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## Chapter 4

# Potential of microalgae

*Hubert Bonnefond, Charlotte Combe, Jean-Paul Cadoret, Antoine Sciandra, Olivier Bernard*

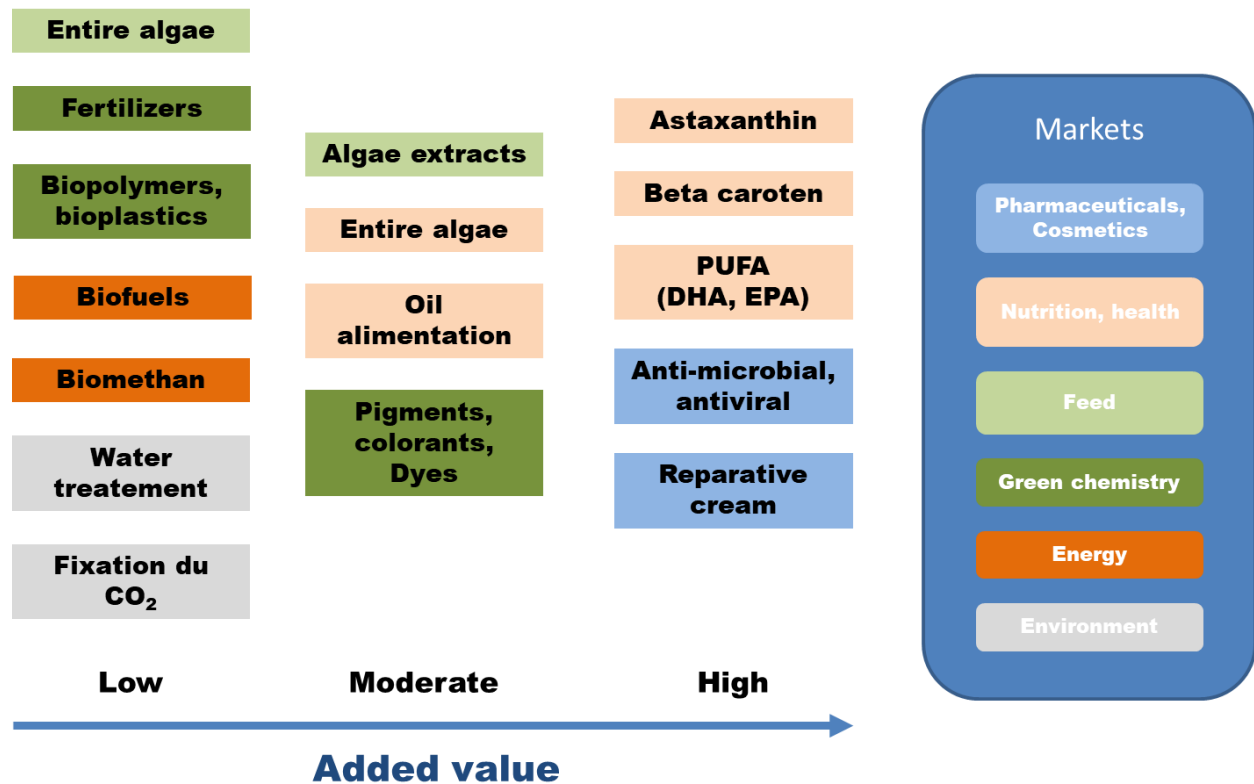
### 1. Introduction

The first time the use of microalgae was mentioned dates back to the conquistador Cortes, who described in his memoirs the Inca habit of dragging very tight nets across the surface of lakes to harvest a green mud that they consumed in the form of dried cakes. More recently, large-scale industrial cultivation of microalgae began in Japan in the 1960s for aquaculture purposes. Today, global production stands at around 27,000 tonnes, with a 2024 market estimated around 1.1 billion \$ (Amorim et al. 2021). The average price for algal biomass or extracted molecules stands between €40 and €220 kg<sup>-1</sup> (Fernandez et al. 2019).

The products derived from microalgae can be divided into three categories (Figure 4.1):

- products with low added value, for which current production costs do not yet allow industrialisation, but which have great potential,
- medium/high added-value products (US\$10-80.kg<sup>-1</sup>), such as those for human consumption, which are currently under development (whole algae),
- metabolites with very high added value, such as those for cosmetics, which are already produced.

The latter category of very high added-value metabolites includes target molecules such as  $\beta$ -carotene (US\$300-3,000 kg<sup>-1</sup>) and astaxanthin (>US\$2,000 kg<sup>-1</sup>), whose prices depend on quality. However, these sectors are highly competitive, as the existing supply from the petrochemical industry has often saturated the market. On the other hand, the human nutrition/health market for molecules such as long-chain polyunsaturated fatty acids (typically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), with prices ranging from US\$50 to US\$4600.kg<sup>-1</sup>, is strongly increasing, particularly because of the depletion of fish resources, the main supplier of these essential molecules (Sharma *et al.*, 2020; Fernandez et al. 2019).

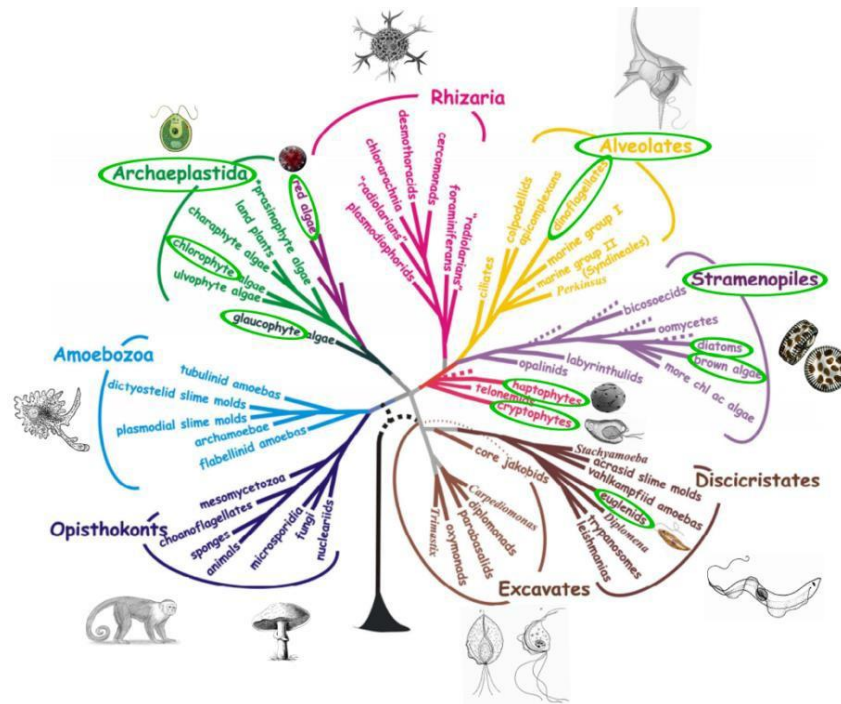


**Figure 4.1.** Main products from microalgae by application sector and added value.

The aim of this chapter is to present these microorganisms, which have been less explored and used than archaea, bacteria or yeasts. The marked development in the green chemistry sector will be detailed for its promising potential in the coming years.

## 2. Microalgae: microorganisms with macro potential

The term microalgae is actually a misnomer for the group of photosynthetic single-celled microorganisms, both prokaryotic and eukaryotic. Prokaryotes are represented by cyanobacteria (sometimes called 'blue-green algae'), while eukaryotes are a diverse group with many classes (Figure 4.2).



**Figure 4.2.** Phylogenetic tree representing the main eukaryotic lineages. Microalgal taxa are shown in green. Modified from Baldauf (2008).

Microalgae are a heterogeneous group with a high diversity, greater than that of terrestrial plants. This diversity is reflected in the variety of behavior (mobile, planktonic or sessile), morphologies, colors and sizes. The size of these organisms range from a few microns to several hundred microns (Sarkar et al. 2020). A number of microalgae live in symbiosis with other organisms (Richmond, 2008), such as bacteria or fungi (lichens), which produce some necessary micronutrients or vitamins.

Because of this diversity, combined with a high degree of metabolic plasticity, microalgae are ubiquitous organisms that occupy almost all of the planet's ecosystems. Inhabiting aquatic, brackish and freshwater environments, they have also conquered soils and a wide range of terrestrial environments. As proof of the diversity of their habitats, some species have colonized hostile ecosystems, such as hyper-salty lakes, glaciers, deserts and even hydrothermal springs.

Several hundred thousand to several million species are thought to live on the planet today. Only a few tens of thousands of them are inventoried. The taxonomic classification (Figure 4.2) of this diversity remains complex and subject to frequent modification. It is currently based on various properties such as pigmentation, chemical nature of storage products, organization of photosynthetic membranes, or morphological characteristics such as the presence of flagella, or the analysis of certain genome sequences (Richmond, 2008). Nowadays, the combination of the analysis of physiological traits and genome analysis makes it possible to classify these organisms

in a reliable manner.

Despite a growing interest in the use of microalgae since the mid-2000s, only a dozen of species are currently produced on an industrial scale (Junior et al. 2020).

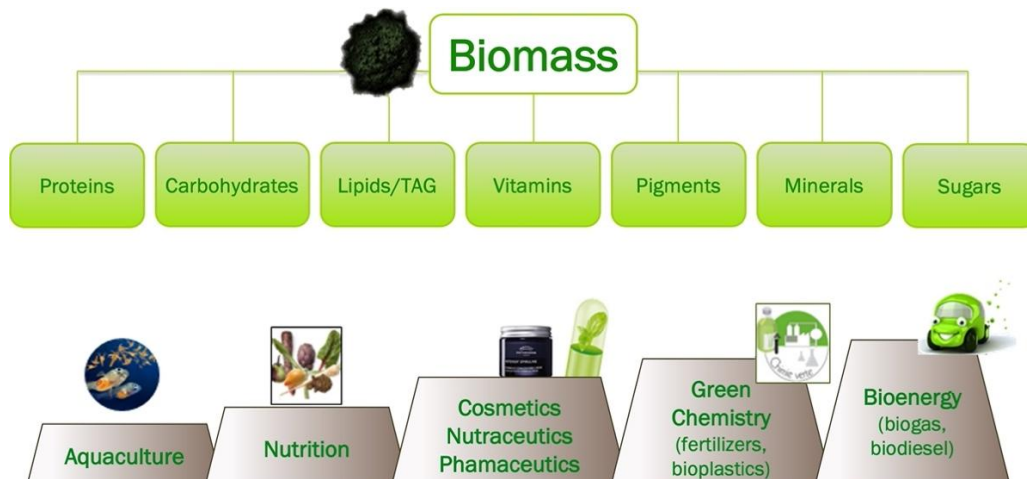
### 3. A reservoir of innovative molecules for numerous applications

The diversity of microalgae, which is hardly exploited, constitutes a real potential for research and industry. A great deal of work has been carried out to highlight this potential. In the years 2010, more than two hundred industrial projects on the production of microalgae for energy purposes have been listed totaling more than 2 billion euros invested, mostly in the United States.

Global production of microalgae is currently estimated at just below 30,000 tones of dry matter per year (Amorim *et al.* 2021). *Arthrospira (Spirulina) platensis* (~10 000 t/year) is the most widely cultivated species on an industrial scale, followed by *Chlorella* sp. (~4000 t/year) and *Dunaliella salina* (~1000 t/year) and *Haematococcus pluvialis* (~200 t/year). Other species of interest, but whose production remains more marginal, are *Nannochloropsis* sp., *Odontella aurita* and *Isochrysis galbana* (Junior et al. 2020).

A wide variety of molecules can be obtained such as proteins, amino acids, sugars and polysaccharides, vitamins, antioxidants, pigments, or long-chain polyunsaturated fatty acids such as DHA (docosahexaenoic acid) or EPA (eicosapentaenoic acid) (Sharma *et al.*, 2020).

This intrinsic richness offers the potential for innovation in a number of rapidly expanding markets, including cosmetics, pharmaceuticals, nutraceuticals, energy, human and animal nutrition (aquaculture), green chemistry and bioremediation (Figure 4.3).



**Figure 4.3.** Composition of microalgae and industrial applications.

The field of human nutrition, which has been expanding steadily over the past 40 years, is the

primary route for the commercialization of microalgae. They can be used as a whole or processed, and are consumed as a food supplement due to their rich composition in proteins, polyunsaturated fatty acids, pigments, vitamins and minerals, or as a natural food coloring (Junior et al. 2020). Their nutritional qualities justifies their applications for animal nutrition, particularly in aquaculture where microalgae are used to feed the larval stages of bivalve mollusks, crustaceans or even certain fish fry. The cosmetics industry then appears to be one of the most lucrative sectors for the sale of microalgae-based products. The bioactive molecules derived from these micro-organisms are used in the composition of suncare, make-up, anti-ageing and moisturizing products, and hair care products (Junior et al. 2020). The chemodiversity of microalgae also offers the possibility of developing new active ingredients and medicines. Many molecules have anti-tumor or anti-viral properties and have protective effects against cardiovascular diseases (Laurienzo, 2010; Ghosh *et al.*, 2015).

In the 2010s, microalgae have received much attention in the field of bioenergy (Novoveská et al. 2023). Firstly, they have high growth rates, much higher than those of terrestrial plants. Secondly, some species have the capacity to store large amounts of lipids, (up to 50% of their dry weight (Morales et al. 2021)). Finally, their production, which does not require fertile soils, does not compete with agricultural production and offers a high potential for CO<sub>2</sub> mitigation from industrial sources (Novoveská et al. 2023). Microalgae are therefore a potential and promising source of biofuels, the so-called 3<sup>rd</sup> generation .

#### Definition and key figures

**Definition of microalgae:** a group of unicellular photosynthetic microorganisms, prokaryotes and eukaryotes.

**Industrial production:** 27,000 t; about ten species cultivated, mainly *Arthrospira (Spirulina) platensis* (~10,000 t/year), *Chlorella* sp. (~4,000 t/year) and *Dunaliella salina* (~1,000 t/year).

**Estimated market:** 1.1 billion dollars forecasted for 2024 (cosmetics, pharmaceuticals, nutraceuticals, energy, human nutrition and aquaculture, green chemistry, bioremediation).

Average cost price of algal biomass or extracted molecules: 40-220 €/kg.

#### Target molecules:

- β-carotene (US\$ 300 – 3000/kg)
- astaxanthin (>US \$2000/kg)

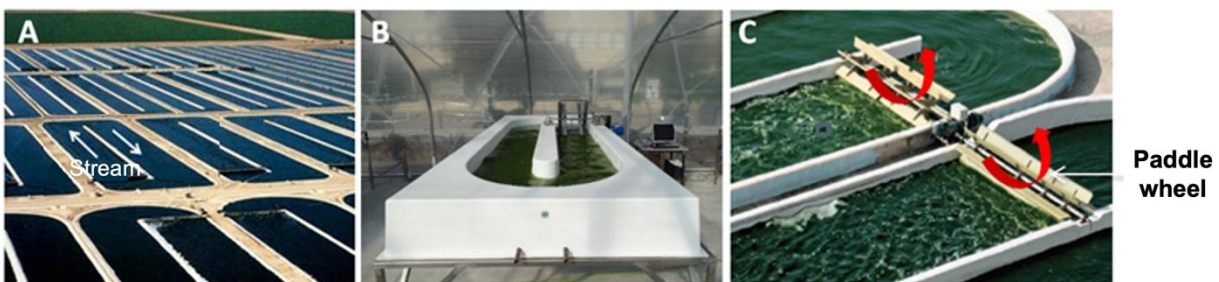
- long-chain polyunsaturated fatty acids (typically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), priced between US\$50 - \$4600/kg

## 4. Culturing systems

The growing interest in microalgae has led to a diversification of culturing systems adapted to the cultivated species and the target molecules of interest: high-yield open ponds (also called 'open ponds' or 'raceways') and closed photobioreactors, each with advantages and drawbacks (Novoveská et al. 2023).

### 4.1 Open, large-scale systems

Over the last few decades, large-scale microalgae cultivation has mainly been carried out in open systems. Although many processes exist, raceways have been used most since their development in the 1950s (Figure 4.4). Raceways are shallow ponds (10-50 cm deep), mechanically agitated by paddle wheels. They are currently considered to be economically viable systems despite their low productivity (maximum  $20\text{-}25\text{gDW}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ ) for a biomass concentration lower than  $0.6\text{gDW}\cdot\text{L}^{-1}$  (Junior et al. 2020). Growth conditions in a raceway are relatively difficult to control. Since they are exposed directly to the ambient atmosphere, they are subject to contamination. Furthermore, they are sensitive to daily and seasonal variations in temperature and light, especially as they are shallow with low thermal inertia. Significant water losses through evaporation are also observed in this type of system (Morales et al. 2019). The low intensity of crop mixing limits energy requirements, but keeps some cells in shaded areas for too long, thus reducing productivity.

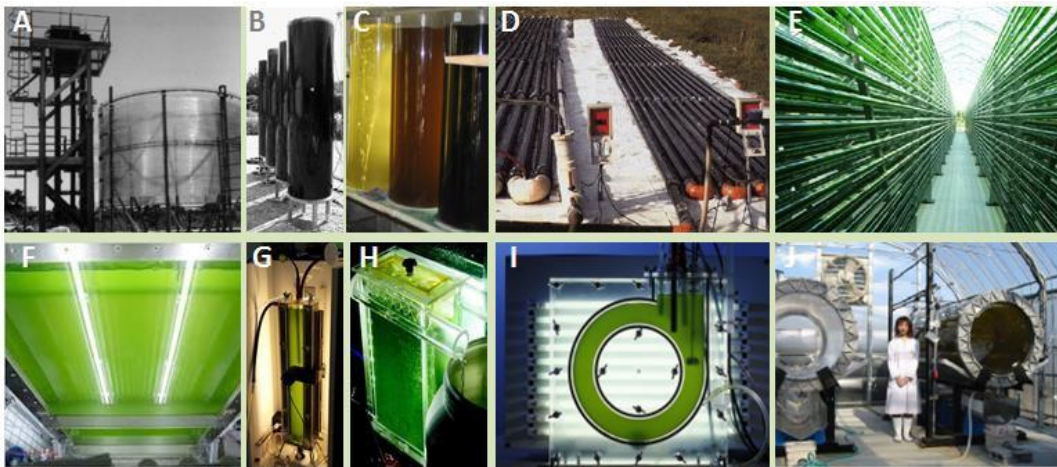


**Figure 4.4. Open microalgae culture systems at different scales.** (A) Raceway fields for large-scale spirulina cultivation (open ponds of 0.4 ha each), Calipatria, California; (B) Pilot raceway ( $6.4\text{ m}^2$ ), for the study of *Dunaliella salina* in a greenhouse, Villefranche-sur-Mer, France; (C) Mechanical paddle wheel system for mixing.

## 4.2 Closed systems

Vertical, horizontal, planar, annular, cylindrical, tubular, toroidal, spiral, bubble column, dome or pyramid shaped, photobioreactors take wide variety of geometries (Figure 4.5). Photobioreactors are defined as closed systems made of transparent materials and illuminated internally or externally by natural (sun) or artificial light. Their design seeks to optimize light distribution and improve mixing efficiency. Since they are closed, they protect cultures from contamination and allow fine control of growth parameters (light, temperature, nutrients, pH, culture volume, biomass level, gas distribution) to maximize productivity.

Photobioreactors are more productive than raceways (Pulz *et al.*, 2013) with productivities above 40 g DW/m<sup>2</sup>/D. However, production costs, including energy costs as well as manufacturing and maintenance costs, are high.



**Figure 4.5.** Examples of photobioreactor geometries for microalgae culture. Photobioreactors (A) "Biocoil"; (B) Vertical annular; (C) Bubble column (Scolabit); (D) Tubular, displayed on a reflective surface, (E) Horizontal tubular; (F, G, H) Planes (I) Toric (J) "Pipe photobioreactor" (tubular photobioreactor delimited by an annular cylindrical closed volume)

## 5. Storage of reserve carbon: from lipids to carbohydrates.

Most algae can build up energy and carbon reserves in the form of both starch (or other polyglucans) and lipids (triglycerides), with ratios that differ according to growth conditions. The accumulation of these energy-rich molecules provides a buffer reservoir essential for living in a fluctuating environment, and in particular for surviving periods of light energy deficit at night, or in poorly lit areas. Both compounds are energy and carbon reserves for cell division processes, such as DNA replication, nuclear division, cytokinesis, and daughter cell formation. Starch is commonly the primary energy storage compound produced from photosynthesis. It is directly



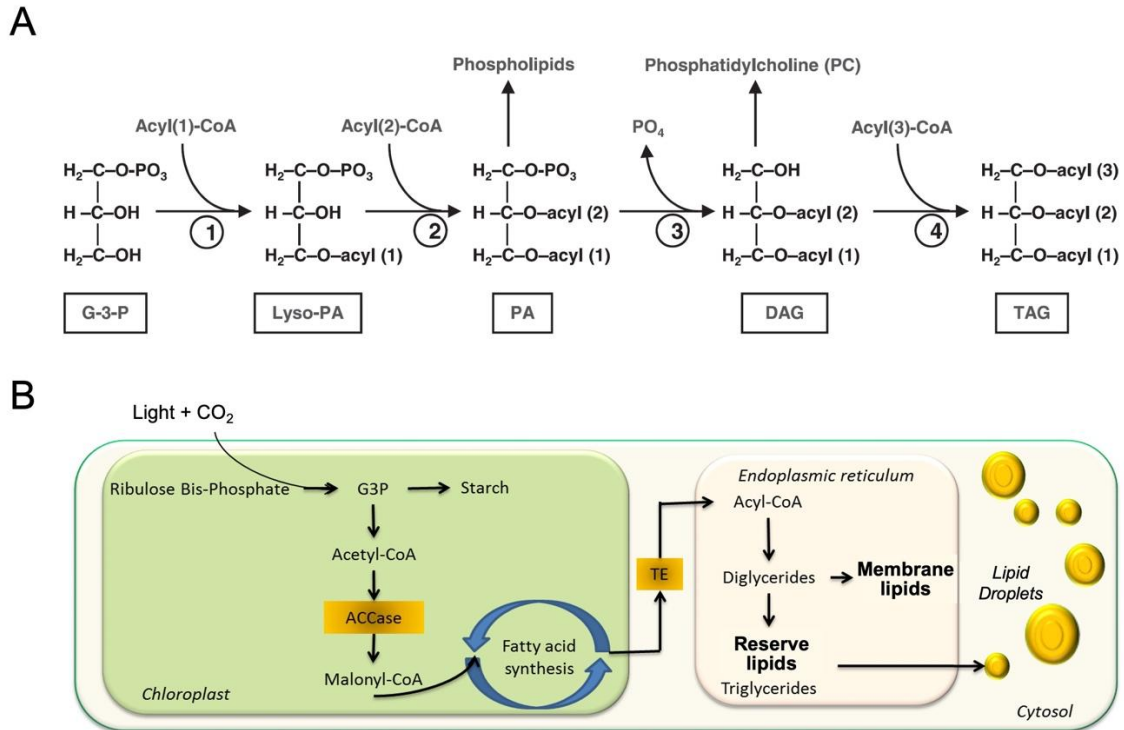
derived from 3- carbon (3C) carbohydrates such as 3-phosphoglycerate (3PG) and glyceraldehyde 3-phosphate (GAP). However, under certain conditions, which are not yet fully understood, this synthesis switches to lipid synthesis by means of an ultra-compact storage of energy and carbon, as it is free from the presence of water (38 KJ.g<sup>-1</sup> against 17 KJ.g<sup>-1</sup> for carbohydrates). Reserve lipids (TAGs), usually serve as a secondary energy source, and constitute an electron sink when their production is more economical for the cell than starch production (usually under nutrient limitation conditions). Figure 4.6 illustrates the current understanding of the relationship between the metabolic pathways of starch and lipid synthesis from a common C3 precursor.

The ability to overproduce starch or lipids is not restricted to specific taxonomic groups, but is widespread across species. In the case of lipid overproduction, starch is often synthesized early on, while lipids begin to accumulate much later. Even in lipid-enriched cells, starch remains in small, though significant, amounts, especially in the form of grains around pyrenoids. The oleaginous algae (*Nannochloropsis* and *Trachydiscus*) are the only known group of algae apparently unable to produce polyglucans (Hildebrand *et al.*, 2013). Diatoms produce large amounts of lipids, especially under nutrient-limited conditions. The marine diatom *Odontella aurita* is used industrially to produce omega-3 fatty acids and chrysolaminarin  $\beta$ -polyglucan.

## 6. Carbon and energy storage

### 6.1 Storage of polysaccharides

The polysaccharides synthesized and then used by microalgae as a source of energy and carbon are typically composed of glucose units (polyglucans) whose nature varies according to the species. Starch is the most common polyglucan. It is produced by most green algae as well as by higher plants. It consists of two different polyglucans, amylose and amylopectin (which differ in the degree of branching), the ratio of which varies between species. Amylose is the linear form of starch while amylopectin is the branched form. Some cyanobacteria, such as *Myxosarcina burmensis* or *Synechococcus sp.*, synthesize particular  $\alpha$ -polyglucans, called semi-amylopectin. Other types of polyglucans are observed depending on the class of microalgae, such as chrysolaminarin in diatoms, cytoplasmic starch (known as floridin) and glycogen in red algae and certain cyanobacteria, and paramylon in Euglenophyceae (for a detailed review, see Hildebrand *et al.*, 2013). These are  $\beta$ -polyglucans that differ from  $\alpha$ -polyglucans in the type of linkages connecting the glucose subunits (except for glycogen, a multi-branched glucose polysaccharide, similar to amylopectin).



**Figure 4.6.** A) Simplified scheme of the synthesis of different complex lipids in eukaryotic microalgae [3]. B) Overview of lipid anabolic pathways (G3P: glyceraldehyde 3 phosphate; ACCase: acetyl-CoA carboxylase; TE: thioesterase; modified from [7]).

## 6.2 Production of polyhydroxyalkanoates

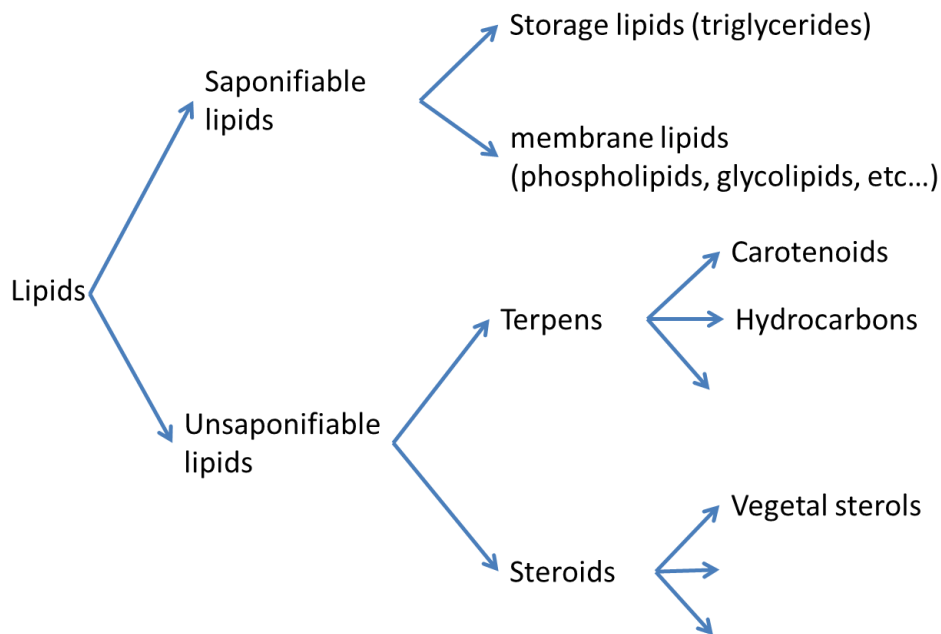
Some species, particularly prokaryotes, accumulate polyhydroxyalkanoates (PHA). These are natural polymers that allow the storage of carbon and energy. The simplest PHA is poly- $\beta$ -hydroxybutyrate (PHB). This natural thermoplastic polyester has similar properties to petroleum-based plastics but is completely biodegradable. The synthesis of PHA and PHB has been observed in several cyanobacteria, such as *Spirulina sp.*, *Nostoc sp* or even *Synechocystis sp.* (Markou and Nerantzis, 2013). *Spirulina maxima* deficient in nitrogen accumulates up to 0.7% of its dry weight in PHB and up to 1.2% when deficient in phosphorus. The highest PHB concentrations (38% of dry weight) were obtained in mixotrophic cultures of *Synechocystis sp.* PCC 6803, which were subjected to phosphorus deficiency, reduced gas exchange and the addition of organic carbon such as glucose or acetate (Panda and Mallick, 2007). Similar results were observed in the nitrogen-fixing cyanobacterium *Nostoc muscorum*, whose PHB concentration increased fivefold when grown in mixotrophy.

## 7. Lipids: molecules abundantly produced by some algal species

### 7.1 Lipids: a family of very heterogeneous molecules

Lipids form a heterogeneous family of rather atypical molecules in that, unlike carbohydrates, they are not defined by a particular molecular structure but by a chemical property: they are insoluble in water. In the living world, lipids have, among other things, two main functions directly resulting from their hydrophobic property. The first class of lipids is the cornerstone of lipid bilayers or membranes, the structural basis of all cells and the barrier between extra- and intracellular environments. These polar membrane lipids are therefore structural lipids. The second class consists of neutral lipids (triglycerides or triacylglycerols) which serve as a reserve for the cell.

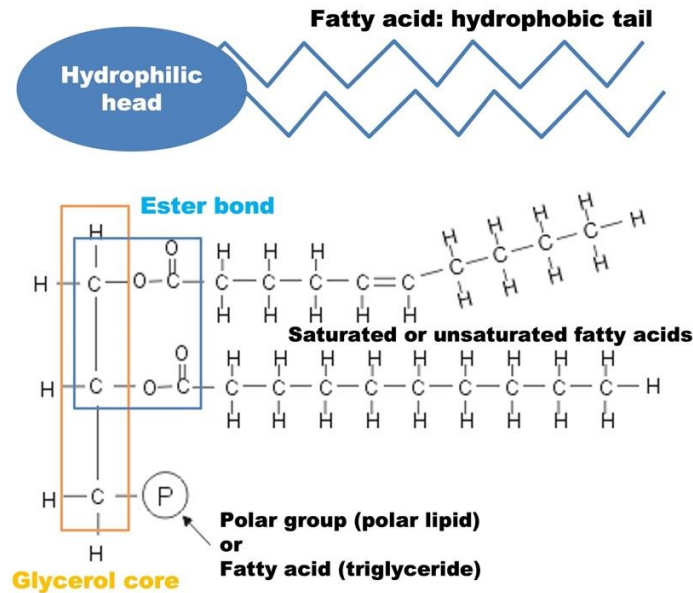
Lipids represent a very large diversity of molecules involved in various biological structures and processes (Figure 4.7). Saponifiable lipids, the main lipids of microalgae, are the basis for biofuel production. It should be noted that carotenoids are a particular family of unsaponifiable lipids.



**Figure 4.7.** Functional classification of lipids.

The basic unit of lipids is the fatty acid. It is a carbonic acid (COOH group) with a carbon chain length ranging from 10 to 22 carbons. In microalgae, the carbon chains are even and mainly made up of C16 and C18, although this composition varies widely from one taxon to another as well as with culture conditions (Sarkar et al. 2020). Saponifiable lipids are said to be amphiphilic. They are characterized by an apolar tail composed of saturated or unsaturated fatty acids and by a hydrophilic

head composed *at least of* a glycerol nucleus and, in the case of membrane lipids, a polar group (sugar, alcohol, phosphate, etc.). The two are joined by an energy-rich ester bond (Figure 4.8).



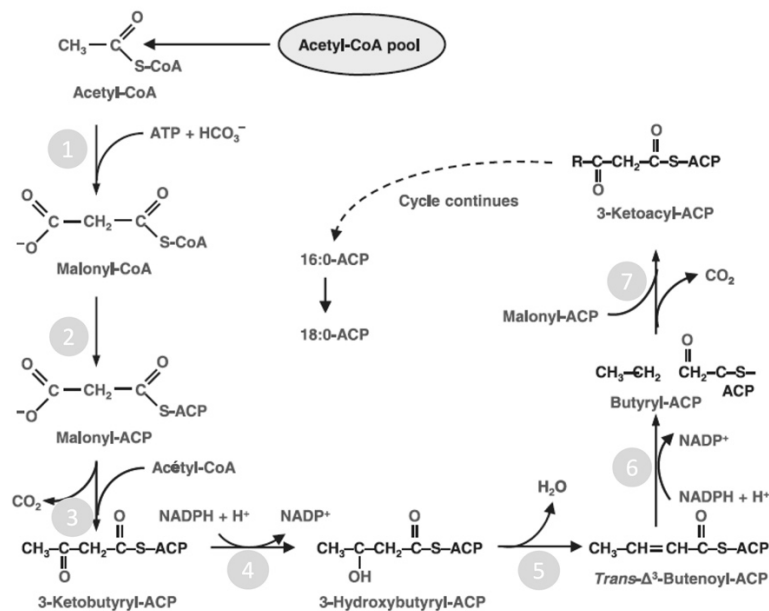
**Figure 4.8.** Schematic and detailed organization of a complex lipid.

Triglycerides are '*reserve*' lipids, a class of fairly homogeneous lipids that differ only in the length and degree of saturation of their fatty acids. This class of lipids is the sourcing for biofuel synthesis. *Structural lipids*, on the other hand, form a more complex class and are differentiated by the polar grouping of their hydrophilic head. Phospholipids, the main constituents of plasma membranes, are subdivided into phosphatidylglycerol (PG), phosphatidylcholine (PC), and phosphatidylethanolamine and phosphatidylserine. In this family, the polar group is a phosphate linked to an alcohol. Glycolipids, mainly located in chloroplast membranes, are divided into monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG). Their polar group is rather complex carbohydrate. These polar lipids play an essential role in the structure and fluidity of membranes, and are modified by changes in environmental parameters (light, temperature, etc.) and associated stress, so that the membranes can retain their biological role of supporting metabolic reactions. If the environmental stress remains moderate, only the length of the fatty acid chains and their degree of establishment are modified. On the other hand, if the environmental parameters move the microalgae too far away from its optimal growth conditions, the proportion of the different polar lipids is modified, at a higher energy cost.

## 7.2 Lipid metabolism in microalgae

Lipid synthesis is a complex process that begins with fatty acid synthesis. Their *de novo*

synthesis takes place in the chloroplast. The first reaction, which converts Acetyl-CoA to Malonyl-CoA, is catalyzed by a key enzyme, acetyl CoA carboxylase (ACCase). Malonyl-CoA is then rapidly converted to Malonyl-ACP. Then, following its association with an Acetyl-CoA and a series of reductions and dehydrations, the fatty acid precursor, butyryl-ACP, is formed. Then, successive elongations of Malonyl-ACP progressively lengthen the chain by two carbons per ring (Figure 4.9. Metabolic pathways of *de novo* fatty acid synthesis (Hu et al., 2008). The result is the formation of a saturated fatty acid, always even in 16 to 22 carbons. To produce an unsaturated acid, a double bond is introduced by a special enzyme, stearoyl ACP desaturase. The control of the different enzymes in this synthesis is not well known. Experiments on *Cyclotella cryptica* have shown that ACCase activity increases 2-4-fold after 4 and 15 hours of silica deficiency respectively, probably due to changes in the covalency of the enzyme (Hu et al., 2008).

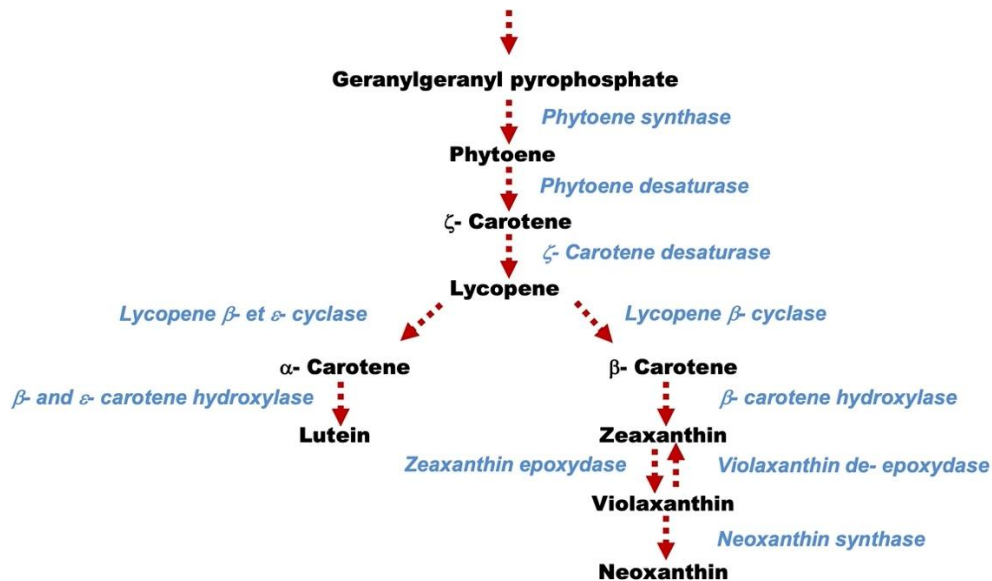


**Figure 4.9.** Metabolic pathways of *de novo* fatty acid synthesis (Hu et al., 2008).

The synthesis of complex lipids most probably uses the glycerol metabolic pathway (glycolysis). The fatty acids produced are sequentially transferred by an acyl transferase from the acyl group of the fatty acid to the 1 and 2 position of glycerol-3-phosphate (G3P), forming a phosphatidic acid (PA) precursor to many phospholipids. This acid can also be dephosphorylated, forming a diglyceride, which is the basic building block for the synthesis of phosphatidylcholine and glycolipids. Finally, the diglyceride can undergo a last transesterification, with a 3<sup>rd</sup> fatty acyl forming a triglyceride (Figure 4.6A). A pathway independent of fatty acid synthesis has also been reported for the synthesis of triglycerides. It consists of using fatty acids from phospholipids (donor) transferred to a diglyceride (acceptor). This pathway could become predominant in the event of stress, and explain the degradation of membranes observed for building reserve

triglycerides (Hu *et al.*, 2008). It should be noted that in cyanobacteria, there is no triglyceride synthesis. Figure 4.6B summarizes the sequence of the different steps from carbon fixation to the synthesis of a triglyceride molecule in eukaryotic microalgae.

As for lipids, the mechanisms triggering  $\beta$ -carotene synthesis in *Dunaliella salina* are still poorly understood. An environmental stress (high light intensity, nutrient deficiency, etc.) is detected by the cell *via* receptor(s) such as a UV photoreceptor, triggering signal transduction according to an intermediary factor, for example singlet oxygen. Free radicals are indeed known to trigger carotenogenesis (Shaish *et al.*, 1993). However, the receptor for such an intermediate factor has not yet been detected in any plant. The oxidation of chloroplast membrane lipids could constitute a second message involved in the signal transduction cascade, triggering the activation of the phytoene synthase and desaturase enzymes. Secondly, the formation of carotenoids is derived from an oxidation set of the formed phytoene molecule (Figure 4.10; Lamers *et al.*, 2008).



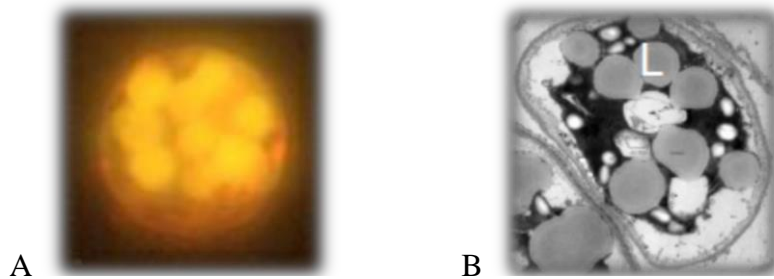
**Figure 4.10.** Metabolic pathways of the synthesis of different carotenoids (Lamers *et al.*, 2008).

### 7.3 Storage structures of triglycerides and $\beta$ -carotene

Reserve lipids are located in particular structures called “lipid bodies or droplets” generally located in the cytosol and visualizable via a fluorochrome such as Nile Red (Figure 4.11; Rumin *et al.*, 2015). These organelles are made up of a monolayer of phospholipids, itself stabilized by proteins with multiple and rather poorly characterized functions (anticoagulant, control of lipid catabolism and anabolism), inside which the triglycerides are located. The idea that these lipid bodies do not only have a storage role but that they constitute real connected organelles is gradually emerging (Davidi *et al.*, 2012; Ladygin, 2014).

In *Dunaliella salina*, there is probably a strong correlation between triglyceride and  $\beta$ -carotene

through these organelles (Bonfond *et al.*, 2017). Lipid droplets are the storage site for these extremely hydrophobic molecules, and could even act as a catalyst for their production. Storage in lipid droplets would shift the equilibria of enzymatic reactions towards  $\beta$ -carotene synthesis.



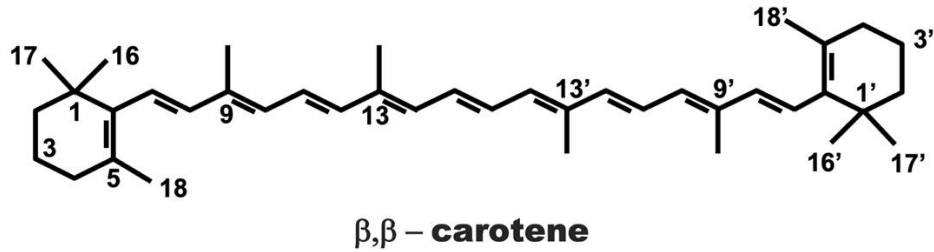
**Figure 4.11.** A) Staining of triglycerides using the fluorochrome Nile red. The red fluorescence is due to chlorophyll, the yellow fluorescence represents lipid droplets. B) Transmission electron microscopy image of *C. reinhardtii*, starchless mutant. (L) represents lipid droplets (Siaut *et al.*, 2011).

#### 7.4 Pigments: molecules with multiple properties

Chlorophyll (Chl) is a well known pigment involved in light energy conversion into chemical energy through photosynthesis. Microalgae also possess a wide range of ‘accessory’ pigments: carotenoids and phycobiliproteins to more efficiently harvest light and get protected from excess of energy.

More than 600 carotenoid molecules have been isolated from various natural sources. This diverse family nevertheless has a common structure: the isoprene unit (5 carbons) which, through a succession of polymerizations, makes it possible to obtain a 40-carbon skeleton. This base is then modified by i) cyclisation of one or both ends, ii) modification of the degree of unsaturation, and iii) addition of oxygen-containing functional groups (Figure 4.12A). If the molecule has one or more oxygen molecules, it is called xanthophyll (e.g., lutein), otherwise carotenes (e.g., carotene).

Carotenoids are pigments with strong antioxidant capacity, which have many applications. Among them,  $\beta$ -carotene has three main functions. Firstly, it protects the photosynthetic antennae, capturing excess light energy for photosynthesis and dissipating it as heat and radiation (conjugated double bond). Secondly, it is an antioxidant. In the event of oxidative stress, it protects the cell’s vital functions by neutralizing free radicals (Figure 4.12B). Finally, in conditions of unbalanced growth (excess carbon fixation compared to nitrogen fixation),  $\beta$ -carotene constitutes a sink capable of absorbing excess carbon and energy (Lamers *et al.*, 2008).



**Figure 4.12.** A) topological formula of a  $\beta$ -carotene molecule. B) simplified reaction between  $\beta$ -carotene and free radicals (Britton, 1995)

$\beta$ -carotene is a fat-soluble orange pigment, with many applications in pharmacology, cosmetics as well as in AFIs (Silva et al. 2020).  $\beta$ -carotene extracted from *Dunaliella salina* (Figure 4.12A) is the main produced carotenoid. This molecule has two stereoisomers: the *trans* and *cis* form, with different biological properties. Chemical synthesis from the petroleum industry produces only an iso-mix of these two isomers. On the other hand, cellular metabolism produces a mixture enriched in one or the other of these fractions depending on environmental conditions (Ben-Amotz *et al.*, 1988). The *trans* form is nevertheless more often found, as it is more stable. It is a precursor of vitamin A (provitamin A), which is essential for vision and the proper functioning of the immune system. In addition, numerous studies have highlighted the effect of  $\beta$ -carotene on the prevention of several types of cancer, atherosclerosis or even heart disease (de Jesus Raposo *et al.*, 2013). Astaxanthin is an anti-inflammatory antioxidant xanthophyll marketed in the pharmaceutical industry for its action against muscle pain, but also as a dietary supplement or as a pink dye in aquaculture. Other xanthophylls, such as lutein, zeaxanthin and canthaxanthin, are also used as food colorings or for pharmaceutical purposes (Naidoo *et al.*, 2013).

Phycobiliproteins (e.g., phycocyanin, phycoerythrin) are present in cyanobacteria and rhodophytes. These pigments are used for food, cosmetic and also pharmaceutical applications (Naidoo *et al.*, 2013). The action of phycocyanins in the defense of the immune system and as an anti-inflammatory agent has recently been demonstrated.

## 8. Factors stimulating the accumulation of reserve carbon

Stressors such as increased light flux, nutrient limitation or deficiency, are the most commonly used approaches to overproduce carbohydrates and lipids. Such treatments effectively block cell



cycle progression, and prevent the consumption of most energy and carbon reserves.

Starch, even under conditions of stress inducing overproduction, keeps the same structure as under standard conditions. In contrast, the composition of lipid reserves can vary not only between species or strains, but also according to the type of stress.

## 8.1 Light as a factor in changing lipid content

Light has a significant impact on total lipid accumulation (Vítová *et al.*, 2015). The change from low to high light intensity causes an increase of more than 40% in total lipids, as well as a strong increase in neutral lipids and a decrease in membrane lipids (Guihéneuf *et al.*, 2010). This also favors the content of saturated fatty acids at the expense of unsaturated fatty acids (Vítová *et al.*, 2015). However, not all microalgae species seem to react in the same way. Moreover, the impact of light intensity on reserve carbon depends on the position of the cell in its division cycle (Vítová *et al.*, 2015). Finally, the positive correlation between light intensity and lipid content is likely to be function of the nutrient status of the cell (itself influencing cell cycle progression). This is particularly true in the growth phase, whereas the effects seem to be less pronounced when the culture is in the stationary phase (Van Wageningen *et al.*, 2012).  $\beta$ -carotene responds very strongly to variations in light intensity as a photoprotectant. An increase in light intensity from 150 to 650  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  results in an increase in  $\beta$ -carotene concentration in *D. salina* of more than 350% (Silva *et al.*, 2020).

## 8.2 Temperature

Temperature is a parameter that influences the kinetics of all cellular proteins, including metabolic enzymes. The response of total lipids to temperature is not very clear (Hu *et al.*, 2008), reflecting the complex effect of this parameter. An increase in total lipids could be observed in response to a decrease in temperature, as well as at extreme temperatures (e.g., in the case of a cold winter). (Ras *et al.*, 2013). In other cases, the total lipid content is maximal at the optimal growth temperature and a strong correlation with the growth rate is observed (Vítová *et al.*, 2015). The fatty acid composition of the lipids is better known and is strongly dependent on temperature. The degree of unsaturation increases with temperature to allow the cell to maintain membrane fluidity (Morales *et al.* 2021). Finally, there is very little information on the response of the different classes of lipids to temperature (reserve lipids, membrane lipids, etc.).

Low temperatures seem to increase the  $\beta$ -carotene content in *Dunaliella salina* (Gomez and Gonzalez, 2005). However, observations are often divergent, and there are too few results to date to identify a significant trend. Moreover, it is very likely that  $\beta$ -carotene production is more a function of the cell's oxidative stress level (high concentration of free radicals) than the specific response of an abiotic parameter (Krol *et al.*, 1997).

### 8.3 Carbon dioxide

The availability of inorganic carbon primarily influences the growth rate. As aeration of dense cultures only with air (0.04% CO<sub>2</sub>) is not sufficient, it is necessary to inject 2-5% CO<sub>2</sub> into the bubbled air (Vítová *et al.*, 2015). Increasingly, the rate of CO<sub>2</sub> injection is generally carried out in a closed loop control loop where pH is regulated a fixed setpoint to meet the carbon demand. In general, lipid accumulation is hindered by CO<sub>2</sub> limitation. Conversely, lipid reserves appear to be positively correlated with increasing CO<sub>2</sub> content of the injected air, this effect being enhanced by nitrogen deficiency (Gordillo *et al.*, 1998). Some studies have shown that basic pH stresses, by reducing growth, lead to an increase in total lipid content. These same stresses would also enhance the effects of mineral deficiency on the accumulation of total or reserve lipids (Morales *et al.*, 2021).

### 8.4 Nitrogen deficiency as a trigger for lipid synthesis

Nitrogen deficiency has been the most studied. Most microalgae respond by overproducing reserve lipids (Morales *et al.*, 2021). The absence of inorganic nitrogen in the medium leads to a drop in protein synthesis and thus in cell division. However, photosynthesis continues to operate on the pre-existing pool of enzymes, resulting in excess energy and carbon fixation. The resulting effects of the imbalance between carbon and nitrogen fluxes are partially compensated by an increased production of energetic, carbon-rich but non-nitrogenous molecules, such as lipids,  $\beta$ -carotene or starch. In *Neochloris oleoabundans*, for example, nitrogen deficiency increases fourfold the concentration of lipids per biomass unit. Nitrogen deficiency also leads to an increase in the ratio of reserve lipids to membrane lipids. Finally, it modifies the distribution of fatty acids, their degree of unsaturation decreasing in response to the decrease in polyunsaturated fatty acids in favor of saturated fatty acids, particularly oleic acid (Morales *et al.*, 2021).

While nitrogen deficiency increases the intracellular reserve of lipids, it also decreases the growth rate of the biomass (Bonfond *et al.*, 2017). Thus, lipid productivity (growth rate, times lipid concentration per unit biomass, times biomass) is not necessarily increased. Industrial lipid production often proceeds in two stages. In the first stage, the algae are first cultured in optimal growth conditions to rapidly reach high biomass levels. In a second step, a nitrogen deficiency is induced, which stops the growth but increases the lipid concentration. Another approach is to induce nitrogen limitation rather than deficiency. This condition is achieved by renewing the culture medium and thus the nitrogen supplied in a sub-optimal way. This makes it possible to stimulate lipid production while maintaining significant growth (Morales *et al.* 2021).

### 8.5 Other deficiencies

Phosphorus deficiency can also lead to lipid accumulation. In *Nannochloropsis sp.*, for example,

this deficiency leads to a 200% increase in lipid content (Morales et al. 2021). Phosphorus is one of the major constituents of nucleic acids. Its limitation triggers, as with nitrogen, a rapid drop in the production of macromolecules (RNA, DNA, proteins) and growth, as well as an imbalance in the flow of carbon and energy leading to an accumulation of non-phosphate reserve molecules. A major restructuring of the plasma membranes then takes place. Phospholipids, their main constituents, disappear in favor of non-phosphated glycolipids and betaines. In some species (*Scenedesmus sp*, *Chlorella ellipsoidea*, *Chlorella vulgaris*, *Dunaliella primolecta*, *Haematococcus pluvialis*...), this deficiency modifies the type of reserve carbon, with microalgae accumulating lipids instead of starch (Vítová *et al.*, 2015).

In diatoms, silica deficiency also leads to a very high accumulation of lipids. In *Cyclotella cryptica*, the total and neutral lipid contents are doubled (Morales *et al.* 2021).

Sulphur deficiency, which has been less studied, has similar effects on the metabolism as phosphate deficiency. Cell division is stopped very rapidly, mainly due to the cessation of DNA replication. Sulphur deficiency seems to have a very strong effect on starch overaccumulation. The effect seems to be less marked on lipids, but few studies have explored this point (Vítová *et al.*, 2015).

### Key points

There are different culture systems adapted to the cultivated microalgae species and the target molecules of interest: **high-yield open ponds** or **raceways** and closed **photobioreactors**.

Photobioreactors are more productive than raceways (**productivity** > 40 gDW/m<sup>2</sup>/D) but the associated production costs are higher.

**Starch** and **lipids** are the main biochemical energy storage constituents in microalgae.

The **metabolism of** microalgae is determined by the species of the microalgae and by the culture conditions.

Overproduction of starch and/or lipids can be promoted by applying **environmental stress** during cultivation.

Factors that can promote **lipid production**:

- increase in light intensity,
- temperature drop,
- increase in the CO<sub>2</sub> concentration,
- nitrogen or phosphorus deficiency.

**Note:** the lipid composition (especially the fatty acid composition) also depends on the growing conditions.

## 9. Conclusion

Microalgae are a barely explored resource. Despite the high biodiversity of these organisms, whose estimated number of species ranges from several hundred thousand to several million, only a few of them are currently cultivated at industrial scale. A key advantage is the ability of many species to fix CO<sub>2</sub> and store it efficiently in the form of polysaccharides and lipids. Microalgae have so far been cultivated for high added value applications, mainly for pigments and antioxidants. Cultivation processes must still progress for reducing the production costs and the associated environmental impact. Numerous barriers still need to be overcome throughout the chain value so that microalgae can offer a varied and abundant resource for green chemistry.

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