Study of Certain Algal Pigmentation

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ABSTRACT
Bioactive commercial pigments derived from algae have long been known. To power their photosynthetic process, algae use pigments that absorb light. Chlorophylls, Phycobilins, and carotenoids are the three main types of pigments found in algae. Among the many roles played by carotenoids, which are notable for their antioxidant, anti-inflammatory, immunoprophylactic, and anticancer properties, are astaxanthin, lutein, fucoxanthin, canthaxanthin, zeaxanthin, and β-cryptoxanthin properties. They protect other molecules from reactive radical-induced oxidative stress in various ways, and their existence of double bonds gives them broad health uses. Depending on the metabolic pathway and genetic potential, some species produce these carotenoids as main products and others as by-products this research presents innovative procedures for the synthesis, extraction, and purification of microalgal pigments.

Key Words: Algal Pigments, Chlorophylls, Phycobilins, Carotenoids

INTRODUCTION
Microalgae are the foundational organisms of a wide variety of freshwater and saltwater aquatic environments. There are 16 distinct categories to which 72,500 of them belong. In the kingdom of algae, the three main orders are diatoms, chlorophyceae, and chrysophyceae. Cyanobacteria, cyanophyceae, and diatoms have been the subjects of the most research due to their biological significance (Garrido, et.al 2018). Because of their resiliency and potential for fast growth, algae may cover the water's surface in a scum. There is a global push to study and cultivate algae for the purpose of extracting valuable chemicals. Microalgal biomass is produced by a number of countries, including Germany, Japan, China, and Taiwan. Annually, these countries generate 19,000 metric tonnes of dehydrated biomass, which is valued at 5.7 billion USD (Lichtenthaler, H.K., 1987). Microalgae have a wealth of valuable products that may be used in medicine and trade. These products include protein, polysaccharide, lipid, pigment, vitamins, and minerals. Numerous noteworthy metabolites, each with its own physiological functions and beneficial health effects, are found in algae. Bioactive compounds, high value goods, and biofuel production are just a few areas that have benefited from algae biomass research (Patel et al., 2021). The pigment stands out among them all due to its many positive effects on health and the environment (Ambati et al., 2019). Chlorophylls, carotenes, xanthophylls, and phycobiliproteins are just a few of the many pigments found in algae that help them absorb light. While certain phyla are exclusive to certain pigments like chlorophylls or phycobilins, all algae include chlorophyll-a, the main photosynthetic pigment, and it efficiently absorbs light throughout the visible spectrum (Deepika et al., 2022). The presence of conjugated ring structures that make up its backbone causes it to absorb light in the visible range. The tetrapyrrole ring that makes up chlorophyll is a biosynthetic variation of protoporphyrin IX and consists of four pyrrole molecules. According to Deepika et al. (2022), the central Mg^{++} atoms of the porphyrin are held in place by covalent and coordinate bonds. Carotenoids are polyene complexes that include orange or yellow "ionone" rings. Carotene is a hydrocarbon based on its molecular structure; it is not called oxycarotene or xanthophyll if it does not include oxygen. The two types of carotene found in algae are α-carotene, which is found in Cryptomonads and a few species of Chlorophytes, and β-carotene, which is found in certain Chlorophytes. Carotene becomes oxycarotenoids when it combines with oxygen. Because of their higher polarity compared to carotenes, xanthophylls, which are oxy-carotenoids, dissolve better in polar solutions. Scenedesmus spp., Haematococcus lacustris (formerly H. pluvialis), Spirulina platensis, Dunaliella salina, Chlorella spp., Botryococcus braunii, Bracteacoccus, dinoflagellates, and diatoms are the main producers of the most well-known xanthophylls derived from algae, which include lutein, astaxanthin, canthaxanthin, and fucoxanthin (Fern´andez, et al., 2021).
Also, certain blue-green algae, such as cyanobacteria, rhodophytes, cryptomonads, and glaucocystophytes, contain allophycocyanins (APCs, blue; absorbs at 540-570 nm), phycocyanins (red; absorbs at 651-655 nm), and phycoerythrins (red; absorbs at 540-570 nm) (Sonani et al., 2017). As part of photosynthesis, these PBPs also take in light and send it on to chlorophylls. The two subunits that make up PBPs that are often seen are α and β. A number of red algae and cyanobacteria rely on phytoerythrin (PE) as their main pigment-binding protein. The presence of phytocyanins is common across all PBP-containing taxa, including cyanobacteria, red algae, glaucophytes, and even some cryptophytes. They are

common among several species of cyanobacteria that live in the wild. Cyanobacteria, glaucophytes, and red algae all contain phycobiliprotein, albeit to a lesser extent than phycocyanins and phycoerythrins. Jiang (2023).

Some examples of biotic and abiotic variables that might affect algal pigment production include Pagels et al. (2020). Photoperiod duration, nutrition availability, temperature, salinity, light irradiance, heavy metals, and pesticides are other aspects to consider. Plants make pigments to help them absorb light while they're in the vegetative development stage. Nevertheless, there are accounts that address the topic of pigment development in response to stress, such as when plants are subjected to salt stress, excessive light, or nitrogen stress. Light and nutrients cause certain species of algae to create additional pigments, according to research by Benavente-Valdés et al. (2016). To maximise the output of biorefinery systems, state-of-the-art technologies may be used to screen different strains of microalgae for their pigment production and biofuel potential (Patel et al., 2022). A novel approach to bioprocess development, the biorefinery concept seeks to sustainably produce several end products from a single biomass feedstock. Some wastes that might benefit from treatment include fume sludge and industrial effluents (Hong et al., 2019). It is possible to enhance pigment output by increasing biomass production via the use of nanobubble technology, mixotrophy, and other emerging trophic modes and technologies. In addition to their long-standing roles in the environment, algal pigments have several positive effects on animal and human health. According to numerous studies, including those by Zittelli (2023), algae pigments have multiple positive health effects, such as lowering inflammation and protecting against cancer, obesity, and angiogenesis. Environmentally friendly and widely used, natural food colourants are derived from a variety of algal pigments (Chakdar and Pabbi, 2017). The current focus of algal pigment research is on developing very costly compounds with possible industrial and medicinal uses. Using naturally occurring pigments instead of manufactured ones is essential for building a reliable nutraceutical company. To enhance the process and boost pigment output, we need more sophisticated technological instruments. There are many food, feed, and pharmaceutical industries that benefit commercially from carotenoids because to their improved colours and health-enhancing properties. Ambati et al. (2019) found that algal pigment may be linked to over sixty potentially deadly disorders. These include cancer, ischaemic heart disease, progeria, and osteoarthritis. Some of the algal carotenoids found in various types of algae are β-carotene, astaxanthin, lutein, and zeaxanthin. These algae may be green, brown, golden, yellow-green, dinoflagellate, or diatom. The majority of the merchandise is composed of pigments that are manufactured chemically. There is a growing need for green pigment that is naturally produced in order to reduce the potential side effects when used for medicinal reasons. Pigments derived from algae are highly sought after because they outperform plant-based pigments that are already available.

Cyanobacteria are typically considered eukaryotic microalgae since they, too, are unicellular prokaryotic creatures capable of photosynthesis and the manufacture of photosynthetic pigment. Latest updates on algal pigment synthesis, extraction, and purification techniques are offered in this research, which aims to standardise and appraise the cost-effective commercial pigment manufacture. There is a sufficient review of the chemistry of the algal pigments found in higher plants elsewhere. However, this account devotes some space to discussing the key aspects of purely algal colouring materials, such as chlorophyll c and d, the phycobilins, and the characteristic algal carotenoids that were already mentioned. An important finding in the study of algal pigments has been the discovery that carotenoids and phycobilins are not only components of chlorophyll or its breakdown products, as was previously believed, but also have some photosynthesis activity. This allows the organism to thrive in environments where chlorophyll is not as effective. Even more astounding, though, is the proof that specific carotenoids influence Chlainjdornonas reproductive processes; a quick overview reveals that this group of pigments is responsible for a wide range of events, including motility, relative scxuality, fertilisation, and the formation of flagella in gametes. There are two main types of algae: cyanobacteria and blue-green algae. We have also reviewed the essentials about its production and storage in microalgal cells, its applications, their marketability, current challenges, and the prospects for major algal pigments in the future.

Algal Pigments- Structure and Function discusses the various pigments found in algae. It begins by introducing that algae range in size and can be single-celled or multicellular organisms. They contain chloroplasts or chromoplasts in their cells that harbor pigments. The main pigments discussed are chlorophyll a, chlorophyll b, xanthophyll, fucoxanthin, phycocyanin, and phycoerythrin. Each pigment has a unique molecular structure and absorbs different wavelengths of light, allowing algae to capture more of the sun's energy for photosynthesis. The pigments also serve protective functions.

	Algal Group	Pigments
1	Chlorophyceae (green algae)	Chl-a, Chl-b, β-carotene, Xanthophylls
2	Xanthophyceae	Chl-a, β-carotene, Xanthophylls
3	Bacillariophyceae	Chl-a, Chl-c, β-carotene
4	Phaeophyceae (brown algae)	Chl-a, Chl-c1, Chl-c2, Fucoxanthin, β-carotene, Xanthophylls
5	Rhodophyceae (red algae)	Chl-a, Chl-d, β-carotene, Phycoerythrin and phycocyanin
6	Myxophyceae	Chl-a, β-carotene, Phycocyanin, phycoerythrin

CLASSIFICATION OF ALGAL PIGMENTS

MATERIALS AND METHODS

PIGMENT EXTRACTION:

The cultured algal samples were air dried and treated with 100% Acetone. 5ml of the algal biomass was grinded with 2 ml of 100% Acetone in a Dounce glass homogenizer (Shekh et,al., 2022).

ANALYSIS OF PIGMENT BY TLC: The extracted pigment was analysed using Thin layer Chromatography. Thin layer chromatography was performed in precoated silica gel sheet (Silica 60 F254). The TLC sheet of length 7 cm and width of 2 cm was taken. The deposit line for loading the sample mixture was marked at the bottom of the TLC sheet. The different algal extracts were loaded at different spots in the deposition line in the TLC sheet. Once the sample gets dried, the TLC plates were placed in the mobile phase. The mobile phase contains the mixture of methanol, distilled water and ammonia in 9: 1: 0.0012µl ratio. Once the mobile phase reaches the solvent front. The Rf value was calculated and the TLC sheets were observed in UV- transilluminator. Rfvalue= Distance moved by the analyte / Distance moved by the solvent.

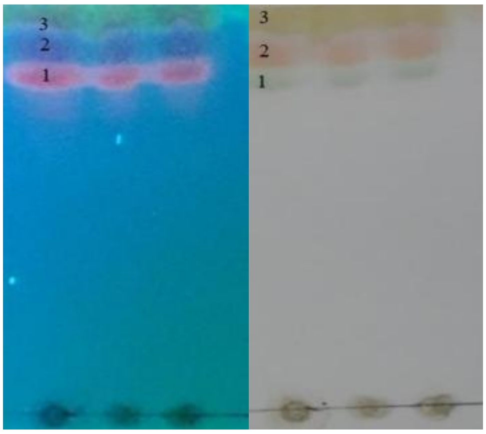


Figure -a, Figure- b

Figure a - TLC Sheet under UV, Figure b- TLC Sheet under Visible light
In Figure a & b Pigment 1- Chlorophyll a Pigment 2- Chlorophyll b Pigment 3- Carotenoids

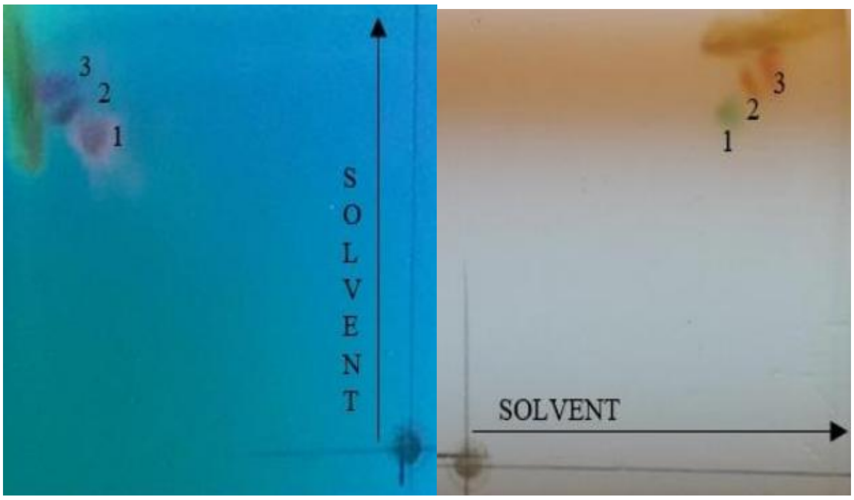


Figure. A Figure. B

Figure. A- TLC sheet in UV, Figure 5B- TLC sheet in visible light Figure A& B Pigment 1- Chlorophyll a Pigment 2- Chlorophyll b Pigment 3- Carotenoid

Compound		Rf value
A	3.6/5.5	0.65
B	3.9/5.5	0.72
C	4.6/5.5	0.83
D	5.3/5.5	0.91

Rf value CALCULATION: Table 1- Rf values of the pigments

Table 1. Maximum Absorbance of the Pigmennts

S.NO	SAMPLE	WAVE LENGTH	ABSORBANCE
A	1µl/ml	320nm, 423nm, 620nm, and 690nm	0.442, 0.338, 0.552, 0.553 and 0.774
B	2µl/ml	320nm, 423nm, 454nm, 620nm, and 690nm	0,559, 0.447, 0.886, 0,992 and 0.998

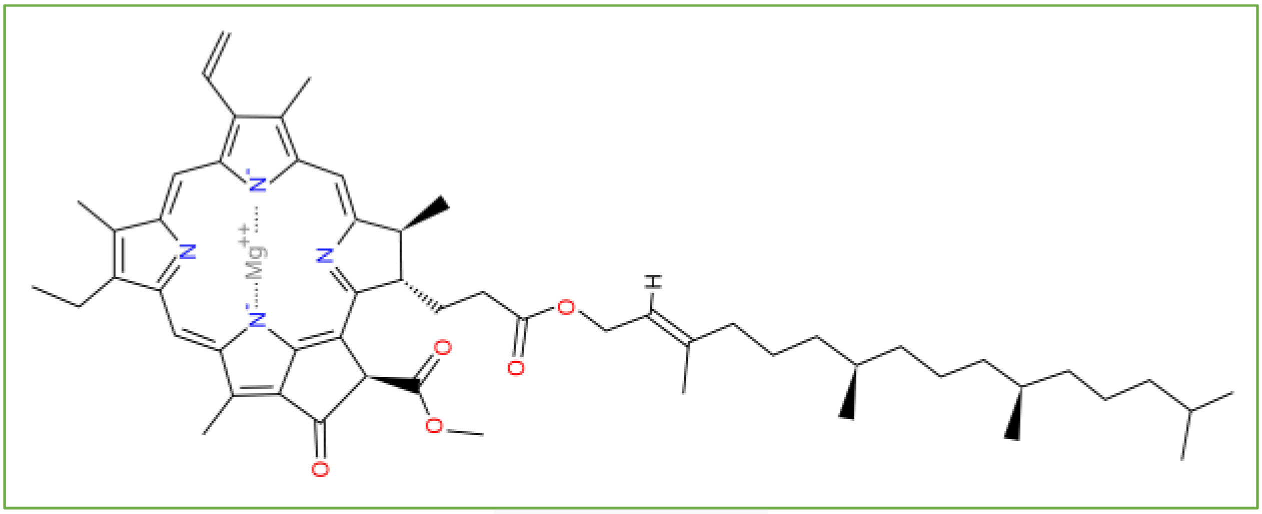
RESULTS AND DISCUSSION

Different types of microalgal pigments

The pigments separated being identified as Chlorophyll a (pigment-1) Chlorophyll b (pigment-2) Carotenoids (pigment-3) through Thin Layer Chromatography (Figure a & b) (Jeffrey, S. W. 1974). Further spectral analysis of the pigments was carried out and based on the absorption spectrum (Fabrowska. J et.al.,2017), it is identified that Chlorophyll a & b fall between 300nm- 400nm (Pointer 5 in the spectrum) (Figure A) and the carotenoids falls between the 400nm- 600nm (Pointer 3,2,1 in the spectrum) (Figure B)

Chlorophyll

In the chloroplast's thylakoid membranes, you may find the photosynthetic pigment chlorophyll in two forms: chlorophyll a and chlorophyll b. Primary pigments are chlorophyll a and accessory pigments are chlorophyll b. Chlorophyll an absorbed light in the blue and orange-red portions of the electromagnetic spectrum (Liang et., al.2023). It moves the energy to the reaction centre in the light-absorbing antenna system and sends two excited electrons to the electron transport chain. The pigment helps to broaden the absorption spectrum in the organism, and chlorophyll b absorbs blue light. An increase in the quantity of light energy that can be converted into chemical energy is made possible by the presence of chlorophyll b in the cell. Chlorophyll a and b vary structurally in that chlorophyll a has a methyl group on the third carbon while chlorophyll b contains an aldehyde group.



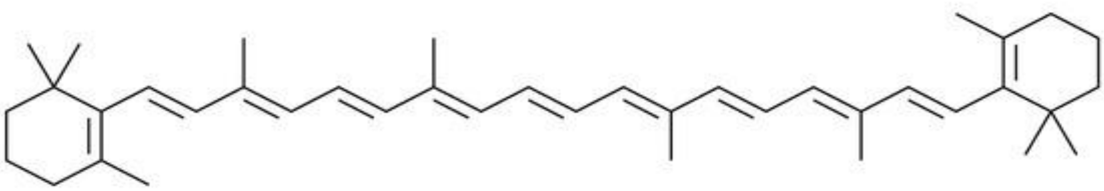
Chlorophyll *a* Structure

All creatures that rely on light for photosynthesis have chlorophylls. Because of their many health benefits, including antioxidant, antimutagenic, and antibacterial properties, green dyes like E140(i) find extensive use in the food industry, medicines, and cosmetics. Chlorella vulgaris and other green microalgae (Chlorophyta) may have a higher chlorophyll content (6.7% of DW, for example) than land plants. On the other hand, the best and most affordable way to get chlorophyll is from plants, especially grass or lucerne (Deepika et al 2022). According to many studies (Nakanishi and Deuchi 2014) the production of chlorophyll by microalgae is not economically valuable and should instead be studied as an aspect of a broader biorefinery process.

There are two main sections of the visible spectrum where chlorophylls absorb light. The first is in the blue region (~u2248400\u2013470 nm) and the second is in the red region (\u2248630\u2013700 nm). The lack of absorption in the green region, which lies between the blue and red spectrums, is responsible for their greenish colour (Li and Chen 2015). According to Deepika et al. (2022), the absorption spectra of various chlorophyll types are affected by their structural variances (Fig. 1). Chlorophyll a, b, c, and f had maximal absorption in the Q band when dissolved in methanol at about 665, 652, 630, 697, and 707 nm, respectively (de Silva and Lombardi 2020). Only chlorophyll f absorbs light in the lower (Soret band) and higher (Q band) wavelength ranges.

Carotenoid pigments

Pigments containing tetraterpenoids are known as carotenoids. The algae contain a variety of carotenoids, including α-carotene, β-carotene, alloxanthin, antheraxanthin, astaxanthin, diatoxanthin, and much more. Carotenoids serve as a photoprotective agent and also absorb light energy for use in photosynthesis, which are two of the most important functions of carotenoids in algae (Rowan, K. S. 1989). Rapid reversal of chlorophyll's excited state is required to prevent the formation of singlet oxygen, which may combine with molecular oxygen to harm other parts of the cell. In order to prevent damage from light, carotenoids reduce the energy of stimulated chlorophyll.



β-Carotene

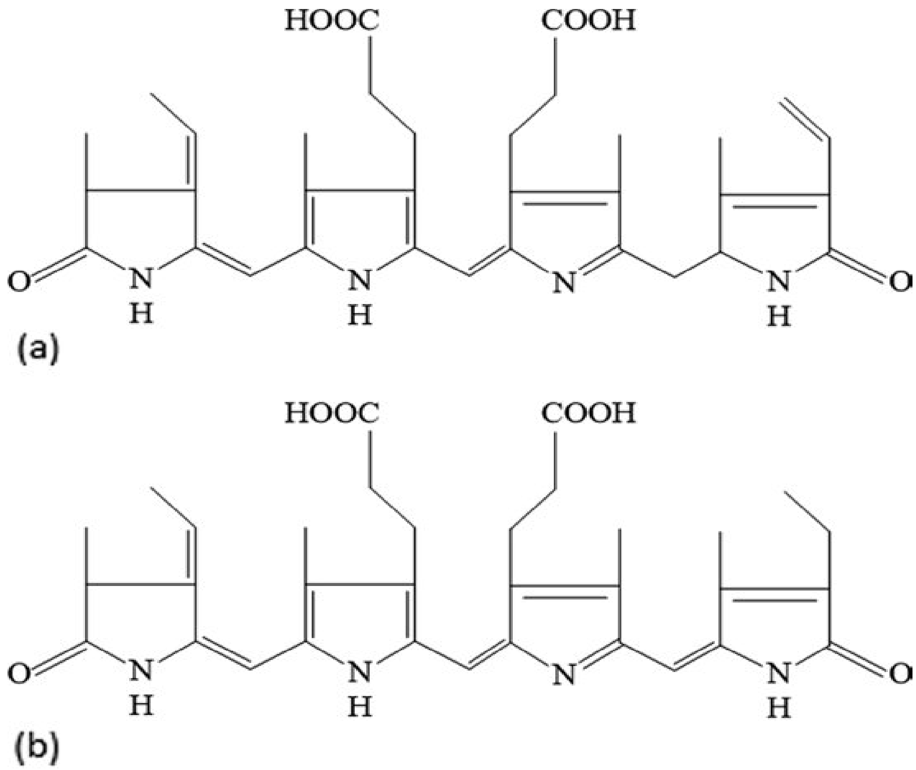
$C_{40}H_{56}$

Carotenoid structure

According to Ashocku et al. (2023) carotenoids are produced by all photosynthetic species, including microalgae, and even certain non-photosynthetic organisms, such as some types of bacteria and fungus. They make up the most diverse category of pigments, with over 600 naturally occurring compounds, 50 of which are present in algae (Levasseur et al. 2020). There are two groups of carotenoids: the carotenes, which include carbon and hydrogen, and the xanthophylls, which contain oxygen as well. According to Abidizadegan et al. (2023) microalgae contain only a handful of commercially available carotenoids, including two carotenes (\u03b2-carotene and lycopene) and four xanthophylls (astaxanthin, canthaxanthin, lutein, and zeaxanthin). According to Gong and Bassi (2016), out of all the carotenoids on the market, astaxanthin and beta-carotene account for almost half. Carotenoids, the most popular class of biological pigments, serve several purposes. To start, for optimal skin health, vision, and immunity, our diets should include beta-carotene, a precursor to vitamin A (Ashokkumar et al., 2023). To further protect against the harmful effects of oxidative stress, carotenoids are used in a number of pharmaceutical formulations (Razzak 2024). A compromised immune system, premature ageing, and certain malignancies are all things that may be avoided with this health benefit. The specific roles of canthaxanthin, lutein, and lycopene in preventing atheromatous, cardiovascular, and blood-related disorders have been recognised. Microalgal carotenoids may be classified as either primary or secondary based on their photosynthetic or non-photosynthetic functions. Lycopene has a special purpose, nonetheless, because it is the building block of all carotenoids (Mulders et al. 2015). Primary carotenoids, such as α and β-carotene, lutein, and zeaxanthin, are essential for proper photosynthesis and cell survival. These carotenoids are abundant throughout normal cell development and serve as structural and functional parts of the photosynthetic machinery. Carotenoids and chlorophyll-binding protein complexes are really present in the thylakoid membranes of cyanobacteria and eukaryotic algae.

Phycobiliproteins

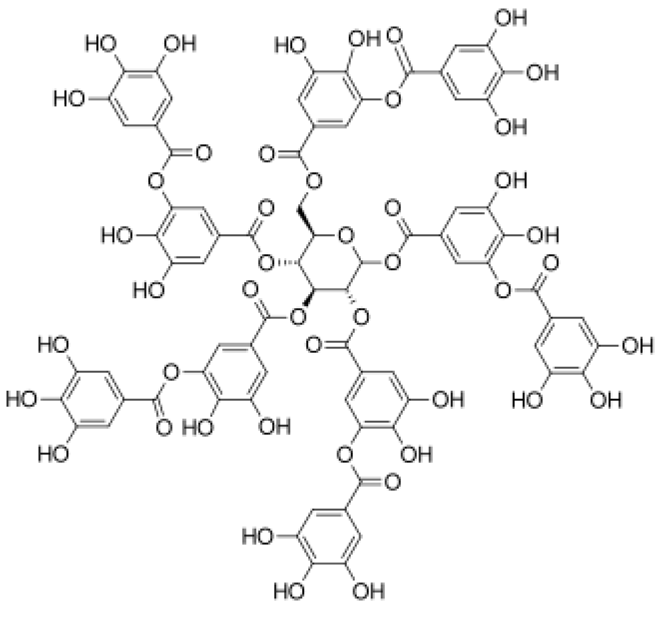
Phycobiliproteins (PBPs) are classified into four groups based on the visual absorption spectra and relative energy levels of light that they absorb. Phycoerythrocyanins (PEC, \u03bbmax: 560\u2013600 nm) and phytoerythrins (PE, \u03bbmax: 540\u2013570 nm) are PBPs that absorb light energy levels that are very high. Phycocyanins (PC) employ intermediate energy levels (610\u2013620 nm), while allophycocyanins (APC) use lower energy levels (\u03bbmax: 650\u2013655 nm) (Tounsi et al. 2023). It is possible for PBPs to be commercially classified into only two categories, PC and PE, depending on whether they are blue or red (Imchen and Singh 2023). Industrial microalgae from PC mostly include two cyanobacteria, Arthrospira sp. and Aphanizome non flos-aquae, while red microalgae from PE include Porphyridium sp. (Levasseur et al. 2020), there has been a notable increase in the commercial production of PC from Arthrospira sp. and PE from Porphyridium sp. Supplemental photosynthetic pigments (PBPs) provide energy to the photosynthetic reaction centre by absorbing light that chlorophylls don't (Deepika et al. 2022). Phycobilisomes, mostly found in Cyanobacteria, red microalgae, and some chromophytes (Cryptista), are light-harvesting antennas, and PBPs are essential components of these complexes (Levasseur et al. 2020). In contrast to carotenoids and chlorophylls, PBPs are soluble in water and do not include phycobilisomes inside their thylakoids. Antennae like these are embedded in the thylakoid lumen of cryptophytes (Tounsi et al. 2023), but they're attached to the thylakoid membrane surface of cyanobacteria and red microalgae. As they approach the thylakoid membrane, phycobilisomes in red microalgae and cyanobacteria are designed to produce a specific sequence of PBPs.



General structure of the major phycobilins: (a) phycoerythrobilin and (b) phycocyanobilin

Polyphenols

Polyphenolic compounds are the most abundant kind of secondary metabolite in terrestrial plants. Flavonoids, tannins, and other water-soluble pigments are part of this group (Bhattacharjya et al., 2020). The wide range of colours seen in flowers is due, in large part, to the anthocyanidins and their glycosylated relatives, the anthocyanins, which are flavonoids. According to Imchen and Singh (2023), anthocyanins exhibit a green absorption band and a UV absorption band; the pigments in question are responsible for the colours red, purple, and blue. There was a lot of debate over whether or not microalgae contain anthocyanins until quite recently (Del Mondo et al. 2022). A red anthocyanin, Cyanidin 3-O-(6'-acetyl-glucoside), was recently discovered in Navicula sp., which is a member of the Amphidinium carterae family of dinophyceae.



Structure of Polyphenols

Most flavonoids have two primary bands in their absorption spectra: one in the near-ultraviolet spectrum (band I > 300 nm) and the other in the ultraviolet region (band II < 300 nm). Isoflavones and flavanones are two examples of non-anthocyanidin flavonoids that do not absorb light in the visible range (380-750 nm) and so do not seem coloured to the human eye (Taniguchi et al. 2023). A bathochromic shift is seen in the absorption spectra of some flavonoids. Flavones and flavonols have an absorption band I that reaches into the visible range of light, which is 400-450 nm. Taniguchi et al. (2023) found that their characteristic yellow tinge is caused by the absorption of blue-purple wavelengths.

Conventional strategies for enhancing pigment content

Finding the sweet spot for microalgae pigment concentration requires fine-tuning a number of parameters. In particular, every single case calls for a custom-tailored combination of temperature and pH. Yet, pigment content may be drastically affected by other crucial variables such as the amount and quality of light reaching the plant, the availability of nutrients, and the presence of other chemicals in the growing media, such salts (Saini et al 2020).

The impact of light quality

Qualitative features of incoming light, in addition to quantitative ones, such as intensity and photoperiod presence or absence, govern pigment synthesis. However, there is no one lighting regime that is optimal for all microalgae. Even within the same species, there is a substantial variation in the effects of different light wavelengths, making it difficult to draw any firm conclusions (Contreras-Ropero et al., 2022). However, light intensities are recognised as crucial parameter in terms of biomass, and β- carotene accumulation. Therefore, these improved growth conditions have shown encouraging results for improved volumetric biomass and pigment generation and provide chance to use them for many uses.

CONCLUSION

Because of their unique properties, algal pigments pose a number of risks to human and environmental health. Algae have recently gained popularity as a natural dye source due to rising awareness of the harmful effects of synthetic dyes and a general shift in consumer preference towards items made from plants and microbes. Due to their much greater bioactive potential compared to other biological pigments, algae pigments are seeing a surge in demand as feed, nutritional supplements, food additives, and hues. The medical, cosmetic, and nutritional fields have all given it positive reviews. The high antioxidative potential of these pigments opens up new avenues for their usage as anti-inflammatory and anti-cancer medications. Because of its bioactivities, algae pigments provide a promising avenue for future product development.

In the biotechnological realm, lithophilic algal pigments find use in many different sectors, including the pharmaceutical, food, and processing industries. Pigments are nutritious, anti-inflammatory, and antioxidant. Another possible application for the pigments is as a natural dye to replace the synthetic ones. More colours, vitamins, and proteins may be made from these algae if their production is increased on a larger scale.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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