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# Synthesis of Antioxidant Carotenoids in Microalgae in Response to Physiological Stress

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Additional information is available at the end of the chapter

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## Abstract

Carotenoids act as potential antioxidants, quenching energy of excited singlet oxygen and scavenging free radicals. Among microalgae, *Haematococcus*, *Chlamydomonas*, *Chlorella*, *Dunaliella* and diatoms and dinoflagellates, such as *Phaeodactylum* and *Isochrysis*, are able to synthesize large amount of carotenoids. The main function of carotenoids consists in absorbing light to perform photosynthesis, and some of them are constitutively present in the cells (primary carotenoids). The main primary carotenoids usually found are neoxanthin, violaxanthin, lutein, and  $\beta$ -carotene. To preserve cells from oxidative damage, their production may be increased, while other carotenoids may be synthesized *de novo*. In particular, under stress conditions such as high light exposure, nutrient starvation, change in oxygen partial pressure, and high or low temperatures, microalgal metabolism is altered and photosynthetic activity may be reduced. In these conditions, photosynthetic electrons transport is reduced, and the intracellular reduction level increase may be associated with the formation of free radicals and species containing singlet oxygen. In order to prevent damage from photooxidation, microalgae are able to adopt strategies to contrast these dangerous oxidant molecules. One of the most active mechanisms is to synthesize large amount of carotenoids, which can act as antioxidants.

**Keywords:** carotenoids, microalgae, antioxidant, stress

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## 1. Introduction

Carotenoids are a class of natural lipid-soluble pigments mainly found in plants, algae, and photosynthetic bacteria. They play a central role in photosynthesis, both as light-harvesting complexes and as photoprotectors. However, it is generally believed that they function as

passive photoprotectors (i.e., as a filter), reducing the amount of light that can reach the light-harvesting pigment complexes of photosystem II (PSII).

For their antioxidant properties, the role of carotenoids in human health has acquired importance in the recent years, mainly due to the attention toward the utilization of compounds obtained from natural sources.

Microalgae and cyanobacteria are photoautotrophic organisms that are exposed to high oxygen and radical stress in their natural environment, and consequently have developed several efficient protective systems against reactive oxygen species and free radicals [1]. They represent an almost untapped resource of natural antioxidants due to their enormous biodiversity, and the value of microalgae as a source of natural antioxidants is further enhanced by the relative ease of purification of target compounds [2].

Microalgae are capable, under stress conditions, of producing significant amounts of substances with high added value (antioxidant carotenoids, phenolic compounds, and polyunsaturated fatty acids), and for this reason, the study of the physiology of the growth of these microorganisms is of particular interest. In particular, carotenoids act by counteracting the effects of the damage caused by an excess of light and protecting the cells from oxidative damage.

Carotenoids are divided into two groups named primary and secondary carotenoids.

The primary carotenoids, such as the xanthophylls and  $\beta$ -carotene, are found in the chloroplast under standard conditions and are directly involved in performing photosynthesis for their role in the absorption of light energy. However, under stress conditions such as high light and nutrient deficiency, the provided energy may not be sustainable, and the content in primary carotenoids may increase, to dissipate the excess energy. Moreover, some photosynthetic microorganisms accumulate large amounts of secondary carotenoids in the cells, as a mechanism of photoprotection, in response to physiological stresses that induce the increase of reduction level inside the cells.

In particular, under high light stress conditions, the dissipation of the excess absorbed light energy occurs via the nonphotochemical quenching (NPQ) of chlorophyll fluorescence, a harmless nonradiative pathway of dissipation of energy. This defensive strategy involves the synthesis of antioxidant carotenoids, such as the secondary carotenoid astaxanthin, the pigment lutein, and the xanthophyll cycle pigments: violaxanthin, antheraxanthin, and zeaxanthin [3–7]. Among the xanthophylls, also luteoxanthin and fucoxanthin, mainly produced by marine strains such as *Phaeodactylum* and *Isochrysis*, have been found to be strong antioxidants.

Diatoms, such as *Phaeodactylum*, have a specific set of pigments with chlorophyll *c*, and they have an additive xanthophyll cycle, consisting in diadinoxanthin (Ddx), which can be deep-oxidized to diatoxanthin (Ddx). These reactions lead to reduction of the singlet oxygen inside the cell, avoiding cellular damage. Among carotenoids, the ketocarotenoid astaxanthin has been shown to have a strong efficacy in quenching singlet oxygen.

Comparing the antioxidant activity of astaxanthin,  $\beta$ -carotene and the xanthophylls zeaxanthin and lutein with the one of  $\alpha$ -tocopherol, a well-known noncarotenoid antioxidant, it has been shown that these carotenoids are among the most powerful antioxidants [8].

Considering the role of carotenoids as quenchers of active oxygen species, they represent a very interesting natural source of antioxidant and antiaging substances.

Among photosynthetic microorganisms, the green unicellular microalga *Haematococcus pluvisialis* is capable of producing a large amount of astaxanthin, a red pigment that starts to accumulate in the central part of the cell until the cell becomes entirely red. The other unicellular green microalga *Dunaliella salina* is well known for  $\beta$ -carotene production. In this microalga, the strong orange pigment is synthesized at one side of the cell, where it starts to accumulate in lipidic bodies, and then it continues to accumulate in the rest of the cell. Another big producer of antioxidant carotenoids is *Scenedesmus*, a colonial microalga able to produce large amounts of lutein, which makes the cells change their color from green to yellow.

Many studies on the physiology of microalgae have been carried out on the unicellular green alga *Chlamydomonas reinhardtii*. This microalga is considered a good model organism as it can be easily manipulated by means of genetic engineering; it has been the source of much information on photosynthetic responses to stress. Concerning the synthesis of carotenoids, particularly interesting were the studies on the xanthophyll cycle induction.

## 2. Physiology of the growth of microalgae

Photosynthetic microorganisms present a great variety of shape and size. Microalgae and cyanobacteria are distributed in a wide spectrum of habitat, having adapted their metabolism to complex and extreme environmental conditions (high salinity, extreme temperature, nutrient deficiency, and UV-radiation). To survive under such different harsh conditions, they have developed several strategies.

Each strain has its own optimal growth conditions, in regards to temperature, pH, salinity, light intensity, nutrient composition of the medium. Among these, one especially important parameter for photosynthetic microorganisms is light intensity.

The photosynthetic efficiency, i.e., transformation of light energy into chemical energy, is first and foremost limited by the fact that photosynthetic cells can only use light in the wavelength range from 400 to 700 nm so that only about 55% of incident solar light is useful to perform photosynthesis.

Moreover, it has to be considered that part of photosynthetic active radiation, about 10%, is reflected by the surface of the cells in the cultures; also, self-shading between cells further reduces the light utilization of each cell. Considering all these limitations, the percentage of light that can be used for photosynthesis is about 41%.

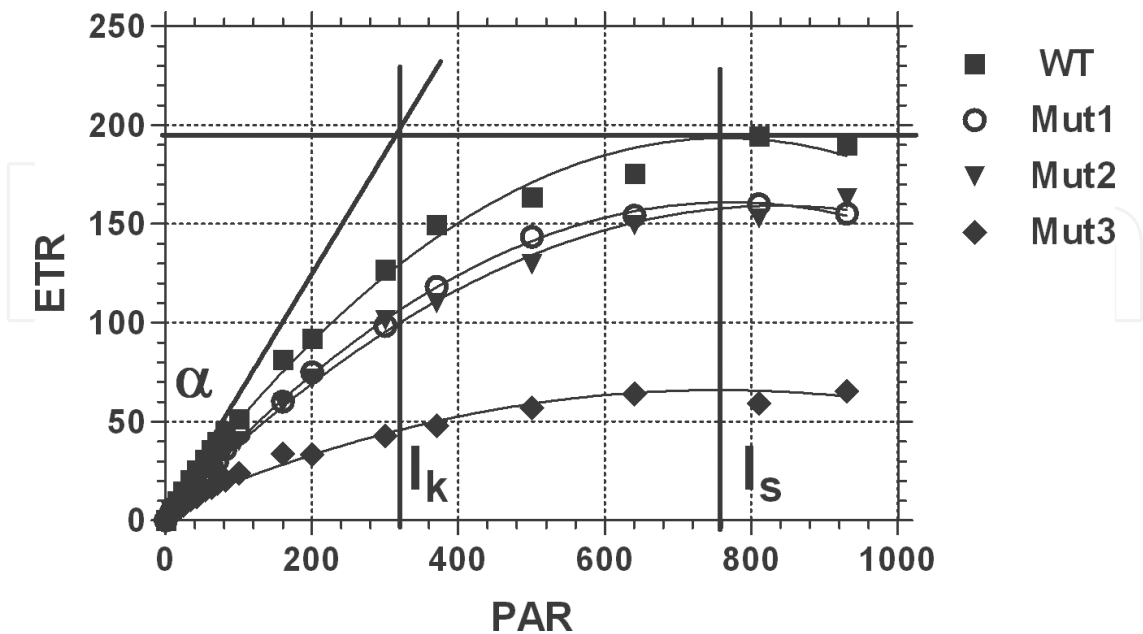
It is also important to consider some physiological limits of the photosynthetic apparatus, which makes it unable to utilize a light irradiation beyond a light intensity. Hence, about 20% of incident solar light is in excess, when it reaches the highest intensities in the central part of the day, and it is dissipated by heat and used to synthesize antioxidant pigments [9, 10].

In **Figure 1**, a typical light-curve response of *C. reinhardtii* is reported, comparing the electrons transport rate (ETR) of different strains with D1 protein mutation affecting photosynthetic performance with the wild type.

In this case, the photosynthetic activity is expressed as the capability to transfer electrons, but it could also be expressed as O<sub>2</sub> evolution, or CO<sub>2</sub> up-take. It is evident that different strains can have different behaviors at increasing light intensities, exhibiting different values of  $\alpha$ , the slope of the first part of the curve, and different  $I_k$  value, i.e., the saturation irradiance, given as an intercept between  $\alpha$  and ETR<sub>max</sub>. According to the light saturation value, the strains can react differently, having different sensitivity to high light stress, and accumulating different levels of photooxidative stress.

For this reason, imposing a light stress inducing the carotenoids synthesis, as well as other stress conditions, such as nutrient limitation-starvation and excessive low or high temperature, is a useful approach in order to accumulate antioxidant compounds, but it is not convenient in terms of culture productivity, as under these limiting conditions, the growth is strongly affected.

One of the main physiological parameters used to monitor stress is the measurement of the photosynthetic activity, by evaluating oxygen evolution and Chla fluorescence measurement. In the presence of stress, the photosynthetic activity usually decreases, and it can be a useful indication on the kind of stress occurring to the cells. In particular, when the photosynthetic apparatus is impaired, light cannot be used efficiently, an accumulation of electrons on the electrons transport chain occurs and cells need to dissipate this excess of energy.



**Figure 1.** Comparison of different light induction curves in *Chlamydomonas reinhardtii* wild type (WT) and D1 protein mutant strains (mutation affecting the photosynthetic activity) Mut1, Mut2, and Mut3.

In response to this overreductive cellular environment condition, microalgae are able to produce a great variety of secondary metabolites, with antioxidant properties, which are biologically active and which cannot be found in other organisms [11, 12].

Among them, antioxidant compounds are the one to have attracted major interest for health and pharmaceutical industry, for their strong efficiency in preventing or delaying the damages caused by free radicals. Several synthetic antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT),  $\alpha$ -tocopherol, and propyl gallate have been used for limiting the oxidative damage, but they are strongly suspected to be responsible for a variety of side effects, such as liver damage and carcinogenesis. For this reason, a strong interest has been focused on finding natural products acting as antioxidants, safe, and effective.

### **3. Carotenoids: function and distribution in photosynthetic cells**

The main functions of carotenoids consist in light absorption, to perform photosynthesis, and photoprotection to preserve the photosynthetic apparatus from photodamage. A role for carotenoids in cell differentiation, cell cycle regulation, growth factors regulation, stimulation of immune systems, intracellular signaling, and modulation of different kinds of receptors has been suggested [13].

However, for their antioxidant properties, they act as quenchers of active oxygen species and physiological stress, such as high light exposure, nutrient limitation or starvation, UV exposure, temperature fluctuation, anaerobiosis, and induce the metabolic pathways for the synthesis of these compounds.

These molecules are constituted by a  $C_{40}$  hydrocarbon backbone liable to structural modifications. According to their structure, carotenoids may be distributed in different ways into the cell compartments. In particular, they can be found within the inner section of the lipid bilayer of cell membranes, only if they are strict hydrocarbons like  $\beta$ -carotene or lycopene, or they can protrude into an aqueous environment from the membrane surface with a hydrophilic portion if they contain oxygen atoms, which confer them a more polar structure [14, 15]. Xanthophylls, such as lutein, fucoxanthin, neoxanthin, and xanthophyll cycle pigments, are among these more hydrophilic carotenoids. The presence of such carotenoids into the membranes may influence the thickness, fluidity, or permeability of them so that they can influence the stability of the cell membrane conferring it resistance, for instance, to ROS.

### **4. Photosynthetic and metabolic processes involved in the photoprotective responses in microalgae**

Damage occurs when the free radical encounters another molecule and seeks to find another electron to pair with. The unpaired electron of a free radical pulls an electron off of a neighboring molecule, causing the affected molecule to behave like a free radical itself.



A range of biochemical and biophysical techniques had provided a good understanding of the events that occur during absorption of the light energy, triggering the primary and secondary electron transfer processes leading to water oxidation. These electron transport pathways involve the redox state of the component of the electron transport chain, the plastoquinone (PQ) pool, which has been widely investigated, for its implication in the regulation of photosynthetic processes.

Under oxidative stress conditions, there is an accumulation of reducing power inside the cells, which increases the reduction of PQ-pool. For this reason, the redox level of PQ-pool play a crucial role in the induction of physiological responses to stress, and it is important also for the synthesis of carotenoids.

It has been shown that there is an involvement of the redox state of PQ pool in the distribution of light energy during photosystem II (PS II) and photosystem I (PS I), i.e., state transitions. State 2 transition is promoted by the reduction of the PQ-pool and consists in the transfer of the light harvesting complex associated with PSII (LHCII) to the PSI, whereas under State 1 transition, which occurs when the PQ-pool is oxidized, the LHCII is associated with the PSII [16, 17]

The degree of reduction of PQ pool is related to a switch between linear and cyclic electron flow. With an over-reduced PQ pool (State 2), the PSI cross section increases and a cyclic electron transport is promoted, by contrast under oxidative conditions (State 1), the cross section of PSII is decreased and linear electron transport can be observed [18–20]. This is one of the strategies that photosynthetic cells employ to reduce the impact of strong light intensity on the photosynthetic apparatus, and it is triggered by the PQ-pool overreduction, and it is commonly associated with induction of carotenoid synthesis. Indeed, under these conditions, the acidification of the thylakoid lumen occurs, and this can activate some enzymes involved in the carotenogenesis. For instance, the deepoxidation of violaxanthin to zeaxanthin, via antheraxanthin, is promoted by low pH in the thylakoid lumen [5, 21, 22].

The synthesis of these carotenoids is important for the cells not only because the deepoxidation is a quenching reaction but also because xanthophylls have the ability to donate electrons [23] and act as inhibitors of the process of oxidation even at relatively small concentrations. Antioxidants also act as radical scavengers and convert radicals to less reactive species.

## 5. Stress-inducing the highest synthesis of antioxidant compounds

Which are the main kinds of stress to induce the carotenoids synthesis?

All those kinds of stress reducing growth and photosynthetic efficiency so that the excess of energy not used for growth (i.e., converted into biomass) is accumulated as reducing power and generates free radicals. Some of the well-known microalgae high producers of carotenoids are reported in **Table 1**. For each microalga the main stress factor inducing the carotenoids synthesis is reported with the respective antioxidant pigment. The detailed explanation is reported below in the text.

Microalgae	Carotenoids	Stress conditions
<i>Haematococcus pluvialis</i>	Asatxanthin; cantaxanthin; lutein	High light Nitrogen starvation
<i>Dunaliella salina</i>	$\beta$ -Carotene	High light High temperature
<i>Scenedesmus</i> sp.	Lutein; $\beta$ -carotene	High light Nutrient starvation
<i>Phaeodactylum tricornutum</i>	Diatoxanthin; fucoxanthin	High light Nutrient starvation
<i>Isochrysis</i>	Diatoxanthin; fucoxanthin	High light Nutrient starvation
<i>Chlamydomonas reinhardtii</i>	Zeaxanthin; lutein	High light Sulfur starvation Anaerobiosis

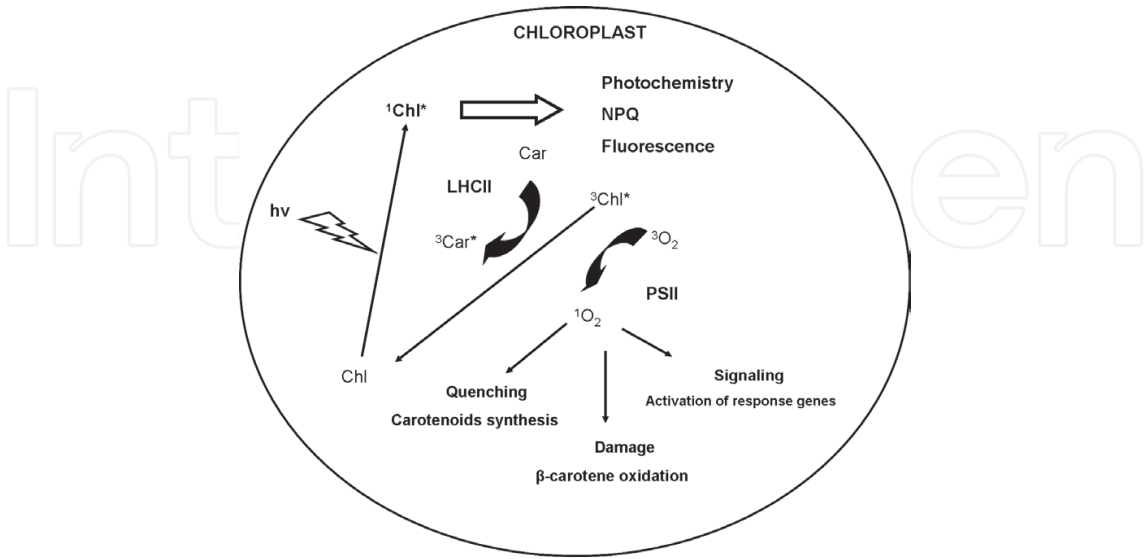
**Table 1.** Microalgae high producers of antioxidant carotenoids and stress conditions inducing their synthesis.

5.1. Light intensity

In particular, the exposure to high light is one of the typical stresses that microalgae may experience under environmental conditions. Indeed, during the central part of the day, the light irradiance may reach and exceed 1800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ .

A schematic explanation of the mechanism is reported in **Figure 2**.

Due to this accumulation of excess energy, leading to ROS formation, the synthesis of antioxidant carotenoids is induced in order to protect the cells from photodamage. Depending



**Figure 2.** Schematic explanation of induction of photoprotection by induction of carotenoids synthesis by high light stress.



on the kind of light and on the strain, the mechanism of induction may follow different metabolic pathways. For instance, in case of sudden exposure to high light intensity, the cells may react with the induction of the xanthophyll cycle, which is known to occur very quickly, within 15–30 min [24]. This phenomenon has been widely reported in the microalga *C. reinhardtii*, which is considered a model organism for physiological and biochemical study on photosynthesis, because it can be easily manipulated for genetic study, and it can grow very easily both under photoheterotrophic and autotrophic conditions [25]. For this microalga, the induction of zeaxanthin synthesis has been detected within 10 minutes of exposure to  $800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , but a partial induction of violaxanthin de-epoxidation to antheraxanthin and then this one to zeaxanthin could be observed already at  $300\text{--}350 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  [26].

The induction of the xanthophyll cycle may affect also the synthesis of diatoxanthin by the de-epoxidation of diadinoxanthin, which represents an additional xanthophyll cycle in diatoms and dinoflagellates, such as *Phaeodactylum* and *Isochrysis*, respectively, among the main producers of this carotenoid. In *Phaeodactylum tricornutum*, a rapid diadinoxanthin to diatoxanthin conversion has been reported, within 15 min, during exposure to sunlight in outdoor cultures in tubular photobioreactors, with the highest diatoxanthin concentration reached in the central part of the day (highest light intensity) [27]. In addition, these microalgae are well known for the synthesis of fucoxanthin and important antioxidant carotenoid. Fucoxanthin is mainly naturally found in marine microalgae, associated with thylakoid membranes, and it works by transferring excitation energy to chlorophyll *a*, driving electrons to the electrons transport chain [28, 29]. Fucoxanthin is usually found to be 0.22–1.82% in the biomass of these microalgae, but it can reach much higher concentrations in *Isochrysis* cultured at proper light intensity, cell density, and mixing. In particular, it has been observed that in this microalga, the effect of self-shading and low light intensity induced an increase in total carotenoid concentration, probably due to the increase of photosystem number under low light, and consequently of the primary carotenoids.

Among the strongest antioxidant carotenoids, the pigment lutein can be overexpressed during high light exposure. It is a very interesting pigment, as it is constitutively present in most of photosynthetic cells, and its synthesis may increase under photooxidative stress. The microalga *Scenedesmus* produced high amounts of lutein (over  $5 \text{ mg m}^{-2} \text{d}^{-1}$ ) in a tubular photobioreactor outdoor, under  $1900 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  and at  $35^\circ\text{C}$  [30]. In this case, the combined effect of high light and high temperature induced the increase of lutein. Indeed, usually, the optimal temperature of growth for microalgal strains is around  $25\text{--}28^\circ\text{C}$ .

Another carotenoid that usually increases during high light exposure is  $\beta$ -carotene. It is a pigment constitutively present in the microalgal cells, which may be oversynthesized under high light. One of the well-known microalgae for production of  $\beta$ -carotene is *D. salina* [31]. In laboratory conditions, it reached a production of  $13.5 \text{ mg L}^{-1} \text{d}^{-1}$  at light intensity in a range of  $200\text{--}1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , at  $30^\circ\text{C}$  [32].

One of the most important secondary carotenoids produced by microalgae is the red pigment astaxanthin. It is a very powerful antioxidant primarily synthesized by *H. pluvialis*, mainly under high light. However, although its synthesis is not so rapid, as it takes 1 day of sunlight

exposure to observe changes in the cells color, from green to red, it can reach a very high content, reaching 5% of the biomass. *H. pluvialis* has been widely studied for its astaxanthin production, due to its high productivity of this carotenoid, and for its robustness. Indeed, most of the studies carried out with *H. pluvialis* have been performed in outdoor cultures, using sunlight to induce astaxanthin production. These studies demonstrated that under environmental conditions, mainly in the summer period, and in very high illuminated areas, this microalga can grow and produce astaxanthin [33, 34].

## 5.2. Nutrient limitation

Nutrient limitation is another important stress condition inducing carotenoids synthesis and it is, like high light irradiance, a situation which can occur under environmental conditions. Macronutrient limitation, or starvation, is more incisive on the induction of protective responses than micronutrient limitation, as it directly affects growth, leading, mainly combined with light exposure, to the increase of reducing power, which is well known to activate defensive strategies such as the induction of the synthesis of certain carotenoids.

Nitrogen limitation is among the most studied nutrient-deprivation stress, as it is one of the most important elements in the cell, for its presence in proteins, enzymes, and because it is directly involved in the growth.

As previously reported in *Dunaliella* for  $\beta$ -carotene under high light stress, carotenoid increases in this microalga and this also occurs under nitrogen starvation. In particular, very interestingly, it has been shown that the increase in  $\beta$ -carotene content is concomitant with the synthesis of total fatty acid occurring under high light exposure and in combination with nitrogen starvation [35]. This can be explained by the fact that  $\beta$ -carotene is accumulated in lipid globules, in the cells, and it is supported by the findings that both lipid globules and  $\beta$ -carotene cannot be found when inhibitors of the fatty acid biosynthetic pathway are present [35]. At light intensity of  $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  under nitrogen starvation, a concentration of  $\beta$ -carotene of 2.7% of the biomass can be reached in *D. salina* [36].

A connection between lipid and carotenoid synthesis has been studied in *H. pluvialis*. In particular, the highest carotenoids accumulation has been observed with high light and nitrogen starvation combined, and under these conditions the astaxanthin content resulted more than two times higher than the control [37].

Under nitrogen starvation, astaxanthin synthesis is higher than in the control culture. Transition from the green stage to the red stage occurs during astaxanthin synthesis, due to the cytoplasmatic accumulation of the red pigment, which is observed within 20 h, reaching 1.4% of dry weight in the starved culture.

## 5.3. Overreduction of PQ-pool: anaerobiosis

Anaerobiosis is a condition that occurs when microalgal cells are cultivated in closed photobioreactors, in growth conditions that limit the photosynthetic activity; the oxygen evolution rate decreases reaching a value equal or lower than the oxygen respiration rate. Under light

exposure, the electrons are driven by light, from water to the electrons transport chain, but if the photosynthetic apparatus is affected, it is not able to use the accumulated electrons, overreducing the cellular environment. Moreover, under anaerobic conditions, the respiration cannot eliminate these reducing electrons, for lack of oxygen that is the final electron acceptor, and therefore, the reduction level of PQ-pool cannot be dissipated.

It has been demonstrated that anaerobiosis has a strongly negative impact on the performance of photosynthetic cells, but on the other hand, it can be a useful means to activate certain metabolic processes sensitive to oxygen, for example, hydrogen production, in some microalgal strains like *Chlamydomonas reinhardtii* [38]. In this microalga, chlorophyll fluorescence and oxygen evolution measurements indicated a strong reduction of photosynthetic activity under sulphur starvation, which leads to the formation of a strongly reductive environment inside the cell compartments. This stress activates an antioxidative response promoting the synthesis of lutein and zeaxanthin [39]. Imposing anaerobic conditions to *C. reinhardtii* in complete medium, it was possible to observe a strong promotion of the xanthophyll cycle; however, under these conditions, the time of induction was not shorter than 5 h, contrary to the short time of induction at high light intensity. After this period, the zeaxanthin content was 12.63 mmol mol<sup>-1</sup> Chl*a*. After 24 h it further increased, reaching 29.51 mmol mol<sup>-1</sup> Chl*a*. Anaerobiosis induced the overexpression of all the xanthophyll pool, which increased by 15%, indicating a *de novo* synthesis of these xanthophylls, in particular violaxanthin, showing that this type of stress is not able to induce a rapid zeaxanthin synthesis but is strong enough to promote mechanisms of photoprotection on a longer time scale, with accumulation of large amounts of xanthophylls. In addition, increases in lutein content, which more than doubled, and of  $\beta$ -carotene, which increased by 90%, were observed. This strategy was able to preserve cells from photodamage. A very interesting aspect of the microalgal metabolism of carotenoids is that pigment composition may be adjusted by the cells according to the environmental conditions, and that some synthetic pathways can be very fast, in order to optimize the cellular performance and to save energy and storage [40]. In *C. reinhardtii* cultures where the xanthophyll cycle had been induced, it has been shown that, after 1 h of aerobic dark adaptation, the pigments antheraxanthin and zeaxanthin decreased, as also did lutein and  $\beta$ -carotene, indicating the occurrence of a recovery. These findings underlined the very interesting peculiarity of microalgae, which consists in the strong capability to adapt to strong changes, in a different manner, according to the order of stress.

## 6. Importance of natural antioxidant compounds from microalgae and application in human health of antioxidants produced by microalgae

There is an increasing interest in the use of natural compounds in preventing and treating several diseases in humans, animals, and plants. For this reason, the research of a natural source of novel compounds with biological activity, in particular new and safe antioxidants, has gained a lot of importance.

Microalgae and cyanobacteria, under stress conditions, are capable of producing significant amounts of substances with high added value (antioxidant carotenoids, phenolic compounds, and polyunsaturated fatty acids), and for this reason, the study of the physiology of the growth of these microorganisms is of particular interest.

The secondary metabolites produced by photosynthetic organisms find numerous applications in the pharmaceutical, cosmetic, and food industries. In particular, the secondary carotenoids are widely used as antioxidants, acting as targets for highly reactive and toxic oxygen species, counteracting the effect of free radicals, and being effective as antiaging and anticancer agents.

Well known is the implication of carotenoids lutein and zeaxanthin in the pathologies of visual function, and the role of  $\beta$ -carotene in protecting the skin during exposure to the sun, and in the treatment of skin diseases.

It is well known that both lutein and zeaxanthin possess antioxidant properties due to their ability to quench singlet oxygen, reactive oxygen species, and free radicals [26, 41]. In particular, studies reported that an important role is played by lutein and zeaxanthin, constituents of the macular pigment, in the prevention of free radicals formation in the human retina, acting as quenchers [42–44]. This protective role against age-related macular degeneration makes these retinal carotenoids suitable for application as dietary supplements [45].

The antioxidant defense systems are important in maintaining good health, and therefore, an antioxidant-rich diet or antioxidant complements may be necessary as a health-protecting factor.

Interest in the employment of antioxidants from natural sources to increase the shelf life of food is considerably enhanced by the consumers' preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants. Dietary antioxidants include three major groups: vitamins (vitamin C or ascorbic acid and vitamin E or tocopherols), phenols, and carotenoids, which are precursors of some vitamins.

## 7. Conclusions

Very interestingly, there is an interconversion among carotenoids, as some of them are precursor of others, and their metabolic pathways are often correlated. For example, in one case, the  $\beta$ -carotene can be the precursor of the xanthophyll violaxanthin. Particularly, under a strong oxidative stress, the induction of the xanthophyll cycle, with the deepoxidation of violaxanthin to zeaxanthin, via antheraxanthin, is concomitant to the decrease of  $\beta$ -carotene that contributes to the de novo synthesis of violaxanthin. This phenomenon has been reported in *C. reinhardtii*.

Moreover, zeaxanthin is reconverted to antheraxanthin and violaxanthin by the enzyme epoxidase. The plasticity of the carotenoid metabolism and the strong induction of their synthesis achievable in microalgae make this argument very interesting in terms of biotechnological applications.

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