Mixotrophy is defined as a growth regime in which CO_2 and organic carbon are simultaneously assimilated, both respiratory and photosynthetic metabolism operating concurrently. The specific growth rate of the mixotrophic culture is approximately the sum of the specific growth rates of cells grown under photoautotrophic and heterotrophic conditions. In some microalgal strains there is a photoihnibition of organic carbon uptake. Only those algal strains which are not sensitive to this photoinhibition are suitable for mixotrophic cultivation.

In mixotrophic cultivation a fraction of light may be used to assimilate organic carbon increasing energetic efficiency and reducing amount of energy dissipated.

Exogenous organic carbon enhances respiration both in light and dark. CO_2 produced by respiration can be re-utilized by photosynthetic activity.

Biotechnology applications

The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine. However the cultivation of algae is only few decade old. In the early 1950's, the increase in the world's population and predictions of an insufficient protein supply led to a search for new alternative and unconventional protein sources. Algal biomass appeared at that time as a good candidate for this purpose.

Interest in applied algal culture continued with studies of the use of algae as photosynthetic gas exchangers for space travel.

The use of microalgae for generating renewable energy sources provoked heightened interest during the energy crisis in the 1970's.

Commercial large-scale culture started in the early 1960's in Japan with the culture of *Chlorella* by Nihon Chlorella (Taiwan). It was followed in the early 1970's by the establishment of an *Arthrospira* harvesting and culturing facility in Lake Texcoco by Sosa Texcoco S.A. (Mexico City). By 1980, there were 46 large-scale factories in Asia producing more than 1000 kg of microalgae (mainly *Chlorella*) per month.

In a short period of about 30 years, the microalgal biotechnology industry has grown and diversified significantly.

In 2006 the microalgal biomass market was estimated in about 5000 t of dry matter/year with a turnover of approximately US$ 1.25×10^9/year.

Human nutrition

Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules and liquids. They can also be incorporated into pastas, snack foods, candy bars or gums, and beverages.
They can act as a nutritional supplement or represent a source of natural food colorants.

TABLE 1. General composition of different human food sources and algae (% of dry matter) (3)

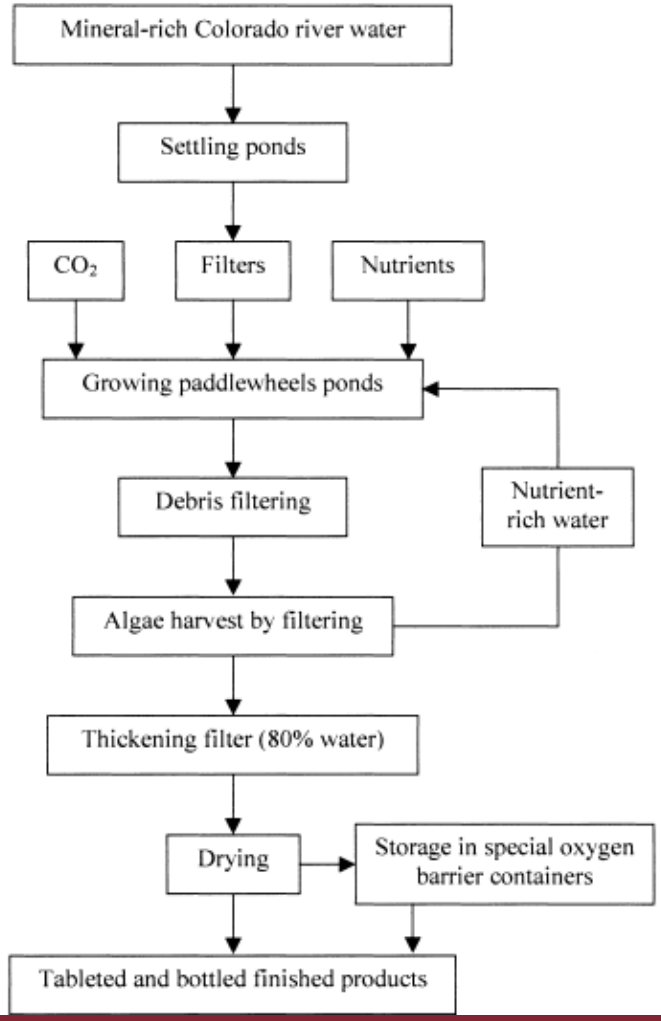
Commodity	Protein	Carbo-hydrate	Lipid
Bakers' yeast	39	38	1
Meat	43	1	34
Milk	26	38	28
Rice	8	77	2
Soybean	37	30	20
Anabaena cylindrica	43–56	25–30	4–7
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51–58	12–17	14–22
Dunaliella salina	57	32	6
Porphyridium cruentum	28–39	40–57	9–14
Scenedesmus obliquus	50–56	10–17	12–14
Spirulina maxima	60–71	13–16	6–7
Synechococcus sp.	63	15	11

The commercial applications are dominated by four strains: *Arthrospira, Chlorella, D. salina* and *Aphanizomenon flos-aquae*.

Should be considered that algal composition can greatly vary in base to strategy of cultivation.

Earthrise Farms

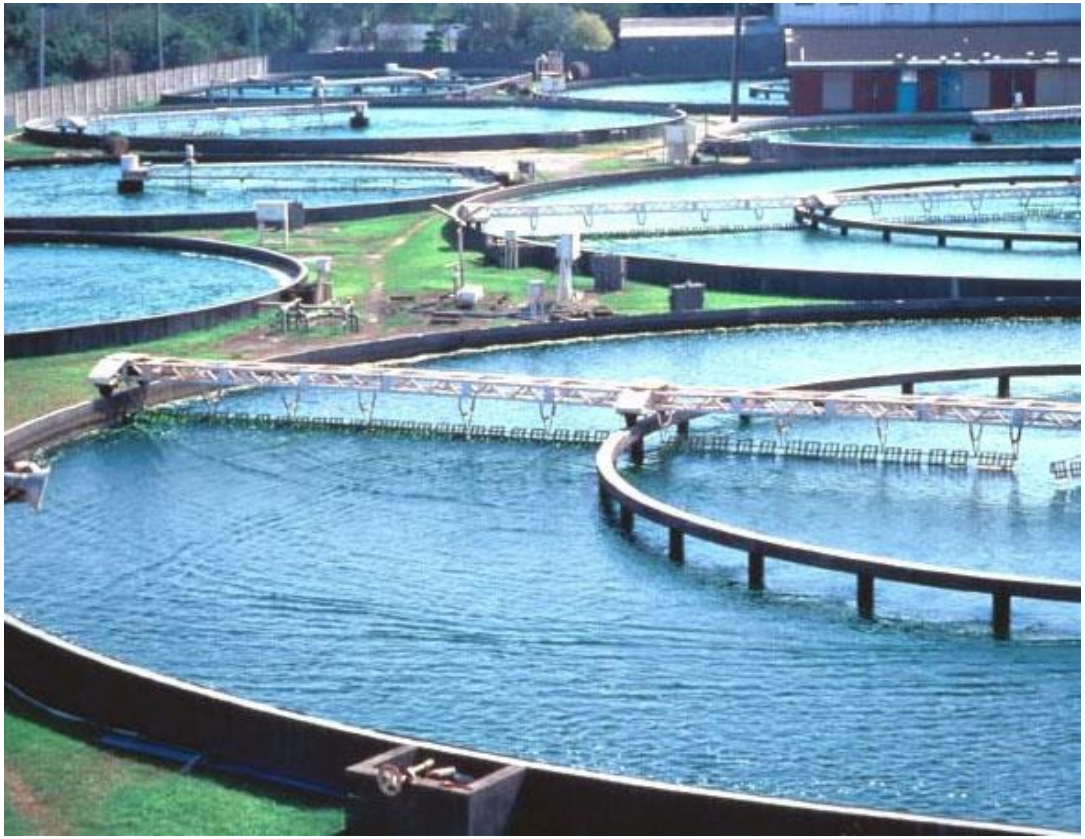
Earthrise Farms is the largest plant in the world for Spirulina production. It covers an area of 440000 m^2 and it is located in the Sonoran Desert of southeastern California.



Earthrise Farms produces mainly tablets and powder *Arthrospira*-based.

Chlorella Manufacturing and Co.

It is the largest producer of *Chlorella* with 400 t of dried biomass produced per year. The most important substance in Chlorella is β-1,3-glucan, which is an active immunostimulator, a free radical scavenger and a reducer of blood lipids.



Chlorella powder

Significant production is also achieved in Klötze, Germany (130–150 t dry biomass per year) with a tubular photobioreactor.
This reactor consists of compact and vertically arranged horizontal running glass tubes with a total length of 500,000 m and a total volume of 700 m^3.

Dunaliella salina is exploited for its β-carotene content that can reach 14% of dry weight.

Dunaliella natural β-carotene is widely distributed today in many different markets under three different categories:
β-carotene extracts,
Dunaliella powder for human use,
dried *Dunaliella* for feed use.

Cyanotech

Cyanotech is located in Hawaii. Main products are BioAstin and Spirulina pacifica. BioAstin is an oleoresin rich in astaxanthin produced from *Haematococcus pluvialis*.

Animal nutrition

Currently 30% of the current world algal production is sold for animal feed applications. Of this 50% is represented by *Arthrospira.*
In 1999, the production of microalgae for aquaculture reached 1000 t (62% for molluscs, 21% for shrimps, and 16% for fish).
Microalgae are import for aquaculture because they are the natural food source of these animals.
In order to be used in aquaculture, a microalgal strain has to meet various criteria. It has to be easily cultured and nontoxic. It also needs to be of the correct size and shape to be ingested and to have a high nutritional qualities and a digestible cell wall to make nutrients available.
Protein content is a major factor determining the nutritional value of microalgae. In addition, highly unsaturated fatty acid (e.g., eicosapentaenoic acid [EPA], arachidonic acid [AA] and docosahexaenoic acid [DHA]) content is of major importance.
Carotenoid pigments like astaxanthin must be supplied in salmon and trout diets.

Arthrospira is largely used to feed many types of animal: cats, dogs, aquarium fish, ornamental birds, horses, cows and breeding bulls.

Cosmetics production

Microalgae are cultivated also in the skin care market. Microalgae extracts can be found in several face and skin care products as anti-aging cream, refreshing or regenerant care products, emollient and in anti-irritant.

High value molecules

Pure high value molecules can be extracted from microalgae when their concentration are sufficiently high. Mainly three classes of compounds are actually produced from microalgae: **fatty acids**, **pigments** and **stable isotopes**.

Lipids of microalgae are generally rich in polyunsaturated fatty acids (PUFAs). Their composition is similar to fish oil, with an high ratio ω3/ω6.

PUFA	Structure	Potential application	Microorganism producer
γ-Linolenic acid (GLA)	18:3 ω6, 9, 12	Infant formulas for full-term infants Nutritional supplements	*Arthrospira*
Arachidonic acid (AA)	20:4 ω6, 9, 12 ,15	Infant formulas for full-term/preterm infants Nutritional supplements	*Porphyridium*
Eicosapentaenoic acid (EPA)	20:5 ω3, 6, 9, 12, 15	Nutritional supplements Aquaculture	*Nannochloropsis*, *Phaeodactylum*, *Nitzschia*
Docosahexaenoic acid (DHA)	22:6 ω3, 6, 9, 12, 15, 18	Infant formulas for full-term/preterm infants Nutritional supplements Aquaculture	*Crypthecodinium*, *Schizochytrium*

Carotenoids and Phycobiliproteins are main pigments produced from microalgae. These molecules can be used as nutraceutical supplement or as natural dye.

Molecule	Origin	Isomer	Market	Price (US$)	Principal producer
β-Carotene	*Dunaliella*	All-*trans* and 9-*cis*		300–3000/kg	Cognis Nutrition and Health (Hutt Lagoon and Whyalla, Australia), Cyanotech (Kona, Hawaii, USA), Inner Mongolia Biological Eng. (Inner Mongolia, China), Nature Beta Technologies (Eilat, Israel), Tianjin Lantai Biotechnology (Tianjin, China)
	Synthetic	All-*trans*	>90%		
Astaxanthin	*Haematococcus*	3S, 3′S			Cyanotech (Kona, Hawaii, USA), Mera Pharmaceuticals (Kailua-Kona, Hawaii, USA), Bioreal (Kihei, Hawaii, USA), Parry's Pharmaceuticals (Chennai, India), Algatech (Kibbutz Ketura, Israel)
	Phaffia yeast	3R, 3′R			DSM (Heerlen, The Netherlands)
	Synthetic	3S, 3′S-3R, 3′R-3R, 3′S (meso)	>95%	≈2500/kg	Hoffman–La Roche (Basel, Switzerland) and BASF (Ludwigshafen, Germany)

Product name	Price (US$)	Distributor
R-phycoerythrin	3.25–14/mg	Cyanotech
Allophycocyanin	6–17/mg	Cyanotech
Streptavidin: B-phycoerythrin	145/mg	Martek
Goat anti-mouse IgG: R-phycoerythrin	165/mg	Martek
Sensilight PBXL1: anti GST	1500/mg	Martek
Mixed fatty acids	60/g	Spectra Stable Isotopes
^{13}C-mixed free fatty acids	200/g	Spectra Stable Isotopes
^{13}C-DHA (>95%)	38000/g	Spectra Stable Isotopes
^{15}N-alanine	260/g	Spectra Stable Isotopes
$^{2}H_7$, ^{13}C, $^{15}N_4$-arginine	5900/g	Spectra Stable Isotopes
dATP-CN	26000/g	Spectra Stable Isotopes

Microalgae are suitable for the production of stable isotopically labeled compounds. They can fix ^{13}C ^{15}N and ^{2}H from relatively inexpensive inorganic molecules as $^{13}CO_2$, $^{15}NO_3$, $^{2}H_2O$ through photosynthetic activity.

Production of BioOil

Microalgae are receiving an increasing interest for oil production because they can store lipids in high content (until to reach 90% of biomass dry weight). This interest is increased in latest years because this oil is seen as a promising potential source for biofuel production.

Why it is a promising source?

- Microalgae are a renewable source.
- Fixation of carbon dioxide can reduce concentration of greenhouse gases.
- Microalgae can grow in lands which are not suitable for farming.
- BioOil productivity per hectare is higher then productivities obtained with oleaginous crops.

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity (kg biodiesel/ha year)
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalgae (low oil content)	30	58,700	0.2	51,927
Microalgae (medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

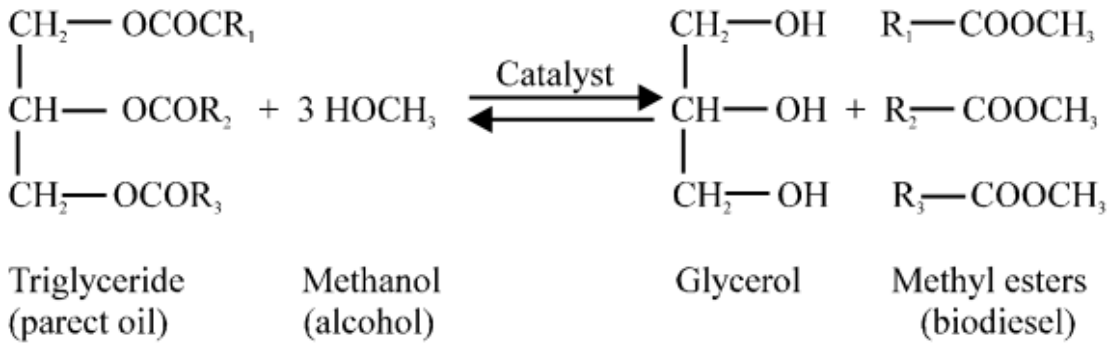
Oil extraction

- Dewatering: sedimentation, centrifugation, filtration, flotation, agglomeration, flocculation.
- Pretreatments: microwave, bead milling, ultrasonication, chemical lysis.
- Drying: freeze drying, oven drying, spray drying.
- Lipid extraction: organic solvent extraction, supercritical solvent extraction.
- Removal of cell debris: filtration, centrifugation.
- Removal of solvent and residual water: distillation, vacuum evaporation, solid-phase solvent adsorption.

Biodiesel production

- Lipid fractionation: chromatography, acid precipitation, urea crystallization.
- Transesterification: acid catalyzed, base catalyzed.

Transesterification:



Third generation biofuels: Solazyme (US)

Biocarburanti, nutrienti e prodotti per la pelle e la cura del corpo
Dark fermentation con aggiunta di zucchero di canna e sciroppo di mais
Accordi con Sephora e United Airlines

Polysaccharides production

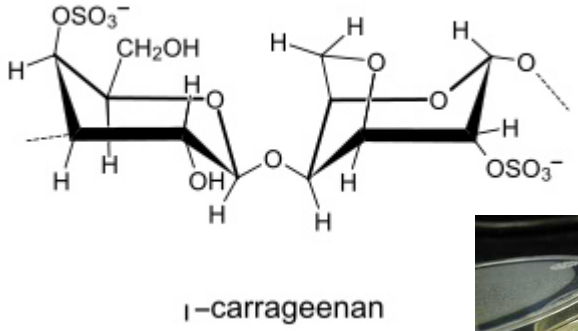
Cell-wall polysaccharides

Cellulose: glucose polymer, chains are formed by 2-3000 glucose unites linked by β(1-4) glycosidic bonds.

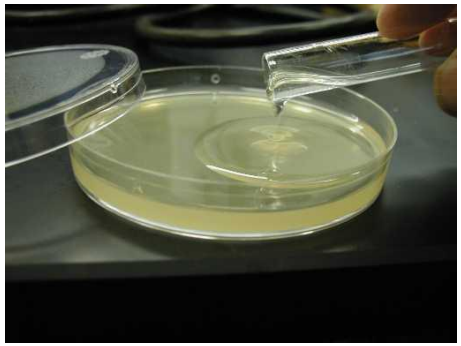
Emicellulose: a mixture of several heteropolymers composed by glucose, xylose, arabinose, mannose, galactose and rhamnose. Chain length are shorter then cellulose.

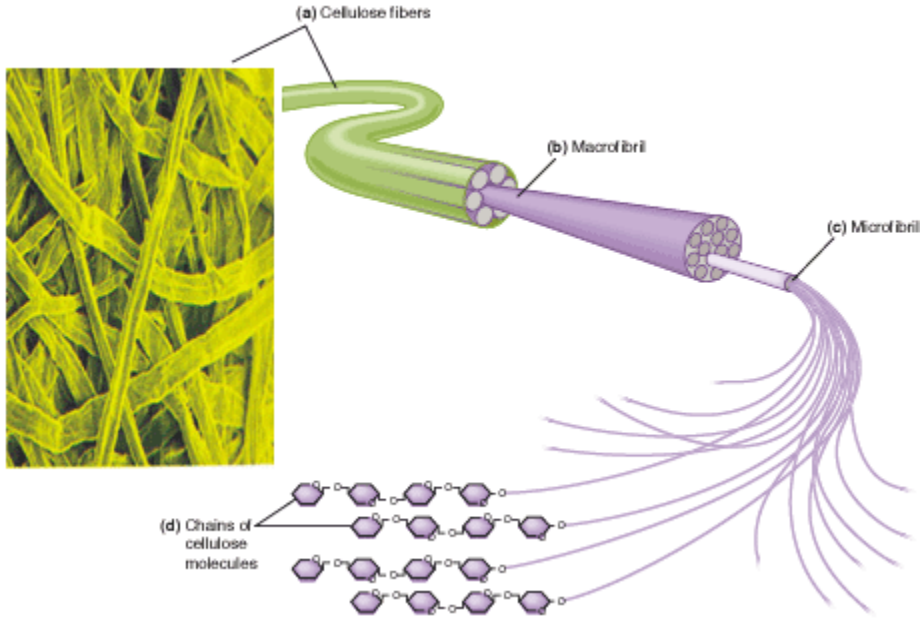
Agar: it is a polymer of D-galactose linked by α(1-3) and β(1-4) glycosidic bonds.

Carrageenans: it is a polymer of D-galactose containing sulfate groups.



ι–carrageenan





Reserve polysaccharides

Starch: it is a polymer of D-glucose units linked by α(1-4) bonds. There are mainly two structures which are amylose (linear molecules) and amylopectin (branched molecules).

Exo-polysaccharides (EPS)

Exo-polysaccharides are a mixture of mucilaginous substances. They are composed by different heterogeneous polymer chains mainly constituted by galactose, glucose, xylose, fucose, arabinose etc..