

# **Algae: A Promising Source of Bioactive Pigments**

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Abstract: Algae have been recognized as natural sources of bioactive commercial pigments. These organisms use pigments to capture sunlight energy for photosynthesis. The pigments in algae are categorized into chlorophylls, phycobilins, and carotenoids. Notable carotenoids include astaxanthin, lutein, fucoxanthin, canthaxanthin, zeaxanthin, and β-cryptoxanthin, which have applications in antioxidant, anti-inflammatory, immunoprophylactic, and antitumor activities, among others. Due to the double bonds in their structures, these carotenoids offer a wide range of health benefits and protect other molecules from oxidative stress induced by free radicals through various mechanisms. These carotenoids are primarily synthesized by certain species, though they also appear as byproducts in several species, depending on their metabolic pathways and genetic capabilities. *Haematococcus pluvialis* and *Chlorella zofingiensis* are particularly suitable for the commercial production of astaxanthin. This review presents the latest information on microalgal pigment production, the factors influencing their production, their applications, and future prospects.

Keywords: Algae, Astaxanthin, Pigments, Carotenoids, Photosynthesis, zeaxanthin

#### 1.Introduction

Microalgae constitute the basis of life in a variety of marine and freshwater ecosystems. Chlorophyceae, Chrysophyceae, and diatoms are the three major groups (Bacillariophyceae) (Guiry, 2012). These algae can grow quickly as a hanging stratum on the surface of the water and can survive with harsh environmental conditions. Microalgae are great sources of value-added products which include proteins, polysaccharides, lipids, polyunsaturated fatty acids, pigments, vitamins, and minerals which have numerous health benefits and trading potentials (Silva et al., 2020). Several cultivation methods have been investigated to enhance product accumulation from various algae, their biomass is explored to investigate numerous bioactive molecules (Patel et al., 2021) high value products. The pigment is the most important among them due to its ecological relevance as well as a wide spectrum of health applications.

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Microalgae form the foundation of life in many marine and freshwater ecosystems. The three major groups include Chlorophyceae, Chrysophyceae, and diatoms (Bacillariophyceae) (Guiry, 2012). These algae can rapidly form a floating layer on the water's surface and thrive under harsh environmental conditions. They are excellent sources of high-value products such as proteins, polysaccharides, lipids, polyunsaturated fatty acids, pigments, vitamins, and minerals, which offer numerous health benefits and commercial opportunities (Silva et al., 2020). Various cultivation techniques have been explored to boost the accumulation of products from different algae, with their biomass being studied for numerous bioactive molecules and high-value products (Patel et al., 2021). Pigments are particularly significant due to their ecological importance and wide range of health applications.

Algae possess a wide range of visible light-harvesting complexes known as pigments, including chlorophylls, carotenes, xanthophylls, and phycobilin proteins. There are two types of carotenes found in algae: α-carotene, present in Cryptomonads and some Chlorophycean members, and β-carotene, found in certain Chlorophytes. The most well-known algal xanthophylls include lutein, astaxanthin, canthaxanthin, and fucoxanthin. These xanthophylls are primarily produced by species such as Scenedesmus spp., *Haematococcus lacustris* (formerly *H. pluvialis*), *Spirulina platensis, Dunaliella salina, Chlorella* spp., *Botryococcus braunii, Bracteacoccus*, dinoflagellates, and diatoms (Lamers et al., 2011; Chivkunova and Lobakova, 2021; Dizaji et al., 2021). Additionally, there are three types of phycobiliproteins (PBPs): phycoerythrins (red; absorbing at 540–570 nm), phycocyanins (blue; absorbing at 651–655 nm), and allophycocyanins (blue; absorbing at 540–570 nm). These phycobiliproteins are also found in certain blue-green algae such as cyanobacteria, rhodophytes, cryptomonads, and glaucocystophytes (Sonani et al., 2017).

Various biotic and abiotic factors can influence the production of algal pigments. These factors include light irradiance, photoperiod length, nutrient availability, pH, temperature, salinity, and the presence of heavy metals and pesticides (Musa et al., 2019; Lopez and Hall, 2021; Yun et al., 2020; Mogany et al., 2021).

## 2. Characteristics of Various Algae-Derived Pigments

Various pigments have been isolated from microalgae, including carotenoid, fucoxanthin, and violaxanthin, and these are extensively used in commercial products such as cosmetics and biomaterials (Martinez Andrade et al., 2018). Microalgal pigments are primarily Cuest.fisioter.2025.54(3):4767-4778



divided into two main groups: carotenes and xanthophylls. Well-known carotenes include β-carotene and lycopene. Xanthophylls, which are oxygen-containing carotenoids known as oxycarotenoids, form the second group. Among these, lutein and zeaxanthin have –OH groups, while canthaxanthin and echinenone have =O groups. Astaxanthin contains both –OH and =O groups. Additionally, diadinoxanthin and violaxanthin include epoxy groups, whereas fucoxanthin and dinoxanthin feature acetyl groups. Both of these carotenoids also contain the C=C=C or allene group along with acetyl groups (Sathasivam et al., 2019). Due to these structural groups, most of these carotenoids are more soluble in water, except for lutein, which behaves like a carotene despite being an oxycarotenoid. Phycobilins are highly water-soluble due to their numerous oxygen-containing structures, making them widely used as dyes in food and as fluorescent markers in molecular biology (Jain and Sirisha, 2020). Additionally, algal chlorophylls are fat-soluble pigments that have various health applications. Table 1 summarizes the production and extraction of pigments from various microalgae strains along with their potential applications.

Table 1. Pigment production and extraction from potential microalgae and their applications.

| Algae                   | <b>Mode of Cultivation</b> | Method of           | Application         | References        |
|-------------------------|----------------------------|---------------------|---------------------|-------------------|
|                         |                            | Extraction          |                     |                   |
| Astaxanthin             |                            |                     |                     |                   |
| Haematococcus           | mixotrophic                | extracted using     | pharmaceutical      | Panutai et al.,   |
| pluvialis               |                            | ethyl               |                     | 2021              |
|                         |                            | acetate             |                     |                   |
| Haematococcus           | autotrophic                | dimethyl sulfoxide; | pharmaceutical      | Le-Feuvre et al., |
| lacustris               |                            | acetone             |                     | 2021              |
| Haematococcus lacustris | mixotrophic                | 90% acetone         | industrial pigments | Han, et al., 2019 |
| α-carotene              |                            |                     |                     |                   |
| Tetraselmis chui        | autotrophic                | acetone, then       | nutraceutical and   | Ahmed et al.,     |
|                         |                            | hexane              | pharmaceutical      | 2014              |
| Dunaniella salina       | autotrophic                | mortar and pestle;  | nutraceutical and   | Ahmed et al.,     |
|                         |                            | acetone, then       | pharmaceutical      | 2014              |
|                         |                            | hexane              | industries          |                   |
|                         |                            |                     |                     |                   |

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| β-carotene                  |                             |   |   |                              |
|-----------------------------|-----------------------------|---|---|------------------------------|
| Scenedesmus<br>quadricauda  | autotrophic                 | extracted with acetone                                | ood processing, animal feeds, pharmaceutical, | Rajput et al.,<br>2021       |
| Nannochloropsis<br>gaditana | mixotrophic                 | ultrasonicated;<br>extracted with<br>acetone: methano | cosmetics and pharmaceuticals                 | Di Lena et al.,<br>2019      |
| Lutein                      |                             |   |   |                              |
| Chlorella sorokiniana       | autotrophic                 | green microwave assisted extraction;                  | industrial applications                       | Mary Leema et al., 2022      |
| Scenedesmus<br>quadricauda  | autotrophic                 | extracted with acetone                                | pharmaceutical, and cosmetics industries      | Rajput et al.,<br>2021       |
| Zeaxanthin                  |                             |   |   |                              |
| Synechococcus sp.           | autotrophic                 | freeze dried, zirconium beads added with methano      | pharmaceuticals, and antioxidant production   | Bourdon et al.,<br>2021      |
| Synechocystis sp.           | autotrophic                 | freeze dried,   | pharmaceuticals, vitamin,                     | Bourdon et al.,<br>2021      |
| Canthaxanthin               |                             |   |   |                              |
| Bracteacoccus aggregatus    | autotrophic                 | extracted with dimethyl sulfoxide                     | industrial pigment production                 | Checanov et al., 2021        |
| Neoxanthin                  |                             |   |   |                              |
| Desmodesmus sp.             | autotrophic                 | ultrasound; hexane:<br>ethanol<br>extraction          | commercial production                         | Soares et al.,<br>2016       |
| Phycobiliproteins           |                             |   |   |                              |
| Arthrospira platensis       | Autotrophic/<br>mixotrophic | Ultrasonication/<br>liquid Pressurized<br>extraction  | Food additive/                                | Panutai and<br>Iamtham, 2019 |



## 3. Factors influencing pigment production

Numerous algae are utilized in various commercial sectors including food (Khan et al., 2018; Vigani et al., 2015), cosmetics (Martinez Andrade et al., 2018), pharmaceuticals (Khan et al., 2018), and biofuels (Choi et al., 2019). The rich content of lipids, proteins, and other essential components in microalgae contributes to their widespread use. To achieve high biomass production, it is crucial to develop algae cultivation techniques that are both feasible and economically viable (Khan et al., 2018). Selecting appropriate methods for culturing and extracting is essential to maximize the value of raw materials. Furthermore, optimizing parameters that significantly impact pigment production and yield is necessary to enhance output.

The following parameters can be focused on for optimization.

# **3.1. Light**

The duration and intensity of light directly influence photosynthesis (Metsoviti et al., 2019), impacting the biochemical composition of microalgal biomass and serving as a growth-limiting factor (Musa et al., 2019). While light is essential for photosynthesis, excessively high light levels can have detrimental effects, damaging the photosynthetic machinery beyond its tolerance limit (Sim et al., 2019).

Blue and green light wavelengths penetrate the water column, with water molecules absorbing shorter wavelengths and scattered particles. Green algae possess primary pigments like chlorophyll a and b to capture light, and within their tolerance limits, increasing light intensity enhances their growth rate (Singh and Singh, 2015). Microalgae growth is directly related to light utilization, which is regulated by precise photoperiod and light intensity (Patel et al., 2019). Therefore, light, among other factors, significantly influences algae growth.

Changes in light intensity significantly impact accessory pigments involved in photosynthesis, alongside chlorophyll a. The ratio of chlorophyll-a to lipophilic pigments remains consistent even when light duration rapidly fluctuates with increasing intensity (Wang et al., 2020). Elevated light stress, resulting from intolerable intensities, leads to heightened



pigment accumulation in algae. For instance, β-carotene and phycocyanin production increase in *Dunaliella salina* and *Spirulina platensis* under rising light intensity (Walter et al., 2011). Certain carotenoid pigments not only capture light but also dissipate excess energy. Green and blue light exposure with photoperiods of 8 and 14 hours respectively prompts increased concentrations of phycocyythrin and phycocyanin in *Gracilaria birdiae* (Barufi et al., 2015).

### 3.2. Salinity

Cellular osmosis plays a crucial role in pigment production across all marine microalgae cultures, particularly in hypertonic solutions formed within cells when exposed to higher concentrations of sodium chloride in the external environment (Sikorski, 2021). Consequently, cellular dehydration damages internal components, leading to cell shrinkage (Lopez and Hall, 2021). The broader salinity tolerance range also depends on cultivation methods, such as semi-continuous cultivation, and the degree of salinity increase. Microalgae need to gradually adapt to extreme salinity conditions by exposure to steadily rising salinity levels over an extended period in their habitat (Ishika et al., 2017). Increased salinity tolerance in microalgae is attributed to the accumulation of osmotic organic solutes like glycerol, mannitol, sucrose, and proline (Krist, 1990). For instance, the green alga Chlorella vulgaris exhibits a notable decline in total chlorophyll and carotenoids with increasing salinity concentration, suggesting the necessity to maintain a minimum NaCl concentration (0.0 to 0.1 Molar) for optimal pigment and biomass yield (Kalla and Khan, 2016). Nannochloropsis sp. and Tetraselmis sp. achieve high cell density, protein, lipid, and carbohydrate content at a salinity of 28 ppt (Arkronrat et al., 2016).

Microalgae's ability to tolerate salinity also has practical applications in desalination, where they can help reduce the salinity of seawater, thereby lowering the burden and cost of the reverse osmosis process in the final stages of potable water production (Patel et al., 2021).

#### 3.3. pH

pH plays a crucial role in influencing pigment production during the vegetative growth of algae. Research suggests that pH levels below 5.0 and above 8.5 inhibit the growth of microalgae (Patel et al., 2019). Yongsmith et al. (2000) found that lower pH levels favor the synthesis of yellow pigments, while higher pH levels favor the production of red pigments. pH modification affects the solubility of substances and nutrient absorption by cells.



Nannochloropsis sp. and Tetraselmis sp. achieve high cell density, protein, lipid, and carbohydrate content when cultured at pH levels of 7.5 and 8.5 (Arkronrat et al., 2016). However, sudden pH fluctuations in the microalgae culture medium may hinder chlorophyll and carotenoid production, ultimately impacting growth and biomass yield.

Furthermore, pH also plays a significant role in influencing the extraction yield of products synthesized in algae biomass during the growth phase. Kulkarni and Nikolov (2018) observed variations in pigment extraction yields from frozen and wet Chlorella vulgaris biomass with pH fluctuations. The optimal pH for algal growth and pigment yields varies depending on various factors such as algal species, media type, and laboratory cultivation conditions (Ogbonda et al., 2007). However, alterations in pH did not notably impact the nutritional value of protein fractions. It's worth noting that antioxidant capacity tends to be higher in alkaline environments.

# 3.4. Temperature

Temperature is a critical factor influencing biomass production in phototrophic microalgae, as it affects metabolic processes and biochemical structures within cells (Khan et al., 2018). Studies indicate that elevated temperatures promote pigment synthesis in microalgae. Blue-green microalgae cultures, for instance, often exhibit increased production of carotenoids, particularly β-carotene, in response to higher temperatures (Begum et al., 2016). The optimal temperature for *Dunaliella salina* growth has been identified as 22°C, resulting in the highest β-carotene production levels (Wu et al., 2016). Temperature variations can impact cells differently based on their envelope characteristics; low and high temperatures can alter the cell envelope, making it more rigid and permeable. Drastic temperature changes can induce stress in microalgae, while rising temperatures can disrupt cell hydrophobicity balance (Novosel et al., 2021). The wall-less alga *Dunaliella tertiolecta* exhibited the highest sensitivity to temperature variations. These attributes are significant in the context of pigment extraction and associated costs, which depend on the chosen cell disruption technique

### 3.5. Nutrients and Mixing

The essential nutrients required for the growth of autotrophic microalgae include carbon (C), nitrogen (N), and phosphorus (P). Limiting these nutrients can lead to substantial variations in biochemical components (Perez-Garcia and Bashan, 2015). Studies have shown



that microalgae such as Chlorella vulgaris and Chlorella protothecoides demonstrate higher nutrient uptake efficiencies from aerobic digestion (AD) effluents compared to Bristol medium. Notably, NH4 +-N substrate was extracted within ten days of cultivation, indicating its high availability and utilization potential alongside other potential nutrients.

The algae exhibited enhanced growth when cultivated on AD effluents in mixed-culture conditions alongside distinct bacteria, likely due to the presence of bioavailable nutrients. This improved nutrient uptake from AD effluents led to higher biomass and pigment yields (Yu et al., 2019). Adequate nutrient supply stands as one of the paramount factors influencing pigment yield in algae. Maintaining mixing and aeration in the culture system is crucial for achieving high production. Mixing ensures uniform distribution of nutrients, air, and CO2 throughout the microalgae culture, facilitating even light distribution and preventing biomass settling (Show et al., 2017). Despite meeting other criteria, biomass productivity significantly decreases without proper mixing. Hence, regular mixing of microalgae cultures is necessary to keep all cells suspended, ensuring unrestricted access to light and nutrients. Photobioreactors equipped with effective mixing systems enhance nutrient dissolution, light penetration, and gaseous exchange, promoting optimal growth conditions (Perez-Garcia and Bashan, 2015).

# **Future perspectives**

Given the detrimental effects of synthetic colors and pigments, natural pigments are more appropriate for health-related applications, with algal pigments being particularly effective. However, despite their growing use as a sustainable natural source for bio-pigment production, there remain challenges in microalgal bio-pigment production technology. These challenges include boosting microalgal biomass yield, developing cost-effective and rapid methods for high-yield pigment extraction, and addressing issues related to scaling up the production process.

For commercial production, achieving a higher yield of pigments is crucial, necessitating the bioprospecting of new or previously untapped environments to find high-yielding microalgal strains. However, native microalgal strains typically produce low biomass yields, making their enhancement through 'omics'-based technologies such as proteomics, genomics, and metabolomics highly relevant. Future research should focus on the regulatory mechanisms of pigment production to facilitate the use of genetic engineering, metabolic



engineering, and synthetic biology strategies to enhance existing pathways or develop new pathways for increased pigment production in microalgae. Recently, regulating the trophic mode by switching from autotrophic to mixotrophic cultivation has shown promise in improving biomass yield. Since pigment yield is closely linked to biomass yield, increasing biomass yield by 3-5 times in the mixotrophic mode under optimal conditions is a significant development.

# **Conclusion**

Microalgal pigments, with their significantly higher bioactive potential compared to other biological pigments, are experiencing increasing demand as food additives, feed, nutrient supplements, and dyes. Furthermore, their application as nutraceuticals, cosmeceuticals, and pharmaceuticals has gained traction in recent years. Their high antioxidative potential enables broader applications as anticancer and anti-inflammatory agents. Despite their great diversity, only a limited number of pigments have been characterized for health applications. Further research is necessary to fully explore their untapped potential, requiring more in-depth structural characterization to define their applications.

#### Credit authorship contribution statement

**Jyothi K**: Conceptualization, Writing – original draft, Formal analysis, Investigation, Project administration, **M K Prasad**: Writing – review & editing.

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