

## TECHNOLOGICAL INNOVATIONS IN THE CULTIVATION OF *HAEMATOCOCCUS PLUVIALIS* MICROALGAE: REVIEW

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### ABSTRACT

The cultivation of microalgae presents a great biotechnological potential, mainly to produce natural bioactive substances, which can be used in the pharmaceutical industry and especially in the development of functional foods, thanks to its nutritional properties. Among the commercially important microalgae, *Haematococcus pluvialis* is considered the main source of natural astaxanthin, a carotenoid of high antioxidant action and with wide applications in the nutraceuticals, cosmetics, food and aquaculture industries. This review aimed to cover the most important aspects of biology, biochemical composition, biosynthesis and astaxanthin accumulation in the cells of *H. pluvialis*, in addition to its broad application to humans and animals. The methodology used in this work was a systematic review of the literature, presenting the gaps and opportunities for research. This work provided a broader view of the technologies and methodologies used to produce *H. pluvialis*, providing a direction for future work to be undertaken. During the bibliographic survey, it was observed that information regarding the cultivation of *H. pluvialis*, aiming at the production of astaxanthin, is still very incipient in Brazil, with results observed only on a laboratory scale, making it difficult to really understand the implementation costs for a possible commercial production. This work has started a larger research and will serve as a basis for future activities, mainly to solve possible doubts.

**Keywords:** astaxanthin; carotenoids; nutraceuticals; antioxidant; aquaculture.

## 1. INTRODUCTION

Microalgae can be defined as fast-growing unicellular organisms that present chlorophyll-a, besides other photosynthetic pigments, whose main function is maintenance, development and cellular reproduction through carbon fixation, thus increasing their biomass. Microalgae account for approximately 60% of the planet's primary production, and when cultivated, some factors must be taken into consideration, including nutrients (carbon and salts), light (type and intensity), pH, and temperature (Santos, 2015).

In recent years, there has been an increase in interest in conducting studies on the production of microalgae. This is due to the importance of these microorganisms in the several trophic chains, as well as in the possibility of their use in areas such as human and animal health, nutrition, environment, energy production, and in obtaining compounds of interest to industries, food, chemistry and pharmaceuticals, among others (Bruno, 2001; Derner *et al.*, 2006; Grobbelaar, 2004; Richmond, 2004).

Some countries such as China, Israel, USA etc., already produce microalgae on a commercial scale. Among the various species that are cultivated, we can highlight *Chlorella* Beyerinck (Chlorophyceae) and *Arthrospira* Stizenberger, (Cyanophyceae) for addition to natural foods, and *Dunaliella salina* Teodoresco (Chlorophyceae) and *Haematococcus pluvialis* Flotow (Chlorophyceae), for obtaining carotenoids such as beta-carotene and astaxanthin (Becker, 2007; Derner *et al.*, 2006).

In this work, the main objective was to carry out a bibliographic survey showing the main methodologies and technologies used in the production of *Haematococcus pluvialis* microalgae, aiming at obtaining astaxanthin.

### Haematococcus pluvialis

The microalgae *Haematococcus pluvialis* belongs to the kingdom Plantae, Phylum Chlorophyta, Class Chlorophyceae, Order Chlamydomonadales, Family Haematococcaceae and genus *Haematococcus* (Algaebase, 2020). The microalgae *Haematococcus lacustris* is also considered a taxonomic synonym for *Haematococcus pluvialis*. Currently, 16 species are known within this genus and the first observations of the genus *Haematococcus* date from 1797, made by Girard-Chantrons, having the first description of *Haematococcus pluvialis* made by Flotow, in 1844 (Lorenz, 1999).

The first more detailed description of the life cycle of *H. pluvialis* was published by Hazen in 1899 in the Torrey Botanical Club newsletter. In this description, it was noted that the microalgae were often reddish in color. Thus, he began to describe the life cycle as if, in its first phase, *H. pluvialis* existed in a cyst state, in which the cells would present a reddish coloration and, respectively, a mobile state, with cells presenting a green coloration and flagella, followed again by a cyst state with a red coloration. Hazen did not identify the chemical nature of this reddish dye; thus, he named the substance hemato-chromium, which is currently known as carotenoid astaxanthin (Martins, 2014).

The *Haematococcus pluvialis* microalgae is unicellular mobile, biflagellated and uninuclear. Its cells have an ovoid, ellipsoid or ellipsoid cylindrical shape (Algaebase, 2020). During its growth, it can have both mobile and immobile forms. When mobile, egg cells can reach 8 to 50  $\mu\text{m}$  in diameter (Boussiba, 2000; Martins, 2014), presenting a protoplasm very far from the cell wall, which is relatively thin and separated from the plasmalemma by a mucilaginous space crossed by fine protoplasmic wires (Hoek *et al.*, 1995) (Figure 1).

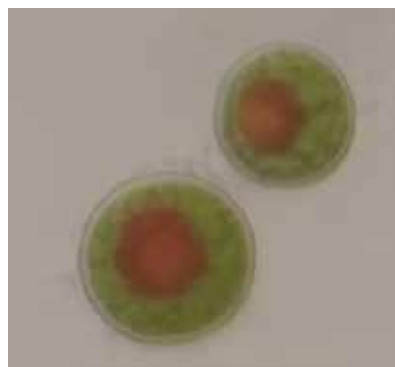


Figure 1. *Haematococcus pluvialis* (Flotow) microalgae.

Source: The Author.

When in its still form, called cyst, it has a thicker wall, with a small periplasmic space limited internally by a rather sinuous plasmalemma. The structural characteristics of protoplasm resemble those of mobile cells, except for the flagellae that are absent; however, no stigma or contractile vacuoles are observed (Figure 2) (Martins, 2014).

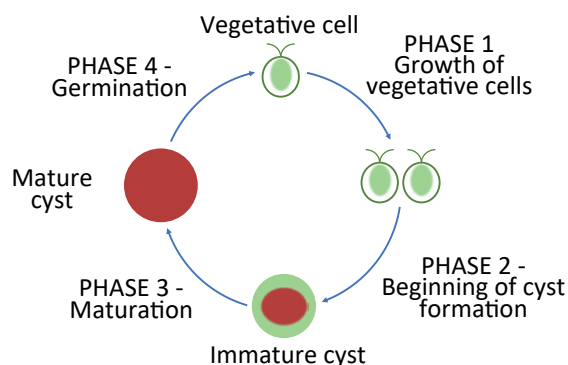


**Figure 2.** *Haematococcus pluvialis* (Flotow) in the form of green cysts.

Source: Elaborated from Martins (2014).

*Haematococcus pluvialis* reproduces asexually, with the formation of biflagellated zoospores, cysts or aplanospores and reproduces sexually by isogamy. Under stress conditions, such as nutrient scarcity, high radiation, saline, among other types, the mobile vegetative form becomes a more resistant property, called cyst (Martins, 2014). In this form, its cells are easily visible due to the reddish coloration they adopt. This coloration is due to the accumulation of the carotenoid pigment astaxanthin (Hoek *et al.*, 1995).

During the life cycle, growth of flagellate vegetative cells occurs, and they predominate as long as there are enough nutrients (Kobayashi *et al.*, 1997b; Martins, 2014). When environmental conditions become unfavorable, cysts are seen more frequently (Lorenz, 1999). Kobayashi *et al.* (1997a, 1997b) studied the life cycle of *H. pluvialis* over two weeks and investigated the mechanisms of morphological changes, dividing the life cycle into four phases: 1- growth of vegetative cells; 2- encystment; 3- maturation; 4- germination (Figure 3).



**Figure 3.** Schematic showing the main phases of the life cycle of *Haematococcus pluvialis* (Flotow). Source: Elaborated from Martins (2014)

In phase 1, initially, the vegetative cells grow. This growth is linked directly to environmental factors such as nutrients,

temperature, light, humidity, among others. In phase 2, the vegetative cells present an ellipsoidal shape and increase in number, besides moving actively due to the two scourges (Kobayashi *et al.*, 1997b).

In phase 3, the cells become immobile spherical cysts; therefore, this phase is named encystment. In this phase, the cyst is initially immature and goes through a series of physical and intracellular content changes (Kobayashi *et al.*, 1997b). The volume of the cells grows drastically and enters a phase of inactivity, in which the cell is enveloped by a wall of very resistant cellulose, composed of substances such as sporopollenin. The protoplast presents a reddish color due to the wide accumulation of astaxanthin (Boussiba, 2000). The biosynthesis of carotenoids in the cysts becomes quite significant, thus occurring the maturation phase, in which the immature cyst becomes mature (Martins, 2014).

When the culture medium becomes free of stressing agents, mobile cells reappear. This fourth phase is called germination (Martins, 2014).

It is worth mentioning that during the life cycle, the vegetative cells have high percentage of chlorophyll and protein, and low levels of carotenoids. During the encystment, there is a decrease in chlorophyll and proteins and an increase in carotenoid biosynthesis. Germination coincides with the synthesis of chlorophyll, proteins and carotenoid degradation (Martins, 2014). Due to the growing interest in natural astaxanthin production, knowledge of the life cycle of *H. pluvialis* becomes of great importance.

The typical natural habitat of *H. pluvialis* is almost always limited to small temporary pools, such as rock cavities periodically filled with rainwater, concrete basins and birdbaths. These sites are prone to rapid and extreme fluctuations of physical parameters, such as light intensity, temperature and salt concentration, or limiting conditions for any form of life (Burchardt *et al.*, 2006). Occasionally, *H. pluvialis* also appears in large numbers in rivers or on the banks of lakes, when exposed to extreme conditions (Canter-Lund; Lund, 1995).

## 2. BIOTECHNOLOGICAL POTENTIAL OF *HAEMATOCOCCUS PLUVIALIS*

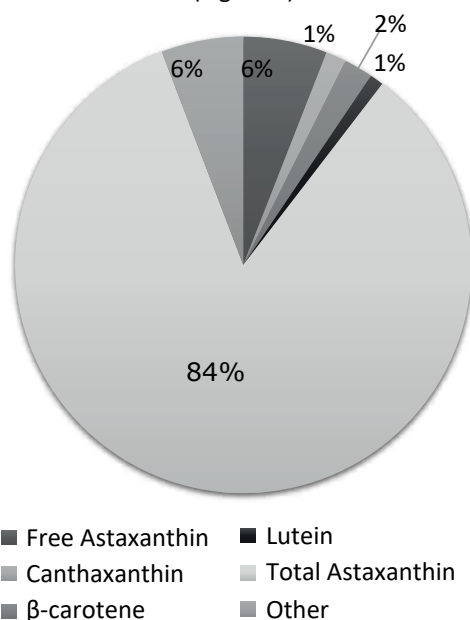
The trends related to the consumption of natural foods to improve human health are changing and the interest in these food products is increasing. Therefore, the food industry is developing new food products enriched with functional ingredients that can provide health benefits in addition to traditional nutrients (Yao *et al.*, 2020). Thus, microalgae can be of great relevance, since they are an important source of functional nutrients with several proven potential biotech-

nological applications (Michalak; Chojnacka, 2015).

Several studies have found that microalgae contain several bioactive compounds that present high added value in the market with important applications, mainly in the food, cosmetics, pharmaceutical and biofuel industries (Amaro *et al.*, 2011; Gouveia *et al.*, 2008). These bioactive compounds are recognized for preventing a variety of diseases and maintaining good health in humans (Plaza *et al.*, 2008; Rao; Rao, 2007).

Of these bioactive compounds, carotenoids are the most extensive class of pigments synthesized by microalgae. About 30 of them play a direct role in the capture of light and energy transfer during photosynthesis (Varela *et al.*, 2015), while others are involved in the defense mechanism against some free radicals that cause photo-oxidative damage (Li *et al.*, 2009). Among the variety of microalgae species, *Haematococcus pluvialis* is widely known to be the best in astaxanthin synthesis, reaching 3 to 5% of natural pigment per dry weight (Saini; Keum, 2018).

Ruiz-Domínguez *et al.* (2019), in a study with *H. pluvialis*, showed that of all the carotenoids found, total astaxanthin (considered the sum of astaxanthin-free esters and astaxanthin) had the highest concentration followed by  $\beta$ -carotene, canthaxanthin and lutein (Figure 4).



**Figure 4.** Concentration (%) of individual and total carotenoids extracted from the microalgae *Haematococcus pluvialis*. Source: Elaborated from Ruiz-Domínguez *et al.* (2019).

Astaxanthin is a carotenoid belonging to the subclass of xanthophylls, abundant in marine animals such as fish, crustaceans, algae and plankton (Hussein *et al.*, 2006). It is the main carotenoid found in wild salmon, giving its unique dark

red color (Higuera-Ciapara *et al.*, 2006; Sébert *et al.*, 2010). Astaxanthin produced from *H. pluvialis* is a natural primary source of astaxanthin for human consumption (Gong *et al.*, 2020; Fassett; Coombes, 2011; Sarada *et al.*, 2002).

Astaxanthin contains 40 carbon atoms and is characterized by the presence of oxygen in its molecular structure. It is structurally similar to  $\beta$ -carotene and other xanthophylls, such as lutein, canthaxanthin and zeaxanthin, which have a long hydrocarbon chain in common, with conjugated double bonds (polygenic chain), containing a carbon ring at each end. It differs from other carotenoids by the presence of hydroxyl (-OH) and ketone (C=O) groups in the end rings (Figure 5), which give the molecule greater polarity and antioxidant activity, when compared to the others (Yang *et al.*, 2013).

In its free form, astaxanthin is considered unstable and susceptible to oxidation and is found in nature in conjugated form with proteins or esterified with one or two fatty acid chains. In *H. pluvialis*, the esterified form predominates in the form of a monoester (Han *et al.*, 2013; Schütz, 2014).

Astaxanthin cannot be produced by higher animals and they absorb it through the consumption of natural sources such as algae, bacteria and fungi (Yuan *et al.*, 2011). Animals such as salmon, lobster, shrimp and trout acquire astaxanthin by consuming algae or bacteria that contain it, and its accumulation in their flesh, skin or exoskeleton is what gives them a rosy or reddish appearance (Kidd, 2011). Therefore, astaxanthin is also used as a food ingredient in aquaculture to produce a reddish color, especially in salmon, trout and shrimp farming (Higuera-Ciapara *et al.*, 2006). Humans can obtain astaxanthin by consuming seafood containing it or food supplements, synthetic or extracted from *H. pluvialis* (Kidd, 2011).

The astaxanthin synthesized by *H. pluvialis* is formed from  $\beta$ -carotene, when under stressing conditions during the biosynthesis of carotenoids (D'Alessandro; Antoniosi-Filho, 2016), and has a reddish color as a special feature (Gouveia *et al.*, 2008; Spolaore *et al.*, 2006; Zhang *et al.*, 2014).

Several organisms have the capacity to synthesize astaxanthin. Of these we can highlight the microalgae *Haematococcus pluvialis*, *Chlorella zofingiensis* and *Chlorococcum sp.*, the yeasts *Xanthophyllomyces dendrorhous* and *Candida utilis* and some bacteria, such as *Agrobacterium aurantiacum*, *Halobacterium salinarum*, *Mycobacterium lacticola* and *Brevibacterium spp.* (Ghiggi. 2007; Ip; Chen, 2005; Liu; Lee, 2000; Miao *et al.*, 2006; Schmidt *et al.*, 2011; Yuan *et al.*, 2002). Table 1 lists the main astaxanthin producing microalgae.

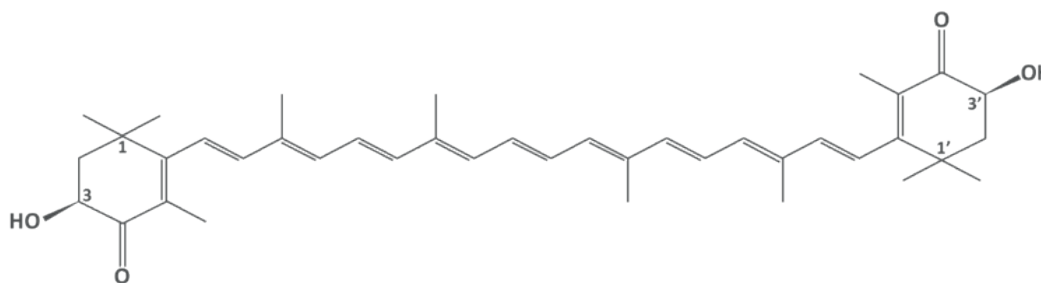


Figure 5. Molecular structure of astaxanthin.

Source: Elaborated from Yang et al., 2013

Table 1. Astaxanthin producing microalgae, according to the concentrations produced by dry biomass (mass % / mass).

Type of Microalgae	Astaxanthin %
Chloromonas nivalis	0.004
Botryococcus braunii	0.01
Chlamydocapsa spp.	0.04
Scenedesmus sp.	0.3
Chlorella zofingiensis	0.7
Chlorococcum sp.	0.7
Scotiellopsis oocystiformis	1.1
Protosiphon botryoides	1.4
Neochloris wimmeri	1.9
Haematococcus pluvialis	4.0

Source: Elaborated from Santos (2015).

Researches carried out with *Chlorella zofingiensis* attract some interests to this microalgae as an alternative producer of astaxanthin, due to its high growth speed in several cultivation conditions: photoautotrophic, mixotrophic and heterotrophic (Del Campo et al., 2007; Ip et al., 2004; Orosa et al., 2000; Sun et al., 2008). However, of the species presented in Table 1, the microalgae with the greatest astaxanthin producing potential in nature is the *Haematococcus pluvialis*, due to its capacity to accumulate large quantities of the carotenoid under stress conditions (Boussiba, 2000; Lemoine; Schoefs, 2010).

For the greater control of the processes, the production of astaxanthin on an industrial scale has usually been carried out by chemical synthesis derived from petroleum (Boussiba et al., 2000; Olaizola, 2003; Yoshihiro et al., 1997). Nonetheless, the synthetic form, besides having a high production cost, may present in its final product an astaxanthin with a structural configuration different from the natural one (Boussiba et al., 2000).

Several astaxanthin isomers have been characterized based on the configuration of the two hydroxyl groups of the molecule. The 3S,3'S stereoisomer is the main form found in *H. pluvialis*, while the synthetic form contains mainly the

3R,3'S isomer (Higuiera-Ciapara et al., 2006; Hussein et al., 2006; Kidd, 2011). This difference increases the instability of the molecule and its effect can be different from the natural astaxanthin, which is esterified, giving it greater stability and preventing against oxidation (Santos, 2015; Schmidt et al., 2011).

Synthetic astaxanthin is produced from petrochemical sources, which raises questions of food safety, pollution and sustainability (Li et al., 2011; Milledge, 2011). Its use is restricted to use as an additive in fish food for pigmentation purposes, and the direct consumption by humans in food or supplements is forbidden (Li et al., 2011). The use of synthetic chemical compounds has been strictly regulated (Yamane et al., 1997), making the natural source of astaxanthin the preferred (Kusdiyantini et al., 1998). Thus, as society stimulates a transition to "green solutions" and natural products, astaxanthin derived from algae seems to be gaining potential in the market (Panis; Rosales-Carreón, 2016; Nguyen; 2013; Pérez-López et al., 2014).

As for the use of astaxanthin produced by *H. pluvialis*, perhaps its greatest potential is in aquaculture, especially in the addition in diets for feeding fish and crustaceans, aiming to enhance the color of meat and skin, thus adding value to the product (Lorenz; Cysewski, 2000).

More than 95% of the aquaculture market consumes chemically synthesized astaxanthin derivatives. In parallel to this reality, the increase in consumption demand for natural products makes the chemically synthesized pigments less and less desirable, and they are acquired only due to greater supply in the market, which provides an opportunity for the production of natural astaxanthin (Lorenz and Cysewski, 2000; Ni et al., 2005; Orosa et al., 2005; Valduga et al., 2009).

Other market segments that natural astaxanthin can undertake are drugs and food, mainly for having known anti-inflammatory and antioxidant properties (Gross et al., 2006; Guerin et al., 2003). Astaxanthin contributes widely to the improvement of the immune system in humans and animals, and has several protective properties against inflammation, ulcer, cancer, neurodegeneration, diabetes and cardiovas-

cular diseases such as arteriosclerosis (Ciccione *et al.*, 2013; Lorenz; Cysewski, 2000), as well as liver protective effects (Yuan *et al.*, 2011). With the current pandemic caused by the coronavirus outbreak, Talukdar *et al.* (2020a, 2020b) suggest that the use of astaxanthin, associated with antiviral drugs, would greatly benefit patients with COVID-19, improving their health and reducing recovery time.

The production of carotenoids using biotechnology and in a natural way, such as astaxanthin, is a field of research of great interest due to its high market value and the growth of demand for natural products (Hui *et al.*, 2005).

The market value of astaxanthin generally ranges between \$2,500 - 7,000/kg, while its global market potential has been estimated at over \$1.5 billion by 2020 (Borowitzka, 2013; Koller *et al.*, 2014; Milledge, 2011; Panis; Rosales-Carreón, 2016; Pérez-López *et al.*, 2014). Of this market, more than 95% refers to astaxanthin produced synthetically, while that produced from algae represents approximately 1% of the quantity traded (Koller *et al.*, 2014; Li *et al.*, 2011; Pérez-López *et al.*, 2014).

### 3. HAEMATOCOCCUS PLUVIALIS CULTIVATION

The production of *H. pluvialis* microalgae provides some unfavorable characteristics when compared to the commercial scale production of other microalgae, mainly due to the complexity of its life cycle and its slow growth (Cifuentes *et al.*, 2003). These same authors show that besides the influence of factors such as nutrients, light intensity and saline stress, cultivation time is also crucial, since younger cells are more sensitive to factors that trigger adverse conditions.

The astaxanthin production process may be limited due to the low productivity of green cells in *H. pluvialis* crops, since astaxanthin is accumulated inside the cysts. Therefore, optimization of the vegetative growth phase is important to achieve good astaxanthin yields (García-Malea *et al.*, 2006; Ghiggi, 2007; Santos, 2015).

Several limiting factors lead to reduction in growth rate and, concomitantly, reflect in fewer astaxanthin producing cells. Both in the natural environment and in controlled crops, the growth of microalgae population is a result of the interaction between biological, physical and chemical factors (Vonshak; Torzillo, 2004).

The manipulation of environmental and nutritional factors, especially the variation in light intensity and nitrogen source, optimize the growth of *H. pluvialis* (Zhang *et al.*, 2009). Cavalheiro *et al.* (2000), in a work carried out for approximately 38 days, submitted *H. pluvialis* to a crop in which the light intensity was  $70 \mu\text{E m}^{-2} \text{s}^{-1}$ , photoperiod of

12 hours, besides the appropriate culture medium with the addition of vitamin B12 and biotin. The authors verified that the mobile cells, which were predominant in the first days, were gradually replaced by cysts, which became dominant from the 12<sup>th</sup> day, reaching a maximum density on the 33<sup>rd</sup> day of cultivation.

After submitting flagellated cells of *H. pluvialis* to the various luminous intensities, Torzillo *et al.* (2005) concluded that  $200 \mu\text{mol m}^{-2}\text{s}^{-1}$  was the ideal intensity for growth, representing a limit above which changes in photochemical parameters and pigment composition would occur.

Goksan *et al.* (2011) analyzed the growth characteristics of *H. pluvialis* when affected by the nitrogen source (sodium nitrate, potassium nitrate, ammonium nitrate, and urea), vitamins and light. The best growth occurred when the sodium nitrate concentration was 1.0 g/L and the potassium nitrate 0.5 g/L, with luminosities between 75 and  $150 \mu\text{mol m}^{-2}\text{s}^{-1}$ .

Biological factors are related to the individual metabolic rates of the cultivated species, as well as its dynamics in the community in which it is inserted, where other organisms can alter the development of the microalgae. Chemical factors related to the composition of the culture medium influence both growth and biochemical composition, which varies with the availability of nutrients, salinity and pH. Physical factors, however, are related to photoperiod, light intensity, and temperature (Devgoswami *et al.*, 2011; Fábregas *et al.*, 2001; García-Malea *et al.*, 2009; Guerin *et al.*, 2003; Hata *et al.*, 2001; Huang *et al.*, 2019; Imamoglu *et al.*, 2007; Rousch *et al.*, 2003).

According to Gladue (1991), most algae species are photoautotrophic, and their production of organic material is a reflection of photosynthetic activity, which can be expressed by the increase in population (Balech, 1977), that is, the growth rate. In crops, the luminosity presents itself as one of the main factors of microalgae growth and varies according to depth, latitude and time (daily and seasonally). Of all the electromagnetic radiation incident on photosynthesis organisms, only the visible spectrum, i.e. wavelengths between 400 and 720 nm (photosynthetically active radiation - PAR), can be absorbed and used for photosynthesis (Lips; Avissar, 1990).

Chlorophylls, carotenoids and phycobilins are the main pigments involved in photosynthesis in microalgae and each one absorbs specific wavelengths. Approximately 40% of the solar energy that falls on the earth's surface on an open day constitutes PAR and is equivalent to about 1800 - 2000  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ . Once captured by the photosynthetic pigments, the luminous energy is transferred to the reaction centers where it will be used for the photochemical reac-

tions (Masojídek *et al.*, 2013; Santos, 2015). The amount of light energy received by the photosynthetic system will impact on the amount of carbon that can be fixed, determining the biomass production and its growth rate (Tzovenis *et al.*, 2003). The light regime is also a critical component in the projection of biomass production (Falkowski; Raven, 2007; Kirk, 1983; Tzovenis *et al.*, 2003).

After studies, Litchman (1998) realized how photoautotrophic phytoplankton growth rates are affected in different conditions of photoperiod and irradiance. A change in the light regime can also influence the rate of nutrient absorption, so the author concluded that excess light is harmful to photoautotrophs, because it can generate inhibition effect on pigments through their photo-oxidation and also the enzymes involved in the photosynthetic process (Boney, 1989).

Hence, not only the intensity of light and its photoperiod, but also its quality interfere with the growth of the microalgae. According to Katsuda *et al.* (2004), *H. pluvialis* showed higher vegetative growth when illuminated with red LED, but when submitted to blue LED, a suppression in vegetative growth and the induction of intense astaxanthin accumulation were observed.

Temperature, irradiation, nitrate and phosphate concentration, and pH of the interaction of these factors are responsible for significant differences in the growth of the microalgae *H. pluvialis* (Borowitzka *et al.*, 1991; Boussiba, 2000; García-Malea *et al.*, 2006; Orosa *et al.*, 2005; Tran *et al.*, 2019).

For nutrition, microalgae need several mineral nutrients, and some species have better performance with the addition of vitamins. The macronutrients required by microalgae are carbon, nitrogen, oxygen, hydrogen, phosphorus, calcium, magnesium, sulfur, and potassium. Micronutrients, trace metals or minority elements are also required, and generally require iron, manganese, copper, molybdenum and cobalt (Barsanti; Gualtieri, 2006).

The microalgae have specific nutritional needs and to optimize the production of green *H. pluvialis* cells a high concentration of nitrate and phosphate (20 and 1 mM, respectively), pH between 6.0 - 7.0, and addition of acetate (0.25% w/v) as additional energy source are necessary (Borowitzka *et al.*, 1991; Boussiba, 2000; Orosa *et al.*, 2005).

Among the macronutrients, carbon is the element used in higher concentrations, including concentrations of mixotrophic species. This demand is due to the presence of carbon in all organic molecules produced, such as proteins, carbohydrates, nucleic acids, lipids and others. Microalgae cultures may become limited in carbon, even at low cell density (Lombardi; Maldonado, 2011; Tran *et al.*, 2019) and,

depending on the growth rate, there will be rapid carbon consumption available with culture pH elevation to pH 9.0 in non-buffered systems (Lourenço, 2006; Santos, 2015).

As for the pH of the crop, it should be controlled in a specific way, since it is important that the nutrients are available in the culture medium so that the microalgae can use them (Han *et al.*, 2020). The addition of carbon dioxide (CO<sub>2</sub>) can serve as a nutrient, but it also helps to control the pH, which naturally increases due to the growth of microalgae (Lourenço, 2006; Santos, 2015; Tran *et al.*, 2019).

In crops grown in closed systems, the pH can reach high values and make the medium unsuitable for growth. Thus, the use of pH buffers can prevent their variation or even make them discrete, besides making them more tolerable for microalgae (Han *et al.*, 2020; Lourenço, 2006; Santos, 2015).

Regarding the extraction of astaxanthin from the microalgae *H. pluvialis*, the most common methodologies are the following: microwave assisted extraction, ultrasound, high pressure homogenization (HPH), pressurized liquid extraction (PLE), enzyme assisted extraction and supercritical fluid extraction (SFE), which is generally based on the use of supercritical carbon dioxide (SC-CO<sub>2</sub>). The latter is more attractive than other methods for the recovery of valuable compounds, even after considering environmental protection (Yen *et al.*, 2015) since many bioactive natural products are thermally unstable and can degrade during the use of traditional extraction methods. Thus, the use of SC-CO<sub>2</sub> has proven to be more effective in the extraction of bioactive compounds (Da Silva *et al.*, 2016; Gałuszka *et al.*, 2013; Reyes *et al.*, 2014), preserving these biomolecules from degradation during the extraction process.

#### 4. FINAL CONSIDERATIONS

In recent years, there has been a growing interest in natural astaxanthin produced by microalgae *H. Pluvialis* to the detriment of artificially produced astaxanthin. In view of this, many scientific improvements have been achieved, mainly in innovation, technology for large-scale production and services, always aiming at a more refined astaxanthin. Nevertheless, its commercial production is still rather expensive because the initial investment depends on state-of-the-art technology.

Despite significant advances in research and development of new technology for astaxanthin production by the microalgae *H. pluvialis*, its cultivation in Brazil is still on a laboratory scale and faces difficulties to be implemented on a commercial scale. We believe that to improve the production capacity of *H. Pluvialis*, we will have to invest in the

development of technological innovations, in the reduction of production costs, good cultivation practices and in the improvement of astaxanthin.

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