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Review

Sustainable Production of Natural Pigments from Microalgae: A Review Study

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Abstract

Microalgae have been identified as a sustainable and versatile source of natural pigments. Their ability to convert carbon dioxide and sunlight into high-value bioproducts make them both an environmentally beneficial and commercially attractive resource. However, the commercialisation of microalgal pigment production remains constrained by several challenges, including high production costs, low pigment yields under standard conditions, and inefficient downstream processing. This review provides all aspects of pigment production from microalgae and not covered in previous reviews. The review encompasses pigment classification, cultivation strategies, factors affecting extraction, as well as extraction and purification processes. Environmental and operational parameters, such as light, temperature, nutrient availability, salinity, and pH, significantly affect pigment biosynthesis and must be optimised for each strain. The choice of cultivation mode (autotrophic, heterotrophic, mixotrophic) and system (open ponds, photobioreactors, hybrid) also plays a crucial role in determining biomass productivity and pigment quality. Downstream processing, including harvesting, cell disruption, extraction, and purification, remains a bottleneck due to high energy costs and pigment instability. Advanced extraction techniques, such as supercritical fluid extraction and microwave-assisted extraction, offer promise for more sustainable and efficient recovery. To advance the field, future research should focus on strain improvement via metabolic engineering, development of energy-efficient processing methods, and implementation of integrated biorefineries. Additionally, pigment stabilisation and public awareness of the benefits of natural pigments will support wider market adoption. With these advancements, microalgae can become a competitive and eco-friendly source of natural pigments.

Keywords: microalgae; natural pigments; carotenoids; chlorophylls; sustainable production

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1. Introduction

Microalgae are a diverse group of photosynthetic microorganisms, including both eukaryotic microalgae and prokaryotic Cyanobacteriophyta. They are recognised as important microbial cell factories to produce a wide range of valuable pigments [1]. Microalgae are a rich source of valuable products, including proteins, lipids, polysaccharides, minerals, vitamins, pigments, and polyunsaturated fatty acids (PUFAs) [2]. Microalgae are widely used in food, cosmetics, and pharmaceuticals industries [1]. The industrial applications of algal pigments are diverse and expanding. Currently, demand for natural, sustainable, and bioactive ingredients is growing [3]. Microalgae convert light energy into chemical energy, which makes them a rich and sustainable source of natural compounds [1]. They utilise

the sunlight to convert carbon dioxide into cellular biomass and coloured compounds through photosynthesis. The utilisation of CO_2 by microalgae for photosynthesis makes them a suitable source of CO_2 abatement. This process offers a significant environmental advantage through the reduction of CO_2 [4]. Microalgae can produce various pigments that offer safe and eco-friendly alternatives to synthetic dyes and artificial colourants [2]. Conventional synthetic dyes are facing a health and environmental risk. This has driven increasing interest in pigments extracted from natural sources like microalgae [1]. The natural pigments from microalgae are not toxic and have no side effects on the human body. Furthermore, they act as nutritional enhancers with various biological activities [2].

Microalgae have a faster growth rate and higher biomass productivity when compared with conventional plant-based sources. Their cultivation can be designed and optimised under controlled conditions [5]. Furthermore, they have the potential to produce a wider spectrum of pigments, including those that are rarely found or absent in higher plants, such as astaxanthin and phycobiliproteins [3]. They can be produced on a large scale without being affected by seasons, weather, or environmental conditions [2].

Microalgae became a valuable source of natural pigments in the late 20th and early 21st centuries. Early research into pigment production from microalgae dates back at least to 1965, with studies reporting the cultivation of microalgae in conventional bacterial fermenters for carotenoid production under both light and dark conditions [6]. Early commercial activity primarily focused on specific carotenoid pigments. β-carotene production from *Dunaliella salina* (*Chlorophyta*) was one of the earliest successes, with significant production established by the 1970s and 1980s, primarily in regions with high salinity environments like Australia and Israel. These open pond systems leveraged the natural stress conditions (high salinity, high light) to induce β-carotene accumulation [7–9]. Astaxanthin from *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) (*Chlorophyta*), recognised for its potent antioxidant and anti-inflammatory properties, is used in aquaculture feed, cosmetics, and health products [2,7,9]. *Haematococcus* cultivation was initially more complex, often requiring closed photobioreactors [10]. Phycocyanin from *Limnospira platensis* (formerly *Arthrospira platensis* (*Spirulina*) (Cyanobacteriophyta) was also investigated early, for its potential demonstrated that Porphyridium (Rhodophyta) polysaccharides can alleviate cyclophosphamide-induced toxicity, used as a natural blue food colourant with its fluorescent properties for diagnostics [4]. Commercial production of *Spirulina* also began in the 1970s [11].

The naturally derived pigments from microalgae sources are favourable products for the environmental market, because of their high productivity and potential for controlled cultivation [5]. The global market for these pigments is growing. This is due to the increasing demand for natural colourants across various industries. It has been estimated that the global microalgae market size was valued at USD 782.59 million in 2024 and is projected to reach USD 1376.42 million by 2032, exhibiting a compound annual growth rate (CAGR) of 7.29% during the forecast period [12].

Chettri et al. [13] recently studied microalgal pigments as a source of natural colours and their application in the food industry. The colouring applications of microalgae have been investigated in recent years [14–16], and studies have demonstrated that pigment extraction from microalgae can provide natural, sustainable, and bioactive colourants with potential applications in food, nutraceutical, and cosmetic industries. Recently, researchers employed new engineering [17–19]. Continuous research is being conducted in this area to develop more efficient cultivation, extraction, and processing technologies for sustainable large-scale production of microalgal pigments [20–22].

Tambat et al. [23] studied the astaxanthin production from *Haematococcus lacustris* and obtained a high astaxanthin yield of 189.2 mg/g. Liu et al. [24] used a newly isolated

microalga for high-titer astaxanthin production and achieved a high astaxanthin yield of 377.67 mg/L. Vadrale et al. [25] optimised lutein yield and improved the lutein to 65.48 mg /L. in another study. Udaypal et al. [26] evaluated biomass production and the lutein yield of three promising microalgal species. Thurakit et al. [27] studied lutein production under different cultivation conditions and carbon sources. These research studies highlight the continuous progress in microalgal pigment, but further investigations are still required to overcome existing challenges and develop efficient, scalable, and sustainable production processes.

However, despite the significant potential and growing market, the industrial-scale production of algal pigments faces certain challenges [2,3]. Cost-effective cultivation to achieve high pigment yields, as well as the development of efficient and sustainable extraction and purification processes that preserve the integrity and bioactivity of these delicate molecules, remain critical areas of focus for research and development. Overcoming these technological bottlenecks is essential to enable algal pigments to effectively compete with their synthetic counterparts and fully realise their potential in the global market [2,3,5].

This research aims to explore and enhance the production of natural pigments from microalgae, focusing on their industrial applications, sustainability, and market potential. It seeks to optimise cultivation conditions, improve pigment yield, and develop cost-effective extraction and purification methods. Additionally, the study addresses challenges related to large-scale production, ensuring that microalgal pigments can serve as viable, eco-friendly alternatives to synthetic dyes while contributing to CO_2 reduction and environmental sustainability.

Table 1 summarises the review articles identified from Google Scholar. The selected papers are organised based on important aspects of pigment production. Each study is classified according to the type of pigment (such as carotenoids, chlorophylls, and phycobiliproteins), factors affecting pigment extraction (including light, temperature, nutrients, and pH), cultivation methods, extraction techniques, and purification processes.

Table 1. Summary of review articles on microalgal pigment production identified from Google Scholar.

Reference	Classification	Factors	Cultivation	Extraction	Purification
[28]				√	
[3]	√	√			
[1]	√	√	√		
[7]	Carotenoids		√	√	√
[8]	Carotenoids	√		√	
[4]	√	√			
[29]				√	
[6]	√	√			
[9]	√				
[30]				√	√
[2]	√	√		√	
[31]	√	√			
[32]		√		√	√
[33]	√	√			
[34]	Lutein		√	√	√

Table 1. *Cont.*

Reference	Classification	Factors	Cultivation	Extraction	Purification
[35]	√				
[36]	√			√	√
[37]		√	√		
This study	√	√	√	√	√

The purpose of Table 1 is to compare the scope and coverage of the present review with previous review articles on microalgal pigment production. The references listed in Table 1 are all review papers, and the tick sign signifies the specific aspects of pigment production investigated in that reviewed article. Furthermore, for some of the review papers, the name of the pigment was inserted which means that only one pigment was reviewed in that particular paper.

The comparison shows that most existing review papers focus on only certain aspects and do not cover all stages together. In contrast, the present review includes all these key topics in one study. This provides a more complete understanding of microalgal pigment production and helps to connect the different parts of the process.

Unlike previous reviews that have mainly focused on specific aspects of microalgal pigment production, this review provides a comprehensive overview covering pigment classification, production influencing factors, cultivation strategies, cell disruption and extraction, and purification technologies. Furthermore, this work critically evaluates the advantages and limitations of existing approaches, discusses sustainability considerations, and highlights the key challenges and future research directions for the large-scale and sustainable production of microalgal pigments.

2. Pigment Classification

Algae pigments are organic molecules and classified into four groups, including chlorophylls, carotenoids, phycobiliproteins, and polyphenols [31]. Chlorophylls are primary photosynthetic pigments that directly convert light energy into chemical energy, contributing to biomass production. They are responsible for the characteristic green colour of algae [3].

Carotenoids expand the range of absorbed solar wavelengths and are known as auxiliary photosynthetic pigments [31]. They are lipid-soluble compounds that display vibrant yellow, orange, and red colours, functioning as both light-harvesting pigments and powerful antioxidants [8]. Phycobiliproteins are water-soluble pigments found in cyanobacteria and certain algae groups. They include phycocyanin and allophycocyanin (blue) and phycoerythrin (red), playing a crucial role in light harvesting, particularly in deeper aquatic environments where red light penetration is limited [3,32]. Recently, polyphenols identified in some microalgae have further expanded the range of bioactive compounds and natural colourants derived from these organisms [31].

These pigments possess various bioactive properties, including antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and potential anticancer activities, which have attracted increasing interest for applications in the food, nutraceutical, pharmaceutical, and feed industries [26].

Table 2 presents the classification of the pigments along with their pigment name and colour. The table summarises the major pigment groups, including their names, colours, mechanisms, and common sources. Chlorophylls are responsible for green coloration due to their role in photosynthesis, while carotenoids exhibit yellow, orange, and red colours depending on their molecular structures. Phycobiliproteins are water-soluble pigments

that produce blue and red colours and function as accessory light-harvesting compounds. Polyphenols, although not considered classical pigments, are bioactive compounds that can contribute to colour properties and provide antioxidant benefits.

Table 2. Classification of pigment-based microalgae and their common sources.

Pigment Group	Name	Colour	Mechanism	Common Source	Reference
Chlorophylls	Chlorophyll *a*	Green	Primary pigment for photosynthesis	All photosynthetic microalgae	[31,38]
	Chlorophyll *b*	Green	Expands light absorption	Green algae (Chlorophyta)	
	Chlorophyll *c*	Green	Accessory pigment in non-green algae	Diatoms, dinoflagellates, brown algae	
Carotenoids	β-Carotene	Orange	Light harvesting, antioxidant (425–480 nm)	*Dunaliella salina*, green algae	[31,32,39]
	Lutein	Yellow	Antioxidant, supports vision health (421–474 nm)	Green algae (*Chlorophyta*)	
	Astaxanthin	Red	Strong antioxidant, protects cells from stress (478 nm)	*Haematococcus lacustris*	
	Zeaxanthin	Orange-yellow	Photoprotection, antioxidant (424–480 nm)	Cyanobacteriophyta, green algae	
	Fucoxanthin	Brown	Enhances light absorption, antioxidant	Diatoms, brown algae	
Phycobiliproteins	Phycocyanin	Blue	Light harvesting in low light conditions (610–620 nm)	Cyanobacteriophyta (*Spirulina*)	[31,40,41]
	Phycoerythrin	Red	Improves light absorption efficiency (540–570 nm)	Red algae (Rhodophyta)	
	Allophycocyanin	Blue green	Accessory pigment in phycobilisomes (650–655 nm)	Cyanobacteria, red algae	
Polyphenols	Flavonoids	Various	Antioxidant, anti-inflammatory properties	*Spirulina*, *Chlorella*	[31,41]
	Phenolic acids	Various	UV protection, antioxidant	Cyanobacteria, diatoms	
	Tannins	Brown	Antimicrobial, stress response	Some microalgae and cyanobacteria	

3. Pigments Production

The production of pigments from microalgae involves several steps. Pigment production occurs in five main steps including microalgae cultivation, cell harvesting, cell disruption, pigment extraction, and purification. The downstream processing contributes the most to the overall cost. The downstream process typically includes cell disruption, pig-

ment extraction, and purification. It is important to note that, for some microalgal strains, these steps can be integrated. In other words, cell disruption and pigment extraction may be performed simultaneously in a single step for certain strains.

3.1. Key Factors

The production of pigments from microalgae is dependent on several key factors, which can be optimised to enhance yield and quality. These factors are discussed in this section. Light is the most important factor that needs to be controlled. The light intensity is a crucial factor affecting photosynthesis and pigment biosynthesis [42]. Different microalgae species have varying light intensity requirements for optimal growth and pigment production [43]. For example, Spirulina's phycobiliprotein production increases with light intensity up to a certain point, while chlorophyll content in several microalgae shows an inverse relationship with light intensity [44].

High light intensity can activate the synthesis of protective pigments like carotenoids and can induce the overproduction of secondary carotenoids like astaxanthin in *Haematococcus lacustris* and β-carotene in *Dunaliella salina* as a photoprotective mechanism [2]. However, excessively high light can also be detrimental, damaging the photosynthetic apparatus [45]. Light quality (wavelength) significantly affects pigment generation [45]. Red light can enhance phycobiliprotein production in some blue-green microalgae, while blue light stimulates it in *Spirulina* and enhances chlorophyll production [2]. Green light can increase C-phycoerythrin levels, and red light can increase C-phycocyanin levels. Blue light has been found optimal for lutein production in some marine microalgae. Different light spectrum proportions (e.g., blue: red, red: far-red) can also influence relative pigment composition [2]. Photoperiod (light/dark cycle) also regulates pigment levels, effectively influencing microalgal chlorophyll levels [3,43]. The 12:12 h light/dark cycle can increase phycocyanin content in *Limnospira platensis* compared to continuous light [46].

Temperature affects metabolic mechanisms and biochemical structures within microalgal cells, influencing pigment production [42]. Generally, high temperatures can enhance pigment synthesis in microalgae [3,42]. Optimal temperatures vary depending on the algal strain and the specific pigment [42]. For instance, 25–28 °C is often cited as optimal for chlorophyll accumulation, while 28 °C is best for astaxanthin in *H. lacustris* [2]. Optimal temperatures for phycobiliprotein production vary by species, ranging from 25 °C to 36 °C [2].

Pigment production is affected by the pH of the cultivation media. The optimum range of pH is between 5 to 8.5, and at levels below 5.0 or above 8.5, the microalgae growth is reduced [45]. Specific pH ranges can optimise the production of certain pigments. For instance, *Limnospira platensis* produced the highest levels of chlorophyll a, carotenoids, C-phycocyanin, and phycobiliproteins at pH 8.5 or 9.0 [3]. Abrupt pH changes can reduce chlorophyll and carotenoid production, slowing growth and reducing the biomass yield [3].

Like all the microorganisms, an adequate supply of nutrients such as carbon (C), nitrogen (N), and phosphorus (P) is essential for pigment yield [3]. The C/N ratio in the culture medium increases the pigment production under heterotrophic conditions [6]. However, the limitation of certain nutrients like nitrogen and phosphorus can favour high pigment accumulation in some microalgae [47]. Nitrogen limitation has been found to induce higher astaxanthin accumulation in *Haematococcus lacustris*, especially under light stress [48]. Nutrient limitation, particularly nitrogen and phosphorus, can induce the synthesis of specific carotenoids such as β-carotene in *Dunaliella salina* [49]. Nitrogen deprivation can slow down cell replication and stimulate the accumulation of secondary carotenoids. It can also upregulate genes involved in carotenoid biosynthesis [10]. Mixing

and aeration are important to ensure even dissemination of nutrients, air, and CO_2 in the cultivation media [3].

Pigment production is altered in stress conditions such as nutrient and light stress. Additionally, the presence of heavy metals and pesticides may change the pigment production [50]. Secondary carotenoids like astaxanthin are often synthesised as a stress response to factors such as increased reactive oxygen species, salt stress, and high light intensity [6,51]. It should be noted that the accumulation of non-photosynthetic pigments (polyphenols and secondary carotenoids) often requires particularly stressful conditions [31].

Salinity plays a crucial role, especially in marine microalgae, impacting cellular osmosis and pigment production [47]. High salinity can increase β-carotene production in some blue-green microalgae while decreasing chlorophyll and total carotenoids in others [8].

Table 3 presents a summary of key factors on pigment production from microalgae. The selection of the microalgae strain is one of the key factors influencing pigment production. It can be concluded that careful control of these factors is needed to optimise pigment yield and quality in microalgal cultures.

Table 3. Key factors influencing pigment production from microalgae.

Factor	Description	Effect on Pigment Production	Typical/Optimal Range	Reference
Microalgae Strain	Species selection determines pigment profile and productivity	Dictates the type and concentration of pigments produced (e.g., chlorophylls, carotenoids, phycobilins)	Strain-dependent; selected based on target pigment	[52]
Light Intensity and Quality	Affects pigment biosynthesis through photosynthetic and photoprotective pathways	Increased intensity and specific wavelengths (blue/red) enhance carotenoid and chlorophyll synthesis	50–300 µmol photons m^{-2} s^{-1}; blue/red light preferred	[43,44,46]
Photoperiod	Controls the photosynthetic rhythm and metabolic activity	Optimised light/dark cycles improve pigment accumulation; prolonged dark periods may reduce yield	12:12 or 16:8 h (light/dark)	[46]
Temperature	Influences enzymatic activity and stress-induced pigment synthesis	Moderate heat can induce pigment formation (e.g., astaxanthin); excessive heat inhibits growth	20–30 °C (some up to 35 °C)	[2,42]
Nutrient Availability	Macronutrient limitation (e.g., nitrogen) can stimulate secondary pigment production	Nitrogen depletion enhances the synthesis of carotenoids and phycobiliproteins	Nitrogen: 0–150 mg L^{-1} depending on strategy	[3]
Salinity	Osmotic stress can act as a trigger for secondary metabolite production	Salt stress induces β-carotene accumulation and other protective pigments	0.5–3 M NaCl (species-dependent)	[47]
pH Level	Affects cell viability and pigment stability	Extreme pH levels may degrade pigments; optimal pH supports pigment biosynthesis	pH 7–9 (up to 10.5 for Spirulina)	[45]
Cultivation System	Determines light exposure, contamination risk, and growth control	Photobioreactors provide better control, sterility, and productivity than open systems	Open raceways or closed photobioreactors	[2,8]

Table 3. *Cont.*

Factor	Description	Effect on Pigment Production	Typical/Optimal Range	Reference
Mixing and Aeration	Ensures uniform light exposure, nutrient distribution, and CO_2 supply	Improves pigment yield by preventing settling, enhancing gas exchange, and reducing gradients	Moderate mixing to avoid shear stress	[3]
Stress Induction	Environmental stress (e.g., light, nutrients) enhances secondary pigment biosynthesis	Stress triggers accumulation of astaxanthin, β-carotene, and other valuable pigments	Induced at late growth phase	[6,50]

3.2. Pigment Diversity Among Different Microalgal Taxa

Microalgae comprise a highly diverse group of photosynthetic microorganisms whose pigment composition varies considerably according to their taxonomic classification, evolutionary history, and photosynthetic apparatus. These differences determine the type, concentration, and commercial value of the pigments synthesised by different microalgal taxa, making taxonomic selection an important criterion for industrial pigment production [53]. In addition to pigment composition, factors such as growth rate, environmental tolerance, cultivation requirements, and biomass productivity strongly influence the suitability of each taxonomic group for large-scale production.

Chlorophyta (green microalgae) are among the most extensively investigated groups owing to their rapid growth, high biomass productivity, and well-established cultivation technologies. Species such as *Chlorella sorokiniana*, *Dunaliella salina*, and *Chlorococcum* spp. accumulate high concentrations of chlorophylls, lutein, β-carotene, and other xanthophylls [54]. *Chlorella sorokiniana* (e.g., UTEX 1230) has demonstrated high lutein productivity under nitrogen-sufficient conditions [55], whereas *Dunaliella salina* (e.g., CCAP 19/18) can accumulate β-carotene to more than 10% of its dry biomass under high irradiance and salinity stress. The major advantages of Chlorophyta include fast growth, adaptability to various cultivation systems, and commercial maturity; however, pigment accumulation often requires environmental stress, which may reduce biomass productivity and increase production costs [7]. *Haematococcus pluvialis* is one of the species of Chlorophyta and regarded as the richest natural source of astaxanthin. Under environmental stresses such as high light intensity, nitrogen starvation, and elevated salinity, this species can accumulate astaxanthin to more than 4–5% of its dry biomass, making it the dominant commercial source of natural astaxanthin [56]. While the exceptionally high pigment content and strong antioxidant properties make H. pluvialis highly attractive for nutraceutical, cosmetic, and aquaculture applications, its two-stage cultivation process, slow growth, and sensitivity to contamination increase production complexity and cost [57].

Cyanobacteriophyta are an important source of phycobiliproteins, particularly phycocyanin and phycoerythrin, which are widely used as natural blue and red colorants in the food, pharmaceutical, and cosmetic industries. Species such as *Arthrospira platensis* (PCC 8005) and *Synechocystis* sp. PCC 6803 have been extensively studied for enhanced phycocyanin production through optimisation of light intensity, nitrogen availability, and cultivation temperature [58,59]. Cyanobacteriophyta generally exhibit rapid growth and relatively simple cultivation requirements, but pigment stability during extraction and storage remains a major challenge because phycobiliproteins are sensitive to temperature, pH, and light exposure.

Bacillariophyta (diatoms) represent one of the most promising taxonomic groups for fucoxanthin production [60]. Species such as *Phaeodactylum tricornutum* (CCAP 1055/1 or CCMP 632) and *Odontella aurita* have attracted considerable attention because of their

high fucoxanthin content, rapid growth, and ability to utilise carbon dioxide efficiently. Original studies reported that fucoxanthin accumulation can be enhanced under moderate light intensity, controlled nitrogen availability, and appropriate photoperiods, with strain-dependent pigment productivity. The principal advantages of diatoms include high photosynthetic efficiency and commercially valuable fucoxanthin production, whereas their requirement for dissolved silica and sensitivity to cultivation conditions can increase process complexity [61].

Heterokontophyta, including haptophytes such as *Tisochrysis lutea* (CCAP 927/14), also constitute valuable sources of fucoxanthin and chlorophyll *c*. Compared with many green microalgae, these species often exhibit higher fucoxanthin content and favourable fatty acid profiles, making them attractive for functional food and nutraceutical applications. However, their relatively slow growth rates and greater sensitivity to environmental fluctuations may limit large-scale commercial cultivation [62].

Euglenophyta, represented by species such as Euglena gracilis (CCAP 1224/5Z), produce chlorophylls together with carotenoids, including β-carotene, lutein, zeaxanthin, and neoxanthin. An important advantage of *Euglena* species is their metabolic flexibility, as they can be cultivated photoautotrophically, heterotrophically, or mixotrophically, enabling higher biomass and pigment productivity under optimised conditions. Nevertheless, heterotrophic cultivation requires an external carbon source, which may increase production costs [63].

Rhodophyta (red microalgae), including species such as *Porphyridium purpureum*, are recognised for producing high-value phycobiliproteins, particularly phycoerythrin, in addition to sulfated polysaccharides. These pigments possess excellent fluorescence properties and are used in food, cosmetics, pharmaceuticals, and biomedical diagnostics. Although red microalgae produce pigments of exceptionally high commercial value, their relatively slow growth rates and more demanding cultivation requirements remain important limitations for industrial-scale production [64,65].

Table 4 summarises representative microalgal taxa, commercially important pigment-producing species, major pigments, and their principal applications. Although substantial progress has been achieved through strain selection, cultivation optimisation, and metabolic engineering, pigment productivity remains highly strain-dependent and strongly influenced by cultivation parameters, including light intensity, temperature, nutrient availability, salinity, and carbon supply. Future research should therefore focus on identifying elite strains from diverse taxonomic groups, standardising cultivation protocols, and integrating sustainable biorefinery approaches to improve the economic feasibility of large-scale production of taxon-specific microalgal pigments.

Table 4. Representative microalgal taxa, major pigments, advantages, limitations, and commercial applications.

Taxonomic Group	Representative Species (Strain/Culture Collection	Major Pigments	Advantages	Limitations	Main Applications	Reference
Chlorophyta	*Chlorella sorokiniana* (UTEX 1230), *Dunaliella salina* (CCAP 19/18), *Haematococcus lacustris*	Chlorophylls, lutein, β-carotene, zeaxanthin, astaxanthin	Fast growth, high biomass productivity, established cultivation	Stress often required for high pigment accumulation	Food colourants, nutraceuticals, cosmetics	[7,54]

Table 4. *Cont.*

Taxonomic Group	Representative Species (Strain/Culture Collection	Major Pigments	Advantages	Limitations	Main Applications	Reference
Cyanobacteria	*Arthrospira platensis* (PCC 8005), *Synechocystis* sp. PCC 6803	Phycocyanin, phycoerythrin, chlorophyll *a*	High phycobiliprotein content, simple cultivation	Pigments sensitive to heat, light and pH	Food colourants, pharmaceuticals, cosmetics	[58,59]
Bacillariophyta	*Phaeodactylum tricornutum* (CCAP 1055/1), *Odontella aurita*	Fucoxanthin, chlorophyll *c*, β-carotene	High fucoxanthin productivity, efficient photosynthesis	Require silica; sensitive cultivation	Functional foods, nutraceuticals	[60,61]
Ochrophyta	*Tisochrysis lutea* (CCAP 927/14)	Fucoxanthin, chlorophyll *c*	High-value fucoxanthin	Slower growth	Functional foods, cosmetics	[62]
Euglenophyta	*Euglena gracilis* (CCAP 1224/5Z)	Chlorophylls, lutein, β-carotene, zeaxanthin	Flexible cultivation modes	Organic carbon needed for heterotrophy	Nutraceuticals	[63]
Rhodophyta	*Porphyridium purpureum* (CCALA 415)	Phycoerythrin, phycocyanin	High-value pigments	Slow growth	Food colourants, diagnostics	[64,65]

4. Microalgae Cultivation

Microalgae cultivation systems are crucial for obtaining biomass and valuable products like pigments. Cultivation of microalgae under different conditions has a great effect on the pigment and biomass productivity. There are several studies to be found in the literature regarding microalgae cultivation methods [66–68].

4.1. Cultivation Techniques

The techniques that have been applied for microalgae cultivation can be classified into four categories, namely, open, closed, dark, and hybrid [66]. Figure 1 shows a schematic representation of various methods of microalgae cultivation.

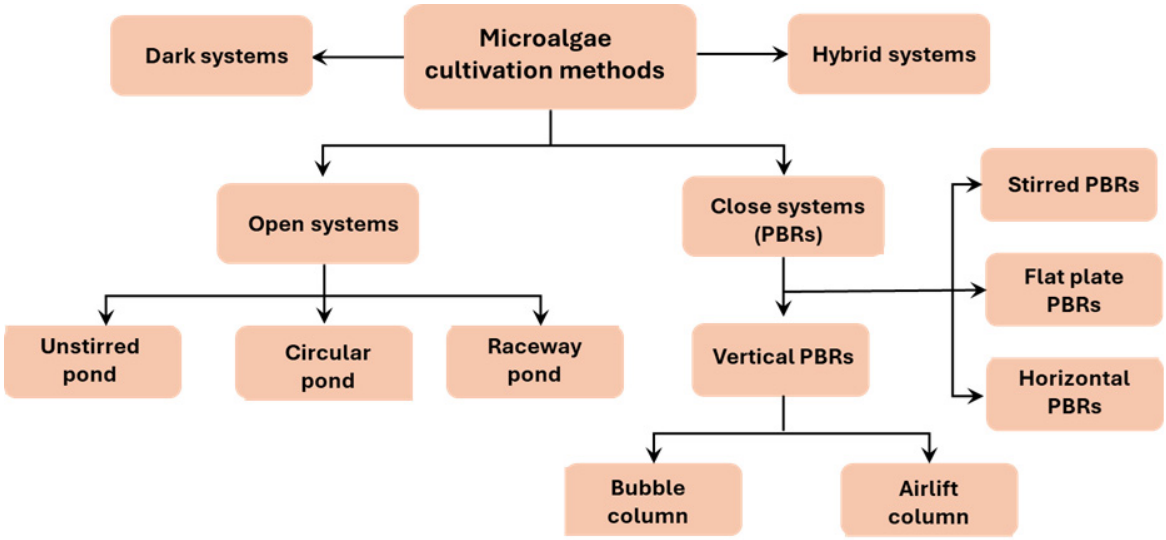

Figure 1. Microalgae cultivation methods.

Open pond systems are classified into unstirred, circular, and raceway ponds. The unstirred ponds are found in natural water systems such as lagoons and lakes without any mixing. Circular ponds are artificial ponds that are used in large-scale microalgae cultivation and consist of a circular-shaped tank/container with a rotating agitator fixed in the centre of the pond to mix efficiently and prevent the sedimentation of the algal biomass [66].

The raceway ponds are a popular and cost-effective method for large-scale cultivation. They are characterised by cheaper construction and maintenance, low operating costs, and ease of scale-up. They utilise sunlight as an energy source and atmospheric air as a CO_2 source [6,66]. Open systems have limitations, including low microalgal cell density, low photosynthetic activity due to limited light availability, and an increased risk of contamination by protozoa, bacteria, and insects. Evaporation losses can also reduce productivity [66]. Mixing in open ponds can be achieved using large rotational arms or aeration with air bubbles to help disseminate CO_2 and sunlight [69]. Open ponds are still used for applications like wastewater treatment and biofuel production, where cost is a major factor. They are suitable for producing low-value algae-based products [66].

Closed systems are photo bioreactors (PBR) for microalgae cultivation that have no direct contact with the external environment. They are preferred for producing high-end pharmaceutical compounds requiring axenic cultures and offer a higher degree of control over the culture, leading to high biomass productivity and quality. PBRs have a higher degree of control on the microalgae culture than open ponds, which leads to higher biomass productivity with higher quality [66]. PBRs are classified into vertical, horizontal, flat plate, and stirred tank. There are two designs for a vertical PBR, namely bubble and airlift columns. The bubble column PBR is used widely in laboratory and commercial scales due to its changeability and flexibility of operation, simplicity in handling, and building structure [70]. Horizontal PBRs are closed tubular reactors, consisting of a parallel set of connected loops of tubes and placed horizontally or inclined at an angle to the horizontal [71].

The stirred PBRs (SPBRs) are derived from the fermentation tank design and improved so as to be suitable for microalgae cultivation. For this reactor, an external light source and agitator for mixing are added to control and enhance the optimal temperature and biomass transfer [72]. These PBRs are applied on a small laboratory-scale only, and are neither suitable nor preferable for larger scale [73]. The most popular type of closed PBR is the flat panel PBR, which is made of two sheets of transparent or semi-transparent glass that are joined in a cascade towards the light source. They can be placed vertically or at specific angles to absorb most of the light intensity from sunlight or different light sources [74].

Some microalgae can be cultivated in the absence of light in a dark system and using organic carbon sources like glucose or acetate for energy. This method overcomes the light limitation issue and can achieve high cell densities in standard fermenters. It is suitable for axenic cultivation and the production of high-quality bioactive compounds. When microalgae are grown heterotrophically, light pigmentation often fades, while volumetric biomass yield, protein, and lipid productivities increase [66].

Hybrid cultivation systems combine a two-stage cultivation to reduce the obstacles of both open and closed systems [75]. The first stage involves cultivation in a photobioreactor where growth parameters are controlled to promote cell division and minimise contamination. The second phase involves shifting the microalgae to open ponds where they are subjected to nutrient depletion and other environmental stresses [66].

From a sustainability perspective, each cultivation technique presents distinct advantages and limitations. Open pond systems generally require lower capital and operational costs but may suffer from lower productivity and higher water losses, whereas closed

photobioreactors provide greater process control and higher pigment yields at the expense of increased energy consumption and infrastructure requirements. Hybrid systems have emerged as a promising approach to balance productivity, resource efficiency, and environmental performance [66].

4.2. Cultivation Systems

The cultivation systems of microalgae significantly influence pigment production. Microalgae can be cultivated using various methods, including autotrophic, heterotrophic and mixotrophic cultivation [9,31].

Autotrophic cultivation uses light as the primary energy source and inorganic carbon (e.g., CO_2). This mode has a low pollution risk, which results in a low biomass concentration and increased harvesting costs [76]. Optimising light intensity, quality, and photoperiod increases photosynthesis and pigment production [31].

Table 5 summarises microalgal species capable of producing valuable pigments under autotrophic cultivation, along with their pigment content and applications. Carotenoids, including lutein, astaxanthin, zeaxanthin, β-carotene, and fucoxanthin, are the main pigments produced by these species. *Chlorella sorokiniana* showed a high lutein content (20.7 mg/g), while *Haematococcus lacustris* produced astaxanthin (2.6 mg/g). These pigments have potential applications in pharmaceutical, nutraceutical, food, cosmetic, and antioxidant industries.

Table 5. Microalgae species that produce various pigments under autotrophic cultivation.

Microalga	Pigment	Pigment Content (mg/g)	Application	Reference
Chlorella sorokiniana	Lutein	20.7	Pharmaceutical, and cosmetics	[77]
Haematococcus lacustris	Astaxanthin	2.6	Pharmaceutical	[78]
Synechocystis sp.	Zeaxanthin	1.6	Pharmaceuticals, vitamins, and antioxidant production	[79]
Rhodosorus sp.	Zeaxanthin	2.16	Pharmaceuticals, vitamins, and antioxidant production	[79]
Chlorococcum amblystomatis	Neoxanthin	0.63	Nutraceuticals	[80]
Tetraselmis suecica	α-Carotene	0.202	Nutraceutical and pharmaceutical industries	[81]
Tetraselmis suecica	β-Carotene	1	Food processing, animal feeds, pharmaceutical, and cosmetics industries	[82]
Tisochrysis lutea	Fucoxanthin	8.8 mg/L	Cosmetic and antioxidant	[83]

Heterotrophic cultivation involves growing microalgae in the dark using organic carbon sources like glucose or acetate [84]. This method enables better control over parameters like pH, temperature, and oxygen levels in conventional industrial fermenters that overcome light limitation and potentially achieve very high cell densities [85,86]. Although it generally produces lower levels of light-dependent pigments, some carotenoids and phycobilins can still be produced [6]. *Auxenochlorella protothecoides* (formerly *Chlorella protothecoides*) (Chlorophyta) is a prototype strain for heterotrophic lutein production, with glucose being a preferred carbon source [87]. *Chromochloris zofingiensis* can also produce astaxanthin heterotrophically [88].

Table 6 summarises microalgal species cultivated under heterotrophic conditions for pigment production, including the carbon sources, pigment types, and pigment content.

Glucose is the most used carbon source, supporting the production of valuable pigments such as lutein, astaxanthin, and phycocyanin. *Auxenochlorella pyrenoidosa* (formerly *Chlorella pyrenoidosa*) showed high lutein production (218 mg/L), while *Haematococcus lacustris* and *Chlorococcum* sp. demonstrated the ability to produce astaxanthin. *Galdieria sulphuraria* (Rhodophyta) exhibited significant phycocyanin accumulation, reaching up to 25–30 mg/g. These findings highlight the potential of heterotrophic cultivation for enhancing pigment productivity and developing commercial applications in food, pharmaceutical, nutraceutical, and cosmetic industries.

Table 6. Microalgae species under heterotrophic cultivation for pigment production.

Microalga	Carbon Source	Pigment	Pigment Content	Reference
Auxenochlorella pyrenoidosa	Glucose 30 g/L	Lutein	218 mg/L	[89]
Auxenochlorella pyrenoidosa	Glucose 40 g/L	Lutein	2.5 mg/g	[90]
Auxenochlorella protothecoides	Glucose 9 g/L	Lutein	16 mg/L	[87]
Haematococcus lacustris	45 mM Acetate	Astaxanthin	9 mg/L	[91]
Chromochloris zofingiensis ATCC30412	50 g/L glucose	Astaxanthin	10.3 mg/L	[88]
Chlorococcum sp.	44 g/L Glucose, 0.1 mM H_2O_2	Astaxanthin	1.8 mg/g	[92]
G. sulphuraria	Glucose 50 g/L	Phycocyanin	3.6 mg/g	[93]
G. sulphuraria	Glucose, fructose, glycerol 5 g/L	Phycocyanin	2–4 mg/g	[94]
G. sulphuraria	Glucose 5 g/L	Phycocyanin	25–30 mg/g	[95]

Mixotrophic cultivation combines light and organic carbon sources. This approach can achieve higher biomass productivity than autotrophic cultivation and can be a viable option for enhancing the production of certain pigments, like lutein, under light stress [6,96].

In many cases, mixotrophic conditions can lead to increased pigment yield compared to autotrophic or heterotrophic cultivation alone [8]. This enhancement can be attributed to a synergistic effect of light energy and organic carbon metabolism [32].

Table 7 presents microalgal species cultivated under heterotrophic conditions for pigment production, including the carbon sources, pigment types, and pigment content. Various organic carbon sources, particularly glucose and acetate, have been applied to enhance the production of valuable pigments such as lutein, astaxanthin, and phycocyanin. *Chlorella sorokiniana* and *Chlorella protothecoides* showed high lutein productivity, while *Chlorococcum* sp. and *Chlorococcum zofingiensis* demonstrated significant astaxanthin production. In addition, *Limnospira platensis* achieved a high phycocyanin content (322 mg/L), highlighting the potential of heterotrophic cultivation for improving pigment production and supporting commercial applications in food, pharmaceutical, and nutraceutical industries.

Table 7. Heterotrophic cultivation of microalgae species for pigment production.

Microalga	Carbon Source	Pigment	Pigment Content	Reference
Chlorella sorokiniana	100 mM glucose 40 mM acetate	Lutein	33 mg/L	[97]
Chlorella sorokiniana	acetate 6 g/L	Lutein	5.86 mg/g	[98]

Table 7. *Cont.*

Microalga	Carbon Source	Pigment	Pigment Content	Reference
Auxenochlorella protothecoides	glucose 40 g/L nitrate 10 g/L	Lutein	6.48 mg/g	[99]
Coccomyxa acidophila	urea 0.67 g/L	Lutein	3.55 mg/g	[100]
Chromochloris zofingiensis ATCC30412	30 g/L glucose	Astaxanthin	12.5 mg/L	[101]
Chlorococcum sp.	44 g/L glucose	Astaxanthin	7.08 mg/g	[92]
Limnospira platensis UTEX	glucose 2.5 g/L	Phycocyanin	322 mg/L	[102]

Autotrophic, heterotrophic, and mixotrophic cultivation each offer distinct advantages and limitations for microalgal pigment production. Autotrophic cultivation is environmentally sustainable because it utilises sunlight and CO_2 as energy and carbon source, respectively, resulting in low operating costs and a reduced risk of contamination. However, its productivity is often limited by light availability, leading to relatively low biomass concentrations and higher harvesting costs [64]. In contrast, heterotrophic cultivation eliminates light limitations by using organic carbon sources in conventional bioreactors, enabling high cell densities and improved process control. Nevertheless, this approach requires expensive organic substrates, is susceptible to microbial contamination, and is generally less suitable for the production of light-dependent pigments such as chlorophylls [73]. Mixotrophic cultivation combines the advantages of both systems by simultaneously utilising light and organic carbon sources, often resulting in higher biomass productivity and enhanced pigment accumulation through the synergistic effects of photosynthesis and heterotrophic metabolism. Although mixotrophic cultivation generally achieves the highest pigment productivity, its commercial implementation is more challenging due to the need to optimise both light conditions and carbon supplementation while minimising contamination. Therefore, the selection of an appropriate cultivation mode depends on the target pigment, the microalgal species, and the economic considerations associated with large-scale production [27].

5. Pigment Extraction from Microalgae

Extraction of pigment from microalgae is a major step in developing a commercially viable and sustainable microalgal bio-industry. The efficient recovery methods have great importance for the pigment production cost [103]. The extraction and purification of pigments from algae involve several key stages: cell harvesting, cell disruption, pigment extraction, and purification [8]. Microalgal pigments are typically intracellular and exist inside the biomass cells. For this reason, the cell wall should be broken to extract the pigments. Therefore, the general process involves an initial biomass pretreatment step to disrupt the cells and then an extraction step to recover the pigments. The final stage of this process is purification to obtain the desired pigment in a suitable form [103].

5.1. Cell Harvesting

The harvesting process alone can account for 20–30% of the total cost to produce microalgae [104]. Currently, there is not a universally suitable method for microalgae harvesting, especially for carotenoid production, as this process usually requires non-toxicity and minimising carotenoid degradation [7]. The difficulty of harvesting suspended microalgae increases as the cell size decreases [105]. Various methods can be employed for harvesting, depending on the microalgae strain and the scale of production [8].

Gong and Bassi [7] studied the cell harvesting methods for the production of carotenoids from microalgae and categorised the cell harvesting methods as physical, chemical, mechanical, and electromagnetic. The main physical method includes sedimentation, centrifugation, and filtration. Sedimentation can be used for large and dense cells. centrifugation is one of the most popular harvesting methods and is often combined with sedimentation to reduce moisture content. Filtration, on the other hand, is highly dependent on the size of the microalgae cells and the characteristics of the biomass, making it more suitable for specific strains [7]. In the chemical methods, flocculation is the most important one, which consumes much less energy and requires lower capital investment compared to mechanical methods. The major cost of this method is for the flocculant chemicals. The electromagnetic techniques need no additional chemicals which make them more environmentally compatible. However, fouling in the electro-cathodes may cause problems in large-scale operation [7].

5.2. Cell Disruption

Microalgae pigments are located within the intracellular matrix and are often protected by rigid cell walls. Therefore, effective cell disruption is essential to enhance the yield and efficiency of pigment extraction. There are several methods for cell disruption, which can be classified as mechanical, chemical, thermal, and enzymatic. Each method is selected based on the microalgae strain, pigment type, the desired scale of operation, and cost considerations [7,103].

Table 8 presents the different cell disruption methods applied for microalgal pigment extraction, including their advantages and limitations. Mechanical techniques, such as bead milling, high-pressure homogenisation, ultrasonication, and grinding, provide effective cell rupture and are suitable for improving pigment recovery; however, they may require high energy input and can cause pigment degradation due to heat generation. Chemical methods, including solvent extraction and acid/alkali hydrolysis, offer simple and efficient cell disruption but may involve solvent toxicity, environmental concerns, and additional purification steps. Thermal and enzymatic approaches provide mild processing conditions and better pigment preservation, although some methods suffer from long processing times, high energy consumption, or increased costs. Emerging technologies, such as pulsed electric field, microwave-assisted extraction, and supercritical CO_2 extraction, have shown potential for sustainable pigment recovery due to their high efficiency, lower solvent consumption, and improved selectivity. However, further optimisation is required to overcome challenges related to scalability, equipment costs, and pigment stability for industrial applications.

Table 8. Cell disruption methods used in microalgal pigment extraction.

Method	Technique	Advantages	Disadvantages	Reference
Mechanical	Bead Milling	High efficiency, scalable, suitable for wet biomass	Generates heat; may degrade sensitive pigments	[7,106]
	High-Pressure Homogenisation	Effective cell rupture, fast, easy scale up	High energy input, cooling may be required	[7,103,107]
	Ultrasonication	Simple setup, efficient for small volumes	Can cause pigment degradation; limited scalability, high energy requirement	[103]
	Grinding	Cost-effective; suitable for wet biomass	Time-consuming, not suitable for scale up	[108,109]

Table 8. *Cont.*

Method	Technique	Advantages	Disadvantages	Reference
Chemical	Solvent extraction	Cheap and easy to scale up, simultaneous disruption and extraction	Solvent toxicity, requires downstream purification	[7,107]
	Acid/Alkali Hydrolysis	Breaks rigid cell walls quickly, requires low energy	May degrade pigments; environmental disposal issues	[107,110]
Thermal	Freeze-Thaw Cycles	Gentle on pigments; low cost, high purity	Time-consuming; not scalable, consumes a lot of energy	[29]
	Autoclaving/Boiling	Simple and effective	Can degrade heat-sensitive pigments	
Enzymatic	Enzyme Treatment	Mild and specific; preserves pigment structure	Expensive; slower process	[111,112]
Emerging	Pulsed Electric Field (PEF)	Low energy, scalable, preserves pigments	Generally, does not result in cell rupture, useful for small molecules, and requires organic solvent	[103,113]
	Microwave-Assisted Extraction	Rapid and efficient; enhances yield	Heat-sensitive pigments may degrade	[7,111]
	Supercritical CO_2 Extraction	Selective; solvent-free; eco-friendly	High cost; needs high-pressure equipment	[107,114]

It is important to note that for microalgae with thick and rigid cell walls, such as *Haematococcus lacustria* (Chlorophyta) and *Nannochloropsis* sp. (Eustigmatophyceae), energy-intensive mechanical treatments or chemical hydrolysis may be required. In contrast, species with less robust or no cell walls, like *Dunaliella salina*, may require less intensive methods, such as freeze-thawing [103]. In some cases, a pretreatment step might not be necessary, as cells can rupture upon solvent contact during extraction. Furthermore, the cell rupture step and the extraction step can sometimes be combined and carried out simultaneously [103]. The selection of an appropriate cell disruption method is, therefore, a crucial decision in the overall process of pigment extraction from microalgae [115].

From a sustainability perspective, cell disruption methods should be evaluated based on their energy consumption, chemical usage, and environmental impact. Mechanical methods are effective but often require high energy inputs, while chemical methods may generate solvent-related environmental concerns [7,24]. Enzymatic treatments offer a greener alternative due to their mild operating conditions and reduced chemical requirements [99]. Emerging technologies such as pulsed electric field, microwave-assisted extraction, and supercritical CO_2 extraction show significant potential for sustainable pigment recovery by reducing solvent consumption, minimising waste generation, and improving process efficiency [99,102].

5.3. Extraction and Purification

After cell disruption, the pigment should be extracted from the mixture of cell debris that contains intracellular and extracellular components. The resulting mixture typically contains pigments, proteins, lipids, carbohydrates, nucleic acids, and cell debris [116]. Extraction of the pigment from this mixture needs to be a selective method. Different techniques are used to extract the pigments based on their solubility [8]. Solvent extraction is a common method for the extraction of pigments. In this method, an organic solvent that can dissolve the pigment is selected, and the pigment is separated from the biomass

mixture. The choice of solvent depends on the polarity, solubility, and chemical stability of the target pigments [8]. The selected solvent for industrial-scale pigment extraction should be relatively volatile and have a low boiling point. For instance, non-polar solvents like n-hexane, dichloromethane, dimethyl ether, and diethyl ether are typically used for carotenoids [117]. The lutein extraction from wet *Chlorella vulgaris* (Chlorophyta) has been investigated using ethanol/hexane (3:1, *v*/*v*) [118]. Fucoxanthin from *Isochrysis galbana* (Haptophyta) can be extracted with ethanol [119], and astaxanthin from *H. lacustrisis* is extracted using chloroform/methanol or acetone/ethyl acetate/ethanol [120]. However, conventional methods are often less efficient, time-consuming, consume large amounts of solvents, and may leave toxic residues, limiting their use in the food industry [28].

To overcome the limitations of conventional methods, various advanced techniques are proposed in the literature. Supercritical fluid extraction (SFE) is one of the efficient methods for this purpose. This method uses supercritical fluids, often CO_2, to extract carotenoids [28]. Supercritical CO_2 is environmentally friendly, non-toxic, and can be easily removed, yielding solvent-free products [28]. It can be used for a wide spectrum of carotenoids by optimising parameters like temperature, pressure, and the addition of co-solvents such as ethanol or vegetable oils [8]. SFE has been used to extract carotenoids from *C. vulgaris* [121], astaxanthin from *H. lacustris* [122], β-carotene from *D. salina* [28], and fucoxanthin from *Undaria pinnatifida* (Phaeophyceae) with ethanol as a co-solvent [123].

Pressurised liquid extraction (PLE), also known as accelerated solvent extraction (ASE), uses high temperatures (50–200 °C) and pressures (35–200 bar) to maintain the extraction solvent in a liquid state, enhancing mass transfer and reducing solvent consumption and extraction time [107,124]. It can employ greener solvents like ethanol and water [103]. PLE has been used for extracting carotenoids from *H. lacustris*, *D. salina*, *C. vulgaris*, and *Tisochrysis lutea* (Haptophyta) [28], as well as high levels of fucoxanthin from *Phaeodactylum tricornutum* [125].

It should be noted that some methods can achieve both disruption and extraction at the same time. Microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and pulsed electric field (PEF) are the methods that can be applied for both cell disruption and pigment extraction.

The choice of extraction method is often a trade-off between efficiency, cost, environmental impact, and the stability of the extracted pigments. Environment-friendly technologies like SFE, MAE, and UAE are gaining importance due to concerns over the high solvent usage in conventional methods [126].

Once the pigments are extracted, further purification is often required to obtain high-purity compounds, especially when the pigments are intended for pharmaceutical or food industry applications. The purification step increases the cost of the pigment, which is why pigments are found only available with other components [29]. Chromatography is a key method for the purification of various microalgal pigments. High-performance liquid chromatography (HPLC) is frequently used for both analytical characterisation and preparative purification of carotenoids and chlorophylls [127]. Hyphenated liquid chromatography (LC) systems with mass spectrometers (LC–MS) are used for the identification and characterisation of individual pigments. C30 reverse phase HPLC has been used for the analysis of algae carotenoids [128]. Expanded-bed adsorption chromatography has been used for the preparative purification of B-phycoerythrin from *Porphyridium purpureum* (formerly *Porphyridium cruentum*) (Rhodophyta) [2].

Other separation techniques may be used in conjunction with or as alternatives to chromatography, depending on the pigment's properties. For example, the use of nanomaterials or nanoparticles is being explored for improved carotenoid recovery and purification [129]. Table 9 presents various methods for pigment purification from microalgae.

Table 9. Some main methods of pigment purification from microalgae.

Method	Principle	Advantages	Limitations	Reference
Liquid–Liquid Extraction (LLE)	Pigment partition between two immiscible liquids based on solubility.	Simple, scalable.	Low selectivity, multiple steps needed.	[28,95]
Aqueous Two-Phase Extraction (ATPE)	Uses two water-based phases to separate pigments by polarity and solubility.	Gentle, good for protein pigments like phycobiliproteins.	Needs optimisation for each pigment system.	[30,103]
Column Chromatography	Separates pigments based on polarity or size using a stationary phase.	Good separation, low-cost for lab-scale use.	Labour-intensive, slow, not scalable.	[8,30]
High-Performance Liquid Chromatography (HPLC)	Separates and quantifies pigments based on retention time.	High precision and resolution, ideal for analytical applications.	Expensive, requires expertise, has limited scalability.	[30,127]
Membrane Filtration (Ultrafiltration)	Uses pressure and membranes to separate by molecular size.	Energy-efficient, good for water-soluble pigments.	Membrane fouling, not suitable for all pigment types.	[30,95]
Precipitation (e.g., Ammonium Sulphate)	Changes solubility to precipitate pigments or associated proteins.	Simple, good for protein-based pigments.	Co-precipitation of impurities, further steps often needed.	[30,103,130]

The sustainability of microalgal pigment production depends not only on cultivation but also on downstream processes, including cell disruption, extraction, and purification. Conventional solvent extraction can achieve high pigment recovery but raises environmental concerns due to solvent toxicity, waste generation, and energy requirements for solvent recovery. Green extraction approaches, such as supercritical CO_2, enzyme-assisted, and ultrasound-assisted extraction, offer more sustainable alternatives by reducing hazardous solvent use, lowering energy consumption, and improving pigment preservation. Similarly, the development of selective and integrated purification methods can minimise chemical usage, waste generation, and processing costs, contributing to more sustainable pigment production [23].

6. Challenges and Future Directions

The demand for pigment from microalgae is increasing as a sustainable natural resource. However, there are still some gaps in the production technology.

A major bottleneck is the high cost associated with both microalgae cultivation and downstream processing, including extraction and purification. This can account for a substantial portion (60–90%) of the overall supply-chain costs. It makes microalgal pigment production often more expensive than synthetic counterparts or pigments from a traditional plant source [7,131].

Many microalgae species produce only a low pigment amount under normal growth conditions, which limits their economic feasibility [66]. However, some species produce higher amounts of pigments under stress, which can inhibit overall biomass production, requiring optimisation [9].

Inefficient extraction and purification methods are an important challenge that needs to be considered. The rigid cell walls of many microalgae hinder the complete recovery of intracellular pigments, making cell disruption a crucial and often energy-intensive step [7]. Existing extraction technologies can be costly to implement and operate, with high energy requirements and solvent consumption. The variability in microalgae composition

necessitates case-by-case optimisation of extraction protocols, making standardisation challenging [29].

Moving from small laboratory scale setups to large-scale industrial systems brings technical and cost-related challenges. In some cases, the lab-scale production is feasible, while on scaling up, maintaining the quality and yield is challenging. Additionally, maintaining optimal growth conditions and efficient extraction at larger volumes can be difficult [3].

Pigment instability is a major challenge, as certain microalgal pigments, such as phycocyanin and chlorophylls, are prone to degradation during extraction, purification, and storage. This degradation can negatively affect their quality, colour properties, and biological activity [32]. The stability of microalgal pigments can be significantly enhanced through several technological approaches. Microencapsulation and nanoencapsulation are among the most effective techniques, providing protection against degradation caused by light, oxygen, moisture, heat, and pH fluctuations while improving shelf life and controlled release [132]. The addition of natural antioxidants, such as ascorbic acid or tocopherols, can further reduce oxidative degradation. Optimising storage conditions by minimising exposure to light, oxygen, and elevated temperatures also plays a critical role in preserving pigment quality. In addition, formulation strategies, including emulsions, liposomes, and biopolymer-based protective coatings, have been widely investigated to improve pigment stability, bioavailability, and compatibility with food, pharmaceutical, and cosmetic products [133]. These approaches are essential for maintaining pigment functionality during processing, storage, and commercial application.

Natural pigments from microalgae face competition from cheaper synthetic colourants [134]. To gain a larger market share, microalgal pigments need to be produced more cost-effectively while highlighting their health benefits and natural origin [3].

Limited understanding of biosynthesis is another challenge that needs to be addressed. Although some pigment biosynthesis pathways are known, a deeper understanding of the regulatory mechanisms controlling pigment production in response to environmental cues is still required for effective metabolic engineering [9].

To overcome current limitations and unlock the full potential of microalgae as a sustainable source of natural pigments, several areas of future research and development are essential. The future direction for pigment production is summarised as follows:

- Research on screening and selecting high-pigment-producing microalgal strains and employing advanced technologies like genetic engineering, mutagenesis, and metabolic engineering to enhance pigment accumulation is crucial [3]. This includes exploring the potential of both photoautotrophic and heterotrophic/mixotrophic cultivation strategies for specific pigments [3].
- Developing more efficient and cost-effective cultivation systems, including optimising growth conditions (light, temperature, nutrients, pH, salinity) and exploring novel trophic modes (e.g., mixotrophy), can significantly improve biomass and pigment yields [66]. Implementing two-stage cultivation processes, where biomass is first maximised followed by stress induction for pigment accumulation, shows promise for certain pigments [10].
- Focusing on the development of low-cost, energy-efficient, and environmentally friendly extraction and purification methods is essential [3]. This includes optimising existing techniques like supercritical fluid extraction (SFE), pressurised liquid extraction (PLE), pulsed electric field (PEF), microwave-assisted extraction (MAE), and ultrasound-assisted extraction (UAE), as well as exploring novel solvent systems like natural deep eutectic solvents (NADES) [135]. Process intensification strategies

that combine extraction and purification steps can also reduce costs and processing time [135].

- Adopting a biorefinery concept where multiple valuable products, including pigments, lipids, proteins, and carbohydrates, are extracted from the same algal biomass can improve the overall economic viability and sustainability of the process [3].
- Metabolic Engineering and Synthetic Biology: Further advancements in understanding and manipulating the metabolic pathways involved in pigment biosynthesis will enable the development of microalgae with enhanced pigment production capabilities [9].
- Research on stabilising pigments during and after extraction through techniques like encapsulation, use of antioxidants, and optimised processing conditions is crucial for maintaining their quality and bioactivity [2].
- Addressing the challenges associated with scaling up production from laboratory to industrial levels through improved bioreactor design and process control is vital for commercial success [3].
- Exploration of Novel Pigments and Sources: Continued exploration of the vast biodiversity of microalgae may lead to the discovery of novel pigments with unique properties and applications [5].
- Evaluating the environmental impact of microalgae-based pigment production using life cycle assessment and focusing on sustainable practices, such as utilising wastewater and flue gas as nutrient sources, is important [8].
- Raising awareness about the benefits of natural microalgal pigments and ensuring their safety and efficacy through comprehensive studies will drive consumer demand and market growth [3].

By addressing these challenges and focusing on these future directions, microalgae can become a more competitive and sustainable source of valuable pigments for a wide range of industries.

7. Conclusions

Microalgae have emerged as viable microbial cell factories capable of converting carbon dioxide and sunlight into high-value, bioactive compounds. However, despite the great potential, several technical, economic, and operational challenges need to be addressed to unlock their full promise.

The process of microalgal pigment production involves several key steps: efficient cultivation, biomass harvesting, cell disruption, pigment extraction, and purification. The cultivation process, being the first step, plays a pivotal role in determining the final pigment production. Environmental factors such as light intensity, photoperiod, temperature, pH, and nutrient availability have great effects on the productivity and composition of the pigments. Light quality and intensity, directly impact photosynthesis and the biosynthesis of primary and secondary pigments. Similarly, temperature and pH affect metabolic activity and pigment accumulation, with deviations from the optimal ranges potentially hindering growth and reducing pigment productivity. Nutrient supply, particularly nitrogen and phosphorus, also affects pigment biosynthesis. While adequate nutrients are essential for growth, nutrient limitation can be intentionally induced to promote the accumulation of stress-related pigments, such as astaxanthin and β-carotene.

In terms of cultivation systems, the choice of method significantly impacts the efficiency, cost, and overall success of pigment production. Open pond systems are cost-effective but face challenges like contamination risks and lower productivity which make them suitable for large-scale production of low-value pigments. Closed photobioreactors, on the other hand, offer better control over environmental conditions, enabling higher

biomass yields and improved quality, especially for high-value pigments. The choice of cultivation method should be aligned with the desired pigment type, production scale, and cost considerations.

After cultivation, the extraction and purification of pigments from microalgae are critical for commercial success. Since microalgal pigments are intracellular, effective cell disruption is necessary to release these compounds. The methods used for cell disruption—whether mechanical, chemical, thermal, or enzymatic—must be selected based on the type of pigment, microalgal strain, and cell wall structure.

The commercialisation of microalgal pigments remains hindered by high production costs, especially due to inefficient downstream processing and pigment instability. Moreover, pigment productivity under normal cultivation conditions is often low, making it less competitive compared to synthetic alternatives. To improve economic feasibility, future research should focus on optimising strain selection through genetic and metabolic engineering, enhancing cultivation systems, and developing cost-effective and environmentally friendly extraction and purification technologies.

The development of new techniques to stabilise pigments during and after extraction is essential in maintaining their quality, bioactivity, and shelf-life, particularly in high-value applications. Furthermore, scaling up from laboratory to industrial systems remains a challenge, requiring improvements in bioreactor design and process control.

In conclusion, the future of microalgal pigment production has immense potential for obtaining sustainable and eco-friendly alternatives to synthetic pigments. By addressing current challenges and focusing on innovation in genetic engineering, cultivation strategies, and downstream processing, microalgae can emerge as a competitive and sustainable source of high-value natural pigments for a wide range of industries.

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