

Review

Carotenoids from Cyanobacteria: Biotechnological Potential and Optimization Strategies

Fernando Pagels ^{1,2} , Vitor Vasconcelos ^{1,2}  and Ana Catarina Guedes ^{1,*} 

¹ CIIMAR—Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Novo Edifício do Terminal de Cruzeiros de Leixões, Avenida General Norton de Matos, s/n, 4450-208 Matosinhos, Portugal; fernandopagels@gmail.com (F.P.); vmvascon@fc.up.pt (V.V.)

² FCUP—Faculty of Science, University of Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal

* Correspondence: acatarinaguedes@gmail.com

Abstract: Carotenoids are tetraterpenoids molecules present in all photosynthetic organisms, responsible for better light-harvesting and energy dissipation in photosynthesis. In cyanobacteria, the biosynthetic pathway of carotenoids is well described, and apart from the more common compounds (e.g., β -carotene, zeaxanthin, and echinenone), specific carotenoids can also be found, such as myxoxanthophyll. Moreover, cyanobacteria have a protein complex called orange carotenoid protein (OCP) as a mechanism of photoprotection. Although cyanobacteria are not the organism of choice for the industrial production of carotenoids, the optimisation of their production and the evaluation of their bioactive capacity demonstrate that these organisms may indeed be a potential candidate for future pigment production in a more environmentally friendly and sustainable approach of biorefinery. Carotenoids-rich extracts are described as antioxidant, anti-inflammatory, and anti-tumoral agents and are proposed for feed and cosmetic industries. Thus, several strategies for the optimisation of a cyanobacteria-based bioprocess for the obtention of pigments were described. This review aims to give an overview of carotenoids from cyanobacteria not only in terms of their chemistry but also in terms of their biotechnological applicability and the advances and the challenges in the production of such compounds.

Keywords: xanthophylls; carotenes; orange carotenoid protein; bioactive potential; production; extraction; purification



Citation: Pagels, F.; Vasconcelos, V.; Guedes, A.C. Carotenoids from Cyanobacteria: Biotechnological Potential and Optimization Strategies. *Biomolecules* **2021**, *11*, 735. <https://doi.org/10.3390/biom11050735>

Academic Editor: Christophe Brunet

Received: 26 April 2021

Accepted: 12 May 2021

Published: 15 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cyanobacteria, as photosynthetic organisms, have a light-harvesting complex for the absorption of light energy for photosynthesis. This harvesting complex is composed of pigments that can be divided into three chemical groups: chlorophylls, phycobiliproteins, and carotenoids [1]. Such compounds are organized in the thylakoid membrane, in parallel to the cell membrane. Specifically, cyanobacteria have a phycobilisome (containing phycobiliproteins) and orange carotenoid proteins (OCP; containing hydroxyechinenone) outside the membrane, while chlorophyll and most carotenoids are located inside the photosystem, in a transmembrane protein complex [2,3].

In cyanobacteria, the main pigments for light absorption are phycobiliproteins, while carotenoids have a huge impact on the non-photochemical quenching (NPQ) as protection agents against saturating light and quencher of reactive oxygen species (ROS) [1].

When it comes to carotenoids, these terpenoids pigments are considered essential for the survival of all photosynthetic organisms and are transversal through these organisms. A large number of pigments are described as carotenoids (ca. 600) and can be divided into two major classes, carotenes, such as α - and β -carotene, and xanthophylls (oxygenised derivatives of carotenes) such as zeaxanthin and echinenone [4,5]. Apart from their biological role, carotenoids attract great interest from the industry due to their bioactive potential as antioxidant, anti-inflammatory, and anti-tumoral, among others [4–6].

The market value of carotenoids surpassed \$1.5 billion (USD) from 2016 to 2019, β -carotene, astaxanthin, and lutein being responsible for 60% of this market [7,8]. The bioprocess of these carotenoids is well established in the microalgae *Dunaliella salina* (β -carotene) and *Haematococcus pluvialis* (astaxanthin) and in the vascular plant marigold *Tagetes erecta* (lutein).

Despite the potential of cyanobacteria as a producer of pigments, the only large-scale market application of these organisms is related to the phycocyanin production from *Arthrospira platensis*. The reason is that the content of carotenoids in cyanobacteria is significantly lower than microalgae or vascular plants [5].

On the other hand, strategies for the use of cyanobacterial carotenoids increased in recent years, translating into a possible *light* for a future *bloom* of cyanobacteria in this market. These strategies go into three main aspects of the bioprocess: (i) in the valorisation of cyanobacterial carotenoids' extracts by exploiting bioactive potential and unique applicability into nutraceuticals, cosmetics, feed, among others; (ii) in the increase of carotenoids content in cyanobacteria by optimising growth conditions, by using pathway triggers/stress stimuli, or by increasing gene expression through genetic engineering; (iii) in the efficient use of the biomass by efficient extraction and by the use of biorefineries, becoming a co-product of phycobiliproteins production.

This paper aims to review the different kinds of cyanobacterial carotenoids as well as the most relevant characteristics for biotechnological application, with a specific focus on their bioactivities. The optimisation of their production, extraction, and purification strategies is also emphasized. Finally, economic considerations and future perspectives in the field are briefly discussed.

2. Chemistry

Carotenoids are tetraterpenoids molecules, i.e., most carotenoids are composed of a C40 hydrocarbon chain containing eight isoprenoids and a series of double-bound conjugations. They can subdivide into carotenes, which are either linear or cyclized molecules with one or two rings at their extremes, lacking oxygen atoms; and xanthophylls, which are oxygenated derivatives of carotenes. Moreover, modifications to carotenes can form glycosylated carotenoids such as myxoxanthophyll or even shortened chain carotenoids (apocarotenoids) [5].

Cyanobacteria are able to synthesize a wide variety of terpenoids due to a complex but well-described biosynthetic pathway known as carotenogenesis (Figure 1). The production of these compounds originates from geranylgeranyl pyrophosphate precursor [9,10]. A series of genes encode synthases, desaturases, cyclases, and hydroxylases responsible for the synthesis of carotenoids. Liang et al. [9] evaluated the presence of the described genes through several cyanobacterial species and found that most of the genes are transversal, although specific genes can be found as a replacement or as redundant to other genes.

The biosynthetic pathway starts with two molecules of geranylgeranyl pyrophosphate condensed into phytoene through phytoene synthase (CrtB); from that, a phytoene desaturase can convert phytoene to ζ -carotene (CrtP), and then a carotene desaturase (CrtQ) converts it to lycopene. A few genera such as *Anabaena* and *Nostoc* can convert phytoene directly to lycopene using a phytoene desaturase (CrtI), although they also produce the regular phytoene desaturase (CrtP) [9]. From lycopene, the major primary carotenoids are formed, α - and β -carotene. α -carotene is formed in a direct conversion by lycopene cyclase (CrtL or CruA), while β -carotene is derived from a γ -carotene in a two-step process performed by a lycopene cyclase (CrtL or CruA) [9,10]. From the three carotenoids, several xanthophylls can be formed as follows.

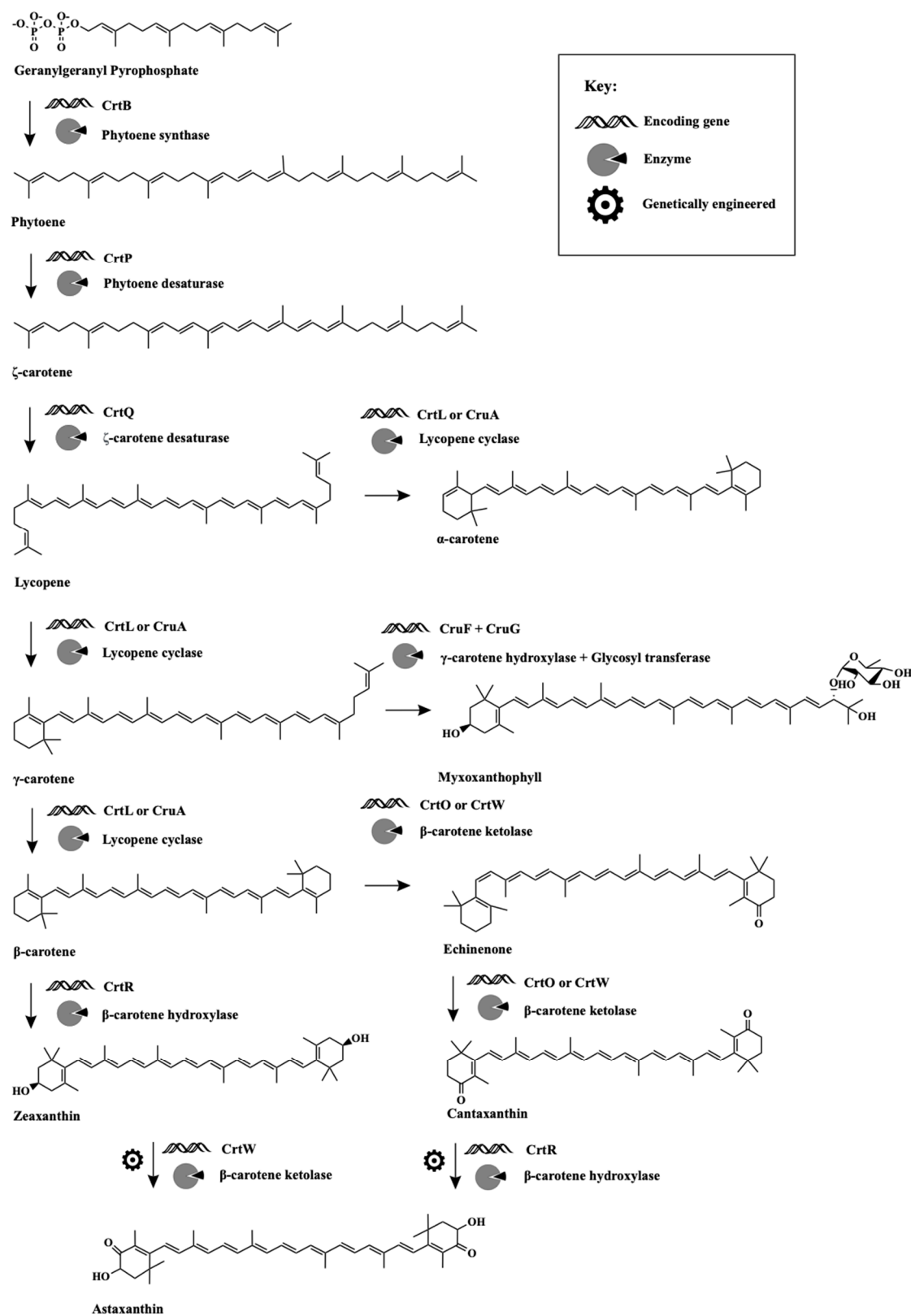


Figure 1. Biosynthetic pathway of carotenoids in cyanobacteria.

From γ-carotene, through a hydroxylation by γ-carotene hydroxylase (CruF) and the addition of a glycoside group by a glycosyltransferase (CruG), a myxoxanthophyll molecule

is formed. Myxoxanthophyll is a yellow glycoside terpenoid exclusive to cyanobacteria, and its production is required for cell wall structure and thylakoid organization [11].

From α -carotene, lutein can be formed by a hydroxylase (CrtR) [12].

From β -carotene, a ketolase (CrtO or CrtW) can convert β -carotene to an echinenone, and a hydroxylase (CrtR) can convert to zeaxanthin. Furthermore, from echinenone, a ketolase (CrtO or CrtW) can form canthaxanthin, and a hydroxylase (CrtR) can form hydroxyechinenone, the main ketocarotenoid in the OCP. From zeaxanthin, both antheraxanthin and violaxanthin can be formed through a revertible activity of epoxidase and de-epoxidase in a process called the violaxanthin cycle. From zeaxanthin, it is also possible to form nostoxanthin by a hydroxylase (CrtG), while from violaxanthin, it is possible to form neoxanthin by a neoxanthin synthase (NSY) [9,10]. Moreover, astaxanthin can be produced using engineered cyanobacteria by the inclusion of a CrtR or a CrtW gene. Astaxanthin can be derived from canthaxanthin and formed by a hydroxylase (CrtR) and derived from zeaxanthin, formed then by a ketolase (CrtW) [13].

Although carotenogenesis genes are conserved in most photosynthetic organisms, the expression and the consequent carotenoid production can be limited to some groups of cyanobacteria [14] and are specifically manipulated by growth conditions [6]. In a biotechnological approach, it is fundamental to take advantage of such regulations to increase the production of specific or total carotenoids.

3. Orange Carotenoid Protein (OCP)

The cyanobacteria light-harvesting occurs mainly through the phycobilisome, a protein component of the extramembrane antenna pigment (phycobiliproteins), which transfers the energy to the photosystem core [15]. On the other hand, the main function of carotenoids in cyanobacteria is energy dissipation and protection against oxidative damage. The NPQ is responsible to reduce excessive light energy to reach the photosystem core. In cyanobacteria, carotenoids can be present in the reaction centre, together with the chlorophyll molecules, or in a protein complex containing a single molecule of carotenoid, which is a key component for the photoprotection—the OCP [16]. The OCP is a water-soluble protein (35 kDa) containing a single molecule of hydroxyechinenone present in an inactive form (orange) that is triggered by blue-green light and converted to an active form (red) (Figure 2). The OCP, although fundamental for cell protection, usually represents only 1% of total carotenoids [2].

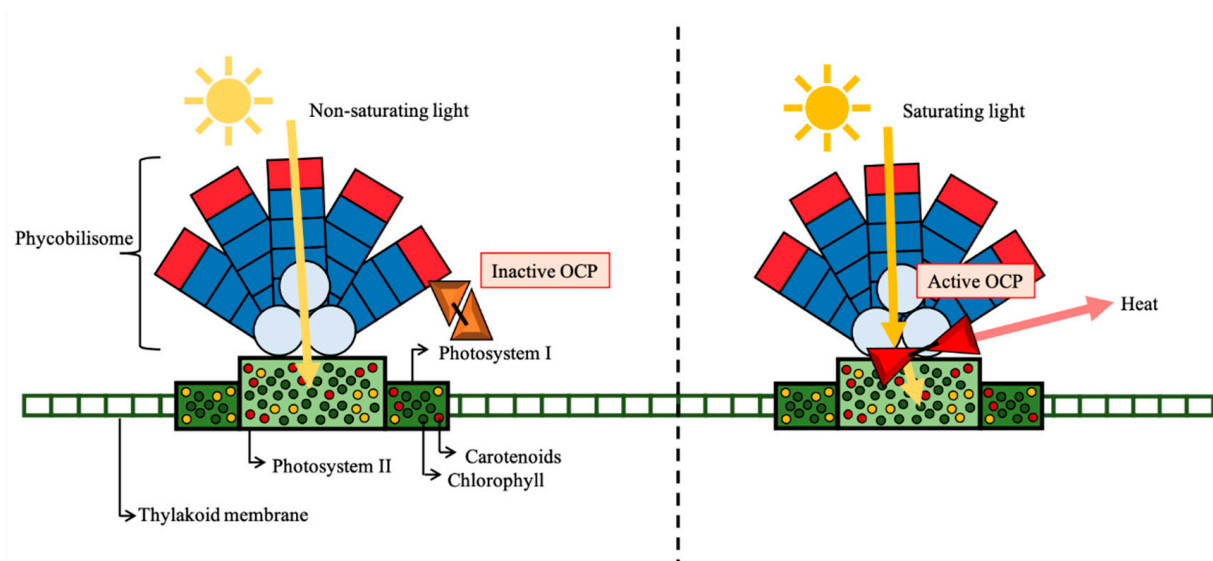


Figure 2. Orange carotenoid protein (OCP) energy dissipation under saturation light.

The OCP is structured by two domains joined by a flexible linker. The first, exclusive to cyanobacteria, is an all-helical N-terminal; and the second, found across all kingdoms, is an α/β -fold C-terminal domain [17]. The OCP is encoded by an *slr1963* gene [18] constitutively expressed, although stress conditions such as high lighting or salt stress can induce a temporary acclimation and an increase of the transcription of the gene [17].

In summary (as observed in Figure 2), the OCP only attaches to the phycobilisome in its red form (activated). In darkness or low-light conditions, the phycobilisome can absorb and transfer all the energy to the photosystem. In saturation light (strong blue-green or white), OCP changes its conformation to an active form and attaches the phycobilisome for energy dissipation, allowing the non-saturating absorption by the photosystem. Besides phycobiliproteins and OCP, other proteins are involved in the process, such as the fluorescence recovery protein (FRP), responsible for inactivating OCP after the NPQ process [17,19].

4. Applications of Carotenoids from Cyanobacteria

Carotenoids overall are widely described as bioactive compounds, such as antioxidants, anti-inflammatory, anti-tumoral, and antimicrobial [6], that can be used in animal feed [4] as a colour enhancer and in cosmetic application as antioxidant and anti-ageing components [20]. However, most carotenoids in the industry come from microalgae and plants, and, as consequence, the studies regarding cyanobacteria carotenoids and their activities are limited [6]. Regarding the bioactive screening of pigments, research is usually performed using targeted extracts with organic solvents, such as ethyl acetate, methanol, ethanol, and acetone. Table 1 summarizes the described application of carotenoids from cyanobacteria.

Table 1. Potential of carotenoids from cyanobacteria for biotechnological applications.

Application	Product	Main Identified Carotenoids	Source	Assay	Reference
Anti-inflammatory	Acetonic extract	β -carotene and echinenone	<i>Nodosilinea (Leptolyngbya) antarctica</i>	LPS-induced macrophages (RAW 264.7)	[21]
Antioxidant	Acetone extract after water extraction	Zeaxanthin and β -carotene	<i>Cyanobium</i> sp.	ABTS ^{•+} and •NO	[22]
	Acetonic extract	β -carotene and echinenone	<i>Arthrospira platensis</i>	DPPH [•] and ABTS ^{•+}	[23]
	Ethyl acetate extract	β -carotene	<i>Trichodesmium</i> sp.	FRAP	[24]
	Methanolic extract	Myxoxanthophyll, zeaxanthin, canthaxanthin and α - and β -carotenes	<i>Lyngbya</i> sp.	DPPH [•]	[25]
	Ethyl acetate/Methanol extract	Zeaxanthin, myxoxanthophyll, β -carotene, echinenone and β -cryptoxanthin	<i>Arthrospira platensis</i> mixed with <i>Dunaliella salina</i>	DMBA-induced tumour in hamster	[26]
Antirolithiasis	Methanol extract	Myxoxanthophyll, zeaxanthin, canthaxanthin and α - and β -carotenes	<i>Pseudanabaena</i> sp., <i>Spirulina</i> sp. and <i>Lyngbya</i> sp.	Calcium oxalate crystallization	[25]
Colour Enhancer (Feed)	Raw biomass	Zeaxanthin, β -carotene and myxoxanthophyll	<i>Arthrospira platensis</i>	In vivo fish and poultry assays	[27–30]
Hyaluronidase inhibitor	Ethanol 70% extract	Zeaxanthin, lutein, canthaxanthin and echinenone	<i>Cyanobium</i> sp. and <i>Tychonema</i> sp.	Hyaluronidase in vitro assay	[31]

ABTS—2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DMBA—7,12-dimethylbenzanthracene; DPPH—2,2-diphenyl-1-picrylhydrazyl; FRAP—ferric antioxidant power; LPS—lipopolysaccharide; NO—nitric oxide.

In terms of antioxidant capacity, Kelman et al. [24] screened extracts from *Trichodesmium* sp., *Anabaena flos-aquae*, *Cyanothece* sp., *Prochlorothrix hollandica*, and *Synechococcus* sp. The extraction was performed using ethyl acetate. The highest antioxidant capacity was found in *Trichodesmium* sp., a bloom-forming marine cyanobacterium and a bioassay-guided fractionation identified β -carotene and retinyl palmitate as main antioxidant compounds.

In another case, methanolic pigment-rich extracts from *Pseudanabaena* sp., *Spirulina* sp. and *Lyngbya* sp. were also suggested as antiurolithiasis (prevention against kidney stone disease) in in vitro assays by Paliwal et al. [25]. Paliwal et al. [25] also evaluated antioxidant capacity, in which *Lyngbya* sp. methanolic extract containing myxoxanthophyll, zeaxanthin, canthaxanthin, and α - and β -carotenes, was the one with the highest IC_{50} for DPPH \bullet scavenging assay (59.56 mg.mg $_{DPPH}^{-1}$).

Patias et al. [23] evaluated the carotenoid composition and the antioxidant capacity of *Aphanothece microscopica* lipophilic extract (ethyl acetate and methanol). The extract contained a substantial amount of total carotenoid (1 mg.mL $^{-1}$) with 14 identified carotenoids (major carotenoids were β -carotene and echinenone) and had an antioxidant capacity relative to 7.3 μ M of α -tocopherol.

Moreover, Park et al. [32] evaluated the carotenoid content and the antioxidant capacity in *A. platensis*. The carotenoid-targeted extract was performed using acetone. Results showed that the biomass contained 4.4 mg.g $^{-1}$ of carotenoids, with major carotenoids being β -carotene and zeaxanthin. Moreover, the antioxidant capacity was evaluated in terms of DPPH \bullet (18.5 μ mol $_{TroloxEquivalent} \cdot g^{-1}$ of dry weight (DW)) and ABTS \bullet^{+} (33.7 μ mol $_{TroloxEquivalent} \cdot g_{DW}^{-1}$) assays, with a positive correlation between the carotenoid content and the antioxidant capacity ($R^2 > 0.8$).

Furthermore, *Cyanobium* sp. were suggested by Pagels et al. [22] as a source of antioxidant carotenoid-targeted extract. The extract was obtained in an acetonic extraction performed after water extraction, which led to a total carotenoid content of 4 mg.g $_{DW}^{-1}$ of extract, with major carotenoids being zeaxanthin and β -carotene. In terms of its antioxidant capacity, the extract was evaluated in ABTS \bullet^{+} and \bullet NO scavenging assays. The IC_{50} were 70 and 162 μ g.mL $^{-1}$, respectively.

Another claimed application of carotenoid-targeted extracts from cyanobacteria is about anti-inflammatory capacity. Lopes et al. [21] screened five cyanobacteria including *Ikalinema aff. pantanalense*, *Cyanobium gracile*, *Nodosilinea (Leptolyngbya) antarctica*, *Cuspidothrix issatschenkoi*, and *Leptolyngbya*-like sp. as sources of carotenoid-targeted extracts for the topical treatment of psoriasis. The extracts were obtained using acetone, and the anti-inflammatory capacity against LPS-induced macrophages (RAW 264.7) was evaluated. *Nodosilinea (Leptolyngbya) antarctica* was the most promising in terms of carotenoids content (64 μ g.g $_{DW}^{-1}$ of extract, β -carotene and echinenone being the major carotenoids) and in terms of anti-inflammatory capacity, with an IC_{50} of 0.3 mg.mL $^{-1}$.

As cosmeceutical products, carotenoid-targeted extracts from cyanobacteria are associated with anti-ageing agents. Morone et al. [31] compared seven different cyanobacteria strains as hyaluronidase inhibitors, including *Phormidium*, *Synechocystis*, *Nodosilinea*, *Cyanobium*, and *Tychonema* genera. The carotenoids-targeted extracts were obtained using ethanol 70%. *Cyanobium* sp. and *Tychonema* sp. were the ones with the highest inhibition capacity, with IC_{50} of 208 and 182 μ g.mL $^{-1}$, respectively. Zeaxanthin and lutein were the main carotenoids identified in *Cyanobium* sp., and canthaxanthin and echinenone were the main carotenoids identified in *Tychonema* sp.

When it comes to the anti-tumoral capacity, Schwartz and Shklar [26] suggested a carotenoid-rich extract of *A. platensis* and *D. salina* (microalgae) as an anti-tumoral agent. The extract was obtained using a mixture of ethyl ether, chloroform, and phosphate buffer saline, 5:1:4. The organic phase was separated and concentrated (1.2 mg.mL $^{-1}$), and the extract contained zeaxanthin (25–30%), myxoxanthophyll (15–20%), β -carotene (10–20%), echinenone (10–15%), and β -cryptoxanthin (5–25%). The extract was applied on 7,12-dimethylbenzanthracene (DMBA)-induced tumour in hamster and led to a local regression of oral squamous cell carcinoma in 4 to 8 weeks. The effect was associated with inhibition

of cytokine tumour necrosis factor (TNF- α). Although promising, it is not possible to determine the specific effect of the cyanobacterium and the microalga individually.

Finally, several studies associated the use of *A. platensis* as feed for a higher accumulation of carotenoids in the animals, as in the cases of fish (*Cyprinus carpio*, *Tilapia nilotica*, *T. mossambica*, and *Plecoglossus altivelis*), shrimp (*Penaeus monodon*), and poultry eggs using the raw cyanobacterium as part of the diet (up to 10%) [27–30].

5. Bioprocess Optimization

5.1. Production of Carotenoids

Cyanobacteria are found almost everywhere due to the unique mechanism of acclimation and adaptation. They are able to survive in a wide range of conditions, including extreme ones. On the other hand, these different conditions lead to changes within the metabolism and the production of compounds; particularly, the photosynthetic apparatus and its pigments composition may change to maximize survival [33].

When it comes to production for industrial applications, the processing parameters are essential to be optimised to increase production. The growth conditions are also fundamental for the extraction, as they can change the cell structure. Both abiotic (e.g., light, temperature, pH) and biotic factors (e.g., intra- and interspecific competition) must be evaluated, and the optimisation is specific to the species, or even within the strain, although some metabolic responses can be shared between species [4].

In terms of carotenoids, light is the most optimised parameter for the high modulation of photosynthetic metabolism [5]. Light can be optimised in terms of source, quality, intensity, or photoperiod. Apart from that, temperature, pH, and salinity can change the metabolism of the organism in terms of nutrient uptake, growth, and photosynthetic efficiency (consequently pigments composition) [6]. Moreover, the medium composition is also an important factor to be considered, as the concentration of macro- and micronutrients can affect directly the health of the culture and cause unwanted stress [33].

Another way to optimise carotenoids production in cyanobacteria is the use of genetic engineering. In recent years, the approach of genetic alteration increased attention, and a few examples are already described in cyanobacteria. As these organisms produce naturally fewer carotenoids than microalgae, the use of genetic engineering provides an alternative for the competition, although the legislation is yet to restrict the industrial use of genetically modified organisms (GMO) [34].

In the following sections, the main factors and strategies that can affect the production of carotenoids by cyanobacteria are discussed in a way to find similarities between species and provide information for further optimisations.

5.1.1. Light

Photosynthetic organisms depend directly on the availability of light for their growth and survival. Changes in light source, intensity, quality (spectra composition), and photoperiod are responsible for the greatest changes in terms of metabolism, and due to the presence of photoreceptors, the organism can acclimate quickly to the surrounding condition [35]. Optimization of carotenoids production in terms of light is summarized in Table 2.

The photosynthetic process is mainly performed by the light-harvesting complex, composed of pigments (carotenoids, phycobiliproteins, and chlorophylls) [1]. However, light quality can stimulate non-photosynthetic photoreceptors and trigger various pathways, as in the case of carotenoids [36].

Pagels et al. [37] evaluated the pigment accumulation in the cyanobacterium *Cyanobium* sp. using different light qualities supplements. Results showed the positive regulation of carotenoids production under the supplementation of red light, increasing the carotenoid content by 10% when compared to the non-supplemented condition. Afterwards, Pagels et al. [38] evaluated the use of two-phase cultivation using white and red LEDs and, under the red phase, the carotenoids content increased by 50% when compared

to the white phase. Moreover, with the optimal cultivation periods of 10 days of white and 4 days of red LEDs, the maximum productivity was of $4.5 \text{ mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. The composition of specific carotenoids was not changed within cultivation times, β -carotene being the major carotenoid present.

A similar positive effect of red light was also observed by Olaizola and Duerr [39], who evaluated the potential of *A. platensis* grown under white, blue, and red lights. Red light provided a similar production as white light ($23.2 \text{ mg}\cdot\text{g}_{\text{DW}}^{-1}\cdot\text{d}^{-1}$), although the amount of myxoxanthophyll decreased while β -carotene increased in red light, providing a possible strategy for composition modulation.

On the other hand, a negative effect of red light was found in *Pseudanabaena* sp. The comparison between white, green, blue, red, and yellow lights showed that red and yellow induced a decrease in carotenoid content when compared to white light, while green increased the carotenoids content by 30% when compared to white [40]. Such differences are well explained regarding phycobiliproteins, where the mechanisms of acclimation are more known [41]. However, it is possible that a green-red photoreceptor is involved also in carotenoids regulation in cyanobacteria. Such regulation can change within species groups, similarly to what happens in phycobiliproteins regulation.

The mixture of blue and red light is common in plants cultivation and microalgae [42], taking advantage of the chlorophyll absorption peak. However, in cyanobacteria, the absorption of blue light is done in a less effective way [43], and that lack of blue light can reduce carotenoids productivity. Lima et al. [44] evaluated the carotenoids content in *A. platensis* grown in several ratios of red and blue lights. The growth under red:blue (70:30, in percentage) increased up to three times the content of carotenoids ($6.91 \text{ }\mu\text{g}\cdot\text{mL}^{-1}$) when compared to 100% red, while no growth was found under 100% blue.

When it comes to UV radiation, cyanobacteria can produce UV-protective compounds such as phenolic compounds, scytonemins, mycosporine-like amino acids, or carotenoids [45]. Under UV radiation, the cell produces carotenoids due to the NPQ ability of these pigments, reducing oxidative stress and increasing the photosystem stability.

Kokabi et al. [45] saw that *Leptolyngbya* cf. *fragilis* doubles the content of carotenoids within 12 h of exposition to UV radiation ($0.29 \text{ mg}\cdot\text{g}_{\text{DW}}^{-1}$). A similar pattern was found in *Lyngbya aestuarii*, which progressively increased the carotenoid production with the UV exposure duration. Thus, by 2 days of irradiation treatment, the content of carotenoids increased by 125% when compared to the control [46].

Regarding carotenoid composition, Llewellyn et al. [47] showed that *Chlorogloeopsis fritschii* produced eight times more canthaxanthin under UV radiation when compared to control with a fluorescent lamp, with no differences in other carotenoids content. On the other hand, Ehling-Schulz et al. [48] observed that UV-B irradiation induced an increase in carotenoids, especially echinenone and myxoxanthophyll in *Nostoc commune* after 1 day of irradiation.

In a genetic evaluation, Huang et al. [49] saw an increase in expression of carotenogenesis genes (*crtE*, *crtP*, and *crtQ*) in *Synechocystis* sp. with the addition of high-intensity UV light when compared to white light. Llewellyn et al. [47] also verified that UV radiation induces upregulation of OCP genes expression.

When it comes to light intensity, optimal conditions provide a more efficient photosynthetic metabolism, increasing biomass production and consequently productivity. However, lower and higher amounts of light can induce photoprotective mechanisms, including carotenogenesis. In cyanobacteria, the carotenoids photoprotection effect is described in high light conditions.

Bañares-España et al. [50] evaluated the carotenoids production in three different strains of *Microcystis aeruginosa*, and all of them produced more carotenoids under high light intensity ($176 \text{ }\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) when compared to low light intensity ($15 \text{ }\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Thus, Walsh et al. (1997) compared the carotenoids production of *Microcystis aeruginosa* under intensities of 20, 40, and $70 \text{ }\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, $40 \text{ }\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ being the optimal carotenoid production in terms of β -carotene ($579.7 \text{ }\mu\text{g}\cdot\text{g}_{\text{DW}}^{-1}$), zeaxan-

thin ($431.2 \mu\text{g}\cdot\text{g}_{\text{DW}}^{-1}$), and echinenone ($143.3 \mu\text{g}\cdot\text{g}_{\text{DW}}^{-1}$), up to three times more than in other intensities.

Moreover, Masamoto and Furukawa [51] compared the accumulation of zeaxanthin in *Synechococcus* sp. grown under 40 and $1300 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and the results showed that under high light intensity, the cyanobacterium produced 4 times more carotenoids than under low light intensity. A positive correlation between carotenoids production and high intensity was also found in *Anabaena cylindrica*, *Anabaena torulosa*, *Anabaenopsis elenkinii* and *Nostoc* sp. grown under 15 and $120 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ [52].

Cyanobacteria cultures respond to light intensity in a curve response, as it reaches the point of saturation and decreases due to photoinhibition. Pagels et al. [53] showed that *Cyanobium* sp. Increased carotenoids productivity under an optimal light intensity of $200 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, with an increase with the intensity from 50 to $200 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, followed by a decrease at $300 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ with a maximum carotenoid productivity of $0.12 \text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$. Regarding the carotenoid's composition, Gris et al. [54] observed that, in *Cyanobacterium aponinum*, zeaxanthin content increased within light intensity from 15 to $650 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, reaching the maximum at $650 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while β -carotene increased from 15 to $100 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, then decreased until $650 \mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Total carotenoids, however, had no significant changes.

Table 2. Effects of light quality and intensity on the production of carotenoids by cyanobacteria. Processing parameters include: light source (LS) and intensity (I), light:dark cycle (LC), temperature (T), pH, and culture media (M). Light intensity is expressed in $\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ unless another unit is indicated.

Cyanobacterium	Tested Conditions ^a	Processing Parameters ^b	Optimal Condition	Carotenoids Content	Reference
Light Quality					
<i>Arthrospira platensis</i>	W, B, R	LS: FL; I: 133; LC: 24:0 h; T: 36 °C; pH: ns; M: Zarrouk	R	$23.2 \text{mg}\cdot\text{g}_{\text{DW}}^{-1}$	[39]
<i>Arthrospira platensis</i>	R + B (0–100% mixtures)	LS: LED; I: 100; LC: 24:0 h; T: 32 °C; pH: 8.0; M: Zarrouk	R + B (70:30, %)	$6.91 \mu\text{g}\cdot\text{mL}^{-1}$	[44]
<i>Cyanobium</i> sp.	R; G; B; UV supplements	LS: SOX + LED; I: 200; LC: 12:12 h; T: 25 °C; pH: 7.5; M: BG11 saline	R supplement	$6.5 \text{mg}\cdot\text{g}_{\text{DW}}^{-1}$	[37]
<i>Cyanobium</i> sp.	W + R in different times	LS: LED; I: 200; LC: 16:8 h; T: 20 °C; pH: 9; M: BG11 saline	10 days of W and 4 days of R	$32 \text{mg}\cdot\text{g}_{\text{DW}}^{-1}$	[38]
<i>Leptolyngbya</i> cf. <i>fragilis</i>	(+/-) UV-B	LS: FL + UV lamp; I: 18 $\text{W}\cdot\text{m}^{-2}$; LC: 12:12 h; T: 25 °C; pH: ns; M: BG11	+UV	$0.29 \text{mg}\cdot\text{g}_{\text{DW}}^{-1}$	[45]
<i>Lyngbya aestuarii</i>	(+/-) UV-B	LS: FL + UV lamp; I: 7.5 $\text{W}\cdot\text{m}^{-2}$ FL or 5 $\text{W}\cdot\text{m}^{-2}$ UV; LC: 24:0 h; T: 25 °C; pH: ns; M: ASN-III	+UV	ns	[46]
<i>Nostoc commune</i>	(+/-) UV-B	LS: FL + UV lamp; I: 1 $\text{W}\cdot\text{m}^{-2}$; LC: ns; T: 30 °C; pH: ns; M: BG11 ₀	+UV-B	ns	[48]
<i>Pseudanabaena</i> sp.	W, B, R, G, Y	LS: FL + colour filters; I: 75–220 lux; LC: 12:12 h; T: 25 °C; pH: ns; M: ASN-III	G	$0.16 \text{mg}\cdot\text{L}^{-1}$	[40]
<i>Synechocystis</i> sp.	(+/-) UV-B	LS: FL + UV lamp; I: 60; LC: ns; T: 30 °C; pH: ns; M: BG11	+UV	ns	[49]
Light Intensity					
<i>Cyanobium</i> sp.	50, 100, 200, 300	LS: FL or SOX; LC: 12:12 h; T: 25 °C; pH: 7.5; M: BG11 saline	200	$0.12 \text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$	[53]

Table 2. Cont.

Cyanobacterium	Tested Conditions ^a	Processing Parameters ^b	Optimal Condition	Carotenoids Content	Reference
<i>Synechococcus</i> sp.	40, 1300	LS: FL; LC: 24:0 h; T: 25 °C; pH: ns; M: BG11	1300	7.59 nmol.A ₇₅₀ ⁻¹ .mL ⁻¹	[51]
<i>Microcystis aeruginosa</i>	20, 40, 70	LS: FL; LC: 12:12 h; T: 25 °C; pH: ns; M: ASM	40	β-carotene: 579.7 µg.g _{DW} ⁻¹ Zeaxanthin: 431.2 µg.g _{DW} ⁻¹ Echinenone: 143.3 µg.g _{DW} ⁻¹	[55]
<i>Anabaena cylindrica</i>	15, 120	LS: FL; LC: 24:0 h; T: 20 °C; pH: ns; M: Juttner	120	ns	[52]
<i>Anabaenopsis elenkinii</i>	15, 120	LS: FL; LC: 24:0 h; T: 20 °C; pH: ns; M: Juttner	120	ns	[52]
<i>Anabaena torulosa</i>	15, 120	LS: FL; LC: 24:0 h; T: 20 °C; pH: ns; M: Juttner	120	ns	[52]
<i>Nostoc</i> sp.	15, 120	LS: FL; LC: 24:0 h; T: 20 °C; pH: ns; M: Juttner	120	ns	[52]
<i>Microcystis aeruginosa</i>	15, 176	LS: FL; LC: 16:8 h; T: 20 °C; pH: ns; M: BG11	176	ca. 0.035 pg.cell ⁻¹	[50]
<i>Cyanobacterium aponinum</i>	15, 40, 70, 100, 150, 300, 500, 650	LS: FL; LC: 24:0 h; T: 35 °C; pH: 8.0; M: BG11	β-carotene: 100 Zeaxanthin: 650	β-carotene: 4.03 mg.g _{DW} ⁻¹ Zeaxanthin: 3.17 mg.g _{DW} ⁻¹	[54]

^a Light quality: R—red; G—green; B—blue; UV—ultraviolet; Y—yellow; W—white; ^b SOX—low-pressure sodium lamp; LED—light emitting diodes; FL—fluorescent lamp; ns—not specified.

5.1.2. Temperature and pH

Both temperature and pH exert a great influence on cyanobacterial metabolism due to nutrient uptake and solubility of CO₂ in the culture medium. These factors can also change enzymatic activity and consequently metabolic pathways of the organism [56]. In the case of cyanobacteria, a wide range of values of the two factors is tolerable, and these organisms are found even in the most extreme environments, such as hot springs, Antarctica, or even saline-alkaline lakes [57,58]. Most of the studies regarding both temperature and pH in cyanobacteria production are related to biomass or phycobiliproteins, and only a few have targeted carotenoids production, summarized in Table 3.

Kłodawska et al. [59] evaluated the effect of temperature (15, 23, 30, and 37 °C) in carotenoids production in *Anabaena* sp., and results showed that optimal temperature for carotenoids production was 23 °C (0.39 mg.g_{DW}⁻¹), although the ratio carotenoids:chlorophyll did not change between 23 °C and 30 °C. However, in terms of composition of β-carotene and echinenone, the optimal temperature was 23 °C, while for keto-myxoxanthophyll and canthaxanthin, the optimal temperature was 30 °C.

Ismaiel et al. [60] evaluated the effect of pH (7.5–11.0) in carotenoids production in *A. platensis*, and the results showed that the content of carotenoids was higher in pH from 8.0 to 9.0 with no statistical differences (ca. 2.4 mg.g_{DW}⁻¹).

Pagels et al. [61] performed a factorial evaluation of temperature (20–30 °C) and pH (6.0–9.0), together with salinity (see Section 5.1.4) on *Cyanobium* sp. for the production of carotenoids. Optimal conditions were set at 20 °C and pH 9.0, with a maximum productivity of 2.04 mg.L⁻¹.d⁻¹. It is noteworthy that the carotenoid content in the optimal condition for productivity is not the maximum content on the cyanobacterium, however, the amount of the final product can have more impact on the decision-making than the biomass composition.

Overall, more studies are required for a better characterization of temperature and pH needs of cyanobacterial cultures as a source of carotenoids.

Table 3. Effects of temperature and pH on the production of carotenoids by cyanobacteria. Processing parameters include: light source (LS) and intensity (I), light:dark cycle (LC), temperature (T), pH, and culture media (M). Light intensity is expressed in $\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ unless another unit is indicated.

Cyanobacterium	Tested Conditions	Processing Parameters ^a	Optimal Condition	Carotenoids Content	Reference
pH					
<i>Arthrospira platensis</i>	7.5–11.0	LS: FL; I: 60; LC: ns; T: 31 °C; M: Zarrouk	8.0–9.0	2.4 mg.g _{DW} ⁻¹	[60]
<i>Cyanobium</i> sp.	6.0–9.0	LS: FL; I: 200; LC: 16:8 h; T: 20 °C; M: BG11 saline	9.0	2.04 mg.L ⁻¹ .d ⁻¹	[61]
Temperature					
<i>Anabaena</i> sp.	15, 23, 30, 37 °C	LS: FL; I: 60; LC: 24:0 h; pH: 7.5; M: BG11 ₀	23 °C	0.39 mg.g _{DW} ⁻¹	[59]
<i>Cyanobium</i> sp.	20–30 °C	LS: FL; I: 200; LC: 16:8; pH: 9.0; M: BG11 saline	20 °C	2.04 mg.L ⁻¹ .d ⁻¹	[61]

^a FL—fluorescent lamp.

5.1.3. Culture Medium Composition

Another important factor to be optimised is the appropriate chemical composition of the growth media. Nitrogen, phosphorous, sulphur, magnesium, and manganese are the most essential nutrients for both growth and carotenoids accumulation. The nutritional needs of cyanobacteria species require case-wise consideration in terms of nutrients concentration or source of nitrogen. Several optimised media are used by laboratory and industrial productions of cyanobacteria, such as Zarrouk and Blue-Green (BG11) media. Table 4 summarizes the optimisation of medium components in cyanobacteria production as a carotenoids source.

Regarding nutrients concentration, Thirumala [62] optimised the medium nutrients to *A. platensis* isolated from Lonar Lake, Mexico. The optimisation used sterilized water from the lake in addition to a mixture of N:P:K (1:1:1) (0–2.5 g.L⁻¹), with an optimal concentration of 2 g.L⁻¹ and with a carotenoid's concentration of 0.0998 $\mu\text{g}\cdot\text{mL}^{-1}$, 14% higher than compared to Zarrouk medium. In another study, *A. platensis* was optimised in terms of NaNO₃ concentration (0.1, 2.5, and 5.0 g.L⁻¹), and the optimal condition (0.1 g.L⁻¹) led to a total of 45.4 mg.g⁻¹ of carotenoids, 60% more than the culture with 2.5 g.L⁻¹ and 750% more than the one with 5.0 g.L⁻¹ [63]. Moreover, Pagels et al. [53] suggested the addition of twice as many nitrates and phosphates than regular BG11 medium suggests for the production of carotenoids by *Cyanobium* sp., and the addition of nutrients induced an increase of 20% in productivity.

When it comes to the source of nitrogen, Erdoğan et al. [64] evaluated the sources of nitrogen (NaNO₃, NaNO₂, NH₄Cl, and CH₄N₂O) in *Prochlorococcus* sp. culture for the production of lutein. The maximum concentration of lutein (3.34 mg.g_{DW}⁻¹) was found in the culture grown with CH₄N₂O.

Another optimisation strategy is to use different laboratory medium. Tarko et al. [65] evaluated the β -carotene content in six strains of *A. platensis* grown with Zarrouk and Revised No. 6 (RM6) media. In all strains, β -carotene was more produced in Zarrouk medium, with a higher concentration (2.26 mg.g_{DW}⁻¹) 15 times more than RM6 medium.

Moreover, a comparison between Chu's No. 10 (CHU10), BG11, and Zarrouk media was performed by Paliwal et al. (2015b) for the production of *Synechocystis* sp., Zarrouk being the optimal medium with a content of 7.99 mg.g_{DW}⁻¹, leading to content 50% higher than BG11 and 80% higher than CHU10. Furthermore, D'Alessandro et al. [66] com-

pared the carotenoids' production by *Geitlerinema amphibium* using Wright's Cryptophyte (WC) and Bold's Basal (BBM) media. The culture grown using BBM medium produced 130% more astaxanthin and 234% more lutein than the one grown using WC medium, 2.74 mg.g_{DW}⁻¹ and 5.49 mg.g_{DW}⁻¹ respectively.

Table 4. Effects of culture medium composition on the production of carotenoids by cyanobacteria. Processing parameters include: light source (LS) and intensity (I), light:dark cycle (LC), temperature (T), pH, and culture media (M). Light intensity is expressed in $\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ unless another unit is indicated.

Cyanobacterium	Tested Culture Media	Processing Parameters ^a	Optimal Condition	Carotenoids Content	Reference
Culture Media Comparison					
<i>Arthrospira platensis</i>	Zarrouk and RM6	LS: ns; I: 2000–3000 lux; LC: 12:12 h; T: 20 °C; pH: 8.2;	Zarrouk	2.26 mg.g _{DW} ⁻¹	[65]
<i>Synechocystis</i> sp.	CHU10, GB11 and Zarrouk	LS: ns; I: 60; LC: ns; T: 25 °C; pH: ns;	Zarrouk	7.99 mg.g _{DW} ⁻¹	[67]
<i>Geitlerinema amphibium</i>	WC and BBM	LS: ns; I: 80; LC: 24:0h; T: 29 °C; pH: ns;	BBM	Astaxanthin: 2.74 mg.g _{DW} ⁻¹ Lutein: 5.49 mg.g _{DW} ⁻¹	[66]
Nutrient's concentration					
<i>Arthrospira platensis</i>	0–2.5 g.L ⁻¹ of N:P:K (1:1:1)	LS: ns; I: 1500 lux; LC: 14:10 h; T: 35 °C; pH: 10; M: Lonar lake water	2 g.L ⁻¹	0.0998 $\mu\text{g}\cdot\text{mL}^{-1}$	[62]
<i>Arthrospira platensis</i>	0.1–5 g.L ⁻¹ of NaNO ₃	LS: FL; I: 475 lux; LC: 24:0 h; T: 25 °C; pH: 9.5; M: Zarrouk	0.1 g.L ⁻¹	45.54 mg.g _{DW} ⁻¹	[63]
<i>Cyanobium</i> sp.	(+/-) NaNO ₃ ; K ₂ HPO ₄	LS: SOX; I: 200; LC: 12:12 h; T: 25 °C; pH: 7.5; M: BG11 saline	+NaNO ₃ ; K ₂ HPO ₄	0.12 mg.L ⁻¹ .d ⁻¹	[53]
Nitrogen source					
<i>Prochlorococcus</i> sp.	NaNO ₃ , NaNO ₂ , NH ₄ Cl, CH ₄ N ₂ O	LS: FL; I: 27; LC: 24:0 h; T: 25 °C; pH: ns; M: BBM	CH ₄ N ₂ O	3.34 mg.g _{DW} ⁻¹	[64]

^a SOX—low-pressure sodium lamp; FL—fluorescent lamp; ns—not specified.

5.1.4. Salinity

When dealing with marine cyanobacteria, salinity is a key point for optimisation. Higher concentrations of salt (NaCl \approx 30 g.L⁻¹), means the use of less potable water, the seawater being a suitable substitute for cultivation. However, the salt concentration in the culture medium influences not only growth and carotenoids accumulation but can also influence biomass harvesting and carotenoids extraction. Table 5 summarizes the optimisation of salinity in cyanobacteria production as a carotenoids source.

Pagels et al. [61] optimised salinity in terms of NaCl concentration (10 to 30 g.L⁻¹), together with temperature and pH (see Section 5.1.2). Optimal concentration was set at 10 g.L⁻¹ (NaCl), with a carotenoid productivity of 2.04 mg.L⁻¹.d⁻¹. From the three studied factors, NaCl concentration had the least impact in terms of carotenoids production.

In the case of *Eubhalothece* sp., a halophilic cyanobacterium that is capable of growing in a wide range of salinity, from freshwater to 70 g.L⁻¹ of NaCl, the carotenoid content was 300% higher in the condition without NaCl addition than the condition with 30 g.L⁻¹. In contrast, the growth was reduced by 400%, meaning that the salt stress-induced a defence mechanism, increasing the carotenoid content but inhibiting biomass production [68].

Table 5. Effects of salinity on the production of carotenoids by cyanobacteria. Processing parameters include: light source (LS) and intensity (I), light:dark cycle (LC), (T), pH, and culture media (M). Light intensity is expressed in $\mu\text{mol}_{\text{photons}}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ unless when another unit is indicated.

Cyanobacterium	Tested [NaCl]	Processing Parameters ^a	Optimal Condition	Carotenoids Content	Reference
<i>Cyanobium</i> sp.	10–30 g.L ⁻¹	LS: FL; I: 200; LC: 16:8; T: 20 °C; pH: 9.0; M: BG11 saline	10 g.L ⁻¹	2.04 mg.L ⁻¹ .d ⁻¹	[61]
<i>Euhalotheca</i> sp.	0 and 30 g.L ⁻¹	LS: ns; I: 75; LC: 14:10; T: 27 °C; pH: 7.5; M: BG11	0 g.L ⁻¹	0.61 $\mu\text{g}\cdot\text{A}_{750}^{-1}$	[68]

^a FL—fluorescent lamp; ns—not specified.

5.1.5. Genetic Engineering

As already referenced, compared to microalgae, cyanobacteria content of carotenoid is generally low and thus has difficulty competing in the market. Another strategy for the increase of the production of carotenoids from cyanobacteria is the use of genetic engineering.

Synechocystis sp. PCC 6803 is the most studied cyanobacteria in terms of genetic engineering. In the case of carotenoids production, Lagarde et al. [69] overexpressed carotenogenesis genes in *Synechocystis* sp. by introducing sequences of the genes encoding the yeast isopentenyl diphosphate isomerase (*ipi*) and the *Synechocystis* β -carotene hydroxylase (*crtR*) as well as the linked *Synechocystis* genes coding for phytoene desaturase and phytoene synthase (*crtP* and *crtB*, respectively). The gene introduction led to an overexpression of *crtP* and *crtB*, increasing the production of myxoxanthophyll and zeaxanthin by 50%. On the other hand, the overexpression of *crtR* increased the production of zeaxanthin by 150% (from 0.39 to 0.98 mg.L⁻¹.OD₇₃₀⁻¹), but it also led to a reduction of echinenone and β -carotene by 50%.

Moreover, Diao et al. [13] induced astaxanthin biosynthesis through genetic engineering in *Synechocystis* sp. PCC 6803, reaching a production of 29.6 mg.g_{DW}⁻¹, representing a 500-fold increase when compared to the wild type strain. Astaxanthin biosynthesis is established in *Synechocystis* by introducing two carotenogenesis enzymes: β -carotenoid ketolase and hydroxylase. The source of the genes and the expression of the chassis determine the efficiency of the process and the astaxanthin content.

Furthermore, Gao et al. [70] overexpressed the *crtO* gene from *Nostoc flagelliforme* into *Nostoc* sp. PCC 7120, inducing production of 16% more echinenone (97.9 mg.L⁻¹) and 80% more canthaxanthin (8.8 mg.L⁻¹).

Genetic engineering of cyanobacteria was also demonstrated to be a viable option for cyanobacteria-derived terpenoids, such as GGPP, a precursor to carotenoids (Section 2). GGPP commercial application also includes pharmaceuticals, nutraceuticals, flavours/fragrances, and industrial chemicals. Terpene production, on the other hand, would inevitably compete with pigment synthesis due to the use of the same precursor pathway, decreasing the final content of carotenoids [71].

5.2. Downstream Process

Downstream processing involves harvesting, extraction, and purification processes. In terms of harvesting, the critical optimisation step is about feasibility on a large scale and its cost, because, generally, the costs of harvesting represent about 20–30% of the total cost of biomass production. Filtration, centrifugation, or even chemical flocculation are some of the options available, but the process must be optimised for individual species and purposes. Optimization of harvesting is targeted to the whole biomass; this review does not cover these aspects, but more aspects of harvesting optimisation for cyanobacteria can be found in Guedes et al. [33]. Moreover, carotenoids are usually commercialized in a form of extract (oleoresin) containing a high concentration of specific carotenoids (e.g.,

β -carotene representing about 20% of the extract). Further purification can be performed, but the cost is hardly justifiable [72].

In terms of extraction, many factors can change the quality of the final product, including target carotenoid, chosen cyanobacteria, available technology, and cost. Most cyanobacteria biomass requires cell disruption associated with solvent extraction, and, in general, extraction of carotenoids is performed by physical disruption of cells (such as pressurized, wave-based, or electric fields technologies). Associated with this is the choice of a compatible organic solvent, preferentially considered safe for industrial use (GRAS solvents), as in the cases of acetone, ethanol, or hexane [22]. The extraction optimisation must consider, if possible, both disruption methodology and compatible solvent (with the system and with the targeted compound). Moreover, is it important to control the temperature through the extraction process due to the thermosensitivity of carotenoids. Carotenoids can be more efficiently extracted under 50 °C to 65 °C, but long expositions to high temperatures (>70 °C) can lead to degradation and loss of the bioactive capacity of the extract [73,74].

Thus, the first step of extraction optimisation must be in terms of solvents. Carotenoids are lipophilic compounds and must be extracted using organic solvents. Table 6 summarizes the solvent optimisations performed in carotenoids extraction from cyanobacteria. Amaro et al. [75] evaluated the effect of different solvents in the carotenoid's extraction in *Gloeothece* sp. and compared the use of ethanol, acetone, ethyl lactate, and a mixture of hexane/isopropanol (60:40, in percentages). The results showed that acetone was the best solvent, with a yield of 1.8 mg.g_{DW}⁻¹, with lutein being the major carotenoid (ca. 80% of total carotenoids), followed by β -carotene, neoxanthin, violaxanthin, and α -carotene. The use of acetone was 40% more efficient than ethanol and hexane/isopropanol (60:40, in percentages) mixture. Noteworthy is that ethyl lactate extracted 70% fewer carotenoids, and α -carotene was not found in this extract.

Another strategy for solvent extraction involves the use of more than one solvent in a successive way, e.g., successive extractions using the remaining biomass from the previous process. This strategy is gaining attention in the last few years and can contribute to the valorisation of carotenoids from cyanobacteria using also the biomass for the extraction of aqueous pigments phycobiliproteins, with high added value in the market [15]. Moreover, the use of successive extraction can increase the purity of both phycobiliproteins and carotenoids extracts, as they are separated into two different extracts.

Tavanandi et al. [76] optimised the extraction of carotenoids from *Arthrospira platensis* after an enzymatic extraction of phycobiliproteins. The first extraction was performed with lysozyme for 20 h at 37 °C and pH 7.0. The remaining biomass was then dried by low humidity drying. Then, the carotenoids extraction was optimised comparing ethanol, acetone, methanol, diethyl ether and DMSO; pH (4.0–10.0); and time of extraction (1–14 h). The optimal condition was ethanol (80%), pH 7.0, stirring for 1 h at 40 °C, with a content of 5.3 mg.g_{DW}⁻¹.

Pagels et al. [22] optimised the successive extraction of pigments from *Cyanobium* sp., comparing the solvent (acetone, ethyl acetate, and ethanol) and if the extraction should be before the aqueous extraction or after (acetone after water extraction). In terms of the composition of the extract, acetone as the first or the second extraction reached the highest concentration of carotenoids in the extract (ca. 4 mg.g_{DW}⁻¹), β -carotene being the major carotenoid, followed by echinenone, zeaxanthin, and lutein.

The successive extraction of carotenoids was also evaluated in *Trichocoleus sociatus* and *Nostoc flagelliforme* by Dorina et al. [77]. The optimisation considered the order of extracted compounds—carotenoids plus chlorophylls, phycobiliproteins, and exopolysaccharides. The optimal condition was found in the extraction of exopolysaccharides prior to biomass drying, followed by a freeze-drying step, an aqueous extraction for phycobiliproteins, and finally a methanol extraction for the obtention of carotenoids, with yields of 2.34 mg.g_{DW}⁻¹ for *Trichocoleus sociatus* and 4.49 mg.g_{DW}⁻¹ for *Nostoc flagelliforme*.

Finally, Assunção et al. [78] evaluated the successive extraction in *Chroococcidiopsis* sp. In this study, acetone, ethanol, and methanol were used for the extraction of carotenoids after a pre-treatment with phosphate buffer, where the extraction with methanol led to the highest content of these pigments ($1.72 \text{ mg.g}_{\text{DW}}^{-1}$), mainly composed of echinenone, β -carotene, α -carotene, lycopene, and zeaxanthin.

Table 6. Optimisation of solvent in the successive extraction of carotenoids from cyanobacteria.

Cyanobacterium	Tested Solvents	Optimal Solvent	Carotenoid Content ($\text{mg.g}_{\text{DW}}^{-1}$)	Main Identified Carotenoids	Reference
<i>Gloeotheca</i> sp.	Ethanol, acetone, ethyl lactate, and hexane/isopropanol (60:40, in percentages).	Acetone	1.8	Lutein, β -carotene, neoxanthin, violaxanthin, and α -carotene	[75]
<i>Arthrospira platensis</i>	Ethanol, acetone, methanol, diethyl ether, and DMSO after enzymatic pre-treatment	Ethanol	5.3	n.s.	[76]
<i>Cyanobium</i> sp.	Acetone, ethyl acetate, and ethanol	Acetone	4.4	β -carotene, echinenone, zeaxanthin, and lutein	[22]
<i>Chroococcidiopsis</i> sp.	Acetone, ethanol, and methanol	Methanol	1.7	Echinenone, β -carotene, α -carotene, lycopene, and zeaxanthin	[78]

n.s.—not specified.

For a better extraction efficiency, the cell disruption methodology must be chosen in terms of the specific cell wall composition and the scalability of the technology. Unlike other Gram-negative bacteria and microalgae, cyanobacteria contain a thick peptidoglycan layer between the inner and the outer membrane, which can increase the resistance of the cell in the extraction process [79]. Regarding cyanobacteria, Table 7 summarizes the main technologies used for carotenoids extraction.

In the case of pressurized liquid extraction (PLE), the extraction is performed at high temperatures ($50\text{--}200 \text{ }^\circ\text{C}$) and high pressure ($100\text{--}140 \text{ bar}$), preventing the solvent from boiling while increasing the solvent flux into the cell. The major limitation is that temperature can induce degradation of pigments [80]. Rodríguez-Meizoso et al. [81] optimised carotenoid extraction from *Phormidium* spp. in terms of solvent (hexane and ethanol) and temperature ($50\text{--}200 \text{ }^\circ\text{C}$) in a constant pressure (100 bar). The optimal condition was ethanol at $150 \text{ }^\circ\text{C}$, the major carotenoid being β -carotene, followed by lutein, violaxanthin, and neoxanthin. Moreover, Amaro et al. [82] used a low temperature pressurized liquid extraction for carotenoids obtention from *Gloeotheca* sp. The optimisation was performed using ethanol in terms of biomass in the system ($50\text{--}150 \text{ mg}_{\text{DW}}$), flow ($1\text{--}4 \text{ mL.min}^{-1}$), temperature ($30\text{--}70 \text{ }^\circ\text{C}$), and cycles of solvent recirculation. The optimal condition was set as $50 \text{ mg}_{\text{DW}}$, $60 \text{ }^\circ\text{C}$, a flow of 3 mL.min^{-1} (180 bar), and three cycles of ethanol recirculation, with contents of $2.9 \text{ mg.g}_{\text{DW}}^{-1}$ of lutein and $1.5 \text{ mg.g}_{\text{DW}}^{-1}$ of β -carotene.

In terms of ultrasound-assisted extraction (UAE), the extraction uses acoustic cavitation for cell disruption. In cyanobacteria, the use of UAE was performed for carotenoid extraction in *A. platensis* [83]. The optimisation was performed in terms of solvent (hexane, n-heptane and diethyl ether), temperature ($10\text{--}50 \text{ }^\circ\text{C}$) and electrical acoustic intensity ($64\text{--}210 \text{ W.cm}^{-2}$). Optimal conditions were found by using heptane at a temperature of $30 \text{ }^\circ\text{C}$ and an electrical acoustic intensity of 167 W.cm^{-2} , with content of ca. $1.0 \text{ mg.g}_{\text{DW}}^{-1}$.

Furthermore, supercritical fluid extraction (SFE) can be an efficient but expensive extraction method for the obtention of carotenoids. In supercritical condition, the solvent acts as gas and liquid at the same time, penetrating the cell and solubilizing the carotenoids. In cyanobacteria, Montero et al. [84] optimised carotenoid extraction in *Synechococcus* sp. in terms of pressure (200–500 bar) and temperature (40–60 °C). Optimal conditions were carotenoid-specific: 358 bar and 50 °C for β -carotene; 454 bar and 59 °C for cryptoxanthin; and 500 bar and 60 °C for zeaxanthin.

Similarly, Macías-Sánchez et al. [85] optimised carotenoids extraction in *Synechococcus* sp. using SFE. The optimal pressure was set at 300 bar (from 100 to 500 bar) and a temperature of 50 °C (from 40 to 60 °C), reaching a content of 1.5 mg.g_{DW}⁻¹.

Other cell disruption methodologies are associated with the extraction of carotenoids in microalgae, and some could be easily applied in cyanobacteria matrices, as in the case of enzymatic extraction [86], high-pressure homogenization [87], microwave-assisted extraction [88], or electroextraction [89]. Moreover, from the described for cyanobacteria, the extraction efficiency of supercritical fluid extraction can be increased by the use of co-solvents, such as ethanol, that increases the solvating power, as observed in *Scenedesmus obliquus* lutein extraction [90].

Table 7. Extraction methodologies used for the obtention of carotenoids from cyanobacteria.

Cyanobacterium	Extraction Method	Carotenoid Content (mg.g _{DW} ⁻¹)	Main Identified Carotenoids	Reference
<i>Phormidium</i> spp.	Pressurized liquid extraction	n.s.	β -carotene, followed by lutein, violaxanthin, and neoxanthin	[81]
<i>Gloeothece</i> sp.	Continuous pressurized solvent extraction	Lutein: 2.9 β -carotene: 1.5	Lutein, β -carotene, neoxanthin, violaxanthin and α -carotene	[82]
<i>Arthrospira platensis</i>	Ultrasound-assisted extraction	ca. 1.0	β -carotene	[83]
<i>Synechococcus</i> sp.	Supercritical fluid extraction	ca. 2.0	β -carotene, zeaxanthin, myxoxanthophyll and β -cryptoxanthin	[84]
<i>Synechococcus</i> sp.	Supercritical fluid extraction	1.5	n.s.	[85]

n.s.—not specified.

6. Economical Perspective

The market for natural pigments is increasing due to an urge to replace synthetic pigments with natural and sustainable sources. This demand is raising as the European Union drives the use of microalgae as a source of carotenoids. As the main consumer of carotenoids (followed by North America and Asia), Europe plays a key role in the carotenoids market [8].

In terms of application, food is the main industrial sector for carotenoids. This market alone is expected to reach over \$2 billion (USD) worldwide in 2026 [8]. Moreover, with the development of fundamental research, carotenoids are being seen as bioactive compounds with high potential to improve human health.

From microalgae, two successful cases of carotenoids bioprocess are well described: astaxanthin from *H. pluvialis* and β -carotene from *D. salina*. From cyanobacteria, the only

successful pigment production is related to phycocyanin from *A. platensis*, with no market application for carotenoids from cyanobacteria [6].

By evaluating all the details through this review, a question is yet to be answered: is it economically feasible to produce carotenoids from cyanobacteria? The answer is not exactly clear and not favourable to cyanobacteria. The content of carotenoids in cyanobacteria is extremely low when compared to microalgae such as *D. salina*, which has a β -carotene content of up to 14% of dry weight. In this review, the highest content of carotenoid was found in *A. platensis* (ca. 4% of dry weight) [63]. However, considering that the phycobiliproteins content in *A. platensis* is up to 20% of dry weight [91], it would be possible, if co-produced, to recover about 24% of dry weight in pigments in two different products.

Mitra and Mishra [92] suggested a biorefinery process using *A. platensis* for the obtention of phycocyanin, β -carotene, polyhydroxyalkanoates (PHA), and biofuel. *A. platensis* biomass production represents more than half of total microalgae and cyanobacteria production, estimating a total market of 780 million (USD) by 2026 [92]. In an optimal scenario, it is possible to use the biomass for this variety of products through a process of several extractions to increase the economic feasibility of cyanobacteria-based bioprocess [93]. Moreover, the reduction of biomass waste and the valorisation of the whole bioprocess makes the exploitation of cyanobacteria a more sustainable and environmentally friendly approach than their market competitor (microalgae).

7. Final Remarks

Carotenoids have been an interest of science and industry for several years. More recently, with increasing demand, new sources are being evaluated, including cyanobacteria, although in a less pronounced way when compared to microalgae or vascular plants. Moreover, the bioactive potential and the application of these carotenoids or carotenoid-rich extracts are promising but are still in a general perspective of screenings and are not conclusive or applied.

When it comes to production, the optimisation of processing parameters was targeted by fundamental and applied research, and great development can be done in the next years if aiming for, for example, a scaling-up process. A similar strategy might be seen in extraction processes as well. Moreover, the development of a biorefinery process of co-production of pigments or even other compounds should be proposed for better use of the biomass and a more sustainable bioprocess.

Overall, cyanobacteria are already seen as potential candidates for numerous applications, and their carotenoids must be considered as a product to be explored. Thus, it is expected that there are other uses, and more studies should be done about these compounds in these organisms.

Author Contributions: Conceptualization, F.P., V.V., and A.C.G.; Investigation, F.P.; Writing—original draft preparation, F.P.; Writing—review and editing, V.V. and A.C.G.; Supervision, V.V. and A.C.G.; Funding acquisition, V.V. and A.C.G. All authors have read and agreed to the published version of the manuscript.

Funding: A PhD fellowship (reference SFRH/BD/136767/2018) for author Fernando Pagels was granted by Fundação para a Ciência e Tecnologia (FCT, Portugal), under the auspices of Programa Operacional Capital Humano (POCH), supported by the European Social Fund and Portuguese funds (MECTES). This work was financially co-supported by the strategical funding from FCT UIDB/04423/2020 and UIDP/04423/2020 and by the projects ALGAVALOR–MicroALGAs: produção integrada e VALORização da biomassa e das suas diversas aplicações (POCI-01-0247-FEDER-035234), supported by the European Regional Development Fund; BioTecNorte operation (NORTE-01-0145-FEDER-000004), funded by the European Regional Development Fund under the scope of Norte2020–Programa Operacional Regional do Norte and BLUEHUMAN–BLUE biotechnology as a road for innovation on HUMAN’s health aiming for smart growth in the Atlantic Area (EAPA_151/2016) of the Interreg Atlantic Area Programme funded by the European Regional Development Fund.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Masojídek, J.; Torzillo, G.; Koblížek, M.; Masojídek, J.; Torzillo, G.; Koblížek, M. Photosynthesis in Microalgae. In *Handbook of Microalgal Culture*; Richmond, A., Ed.; John Wiley & Sons: Hoboken, NJ, USA, 2013; pp. 21–36. [CrossRef]
2. Holt, T.K.; Krogmann, D.W. A carotenoid-protein from cyanobacteria. *BBA Bioenerg.* **1981**, *637*, 408–414. [CrossRef]
3. Mullineaux, C.W. The thylakoid membranes of cyanobacteria: Structure, dynamics and function. *Aust. J. Plant Physiol.* **1999**, *26*, 671–677. [CrossRef]
4. Guedes, A.C.; Amaro, H.M.; Malcata, F.X. Microalgae as sources of carotenoids. *Mar. Drugs* **2011**, *9*, 625–644. [CrossRef] [PubMed]
5. Hirschberg, J.; Chamovitz, D. Carotenoids in Cyanobacteria. In *The Molecular Biology of Cyanobacteria*; Kluwer Academic Publishers: Drive Norwell, MA, USA, 1994; pp. 559–579. [CrossRef]
6. Pagels, F.; Salvaterra, D.; Amaro, H.M.; Guedes, A.C. Pigments from microalgae. In *Handbook of Microalgae-Based Processes and Products*; Jacob-Lopes, E., Maroneze, M.M., Queiroz, M.I., Zepka, L.Q., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; pp. 465–492. [CrossRef]
7. Gong, M.; Bassi, A. Carotenoids from microalgae: A review of recent developments. *Biotechnol. Adv.* **2016**, *34*, 1396–1412. [CrossRef] [PubMed]
8. Markets and Markets. Carotenoids Market by Type (Astaxanthin, Beta-Carotene, Lutein, Lycopene, Canthaxanthin, and Zeaxanthin), Application (Feed, Food & Beverages, Dietary Supplements, Cosmetics, and Pharmaceuticals), Source, Formulation, and Region—Global Forecast to 2026. 2020. Available online: <https://www.marketsandmarkets.com/Market-Reports/carotenoid-market-158421566.html> (accessed on 14 May 2021).
9. Liang, C.; Zhao, F.; Wei, W.; Wen, Z.; Qin, S. Carotenoid biosynthesis in cyanobacteria: Structural and evolutionary scenarios based on comparative genomics. *Int. J. Biol. Sci.* **2006**, *2*, 197–207. [CrossRef] [PubMed]
10. Sugiyama, K.; Takaichi, S. Carotenogenesis in cyanobacteria: CruA/CruP-type and CrtL-type lycopene cyclases. *J. Gen. Appl. Microbiol.* **2020**, *66*, 53–58. [CrossRef]
11. Mohamed, H.E.; van de Meene, A.M.L.; Roberson, R.W.; Vermaas, W.F.J. Myxoxanthophyll is required for normal cell wall structure and thylakoid organization in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* **2005**, *187*, 6883–6892. [CrossRef]
12. Duan, H.K.; Zhu, Y.; Qi, D.D.; Li, W.L.; Hua, X.J.; Liu, Y.X.; Deng, X. Comparative study on the expression of genes involved in carotenoid and ABA biosynthetic pathway in response to salt stress in tomato. *J. Integr. Agric.* **2012**, *11*, 1093–1102. [CrossRef]
13. Diao, J.; Song, X.; Zhang, L.; Cui, J.; Chen, L.; Zhang, W. Tailoring cyanobacteria as a new platform for highly efficient synthesis of astaxanthin. *Metab. Eng.* **2020**, *61*, 275–287. [CrossRef] [PubMed]
14. Takaichi, S.; Mochimaru, M. Carotenoids and carotenogenesis in cyanobacteria: Unique ketocarotenoids and carotenoid glycosides. *Cell. Mol. Life Sci.* **2007**, *64*, 2607. [CrossRef]
15. Pagels, F.; Guedes, A.C.; Amaro, H.M.; Kijjoo, A.; Vasconcelos, V. Phycobiliproteins from cyanobacteria: Chemistry and biotechnological applications. *Biotechnol. Adv.* **2019**, *37*, 422–443. [CrossRef]
16. Zhang, H.; Liu, H.; Niedzwiedzki, D.M.; Prado, M.; Jiang, J.; Gross, M.L.; Blankenship, R.E. Molecular mechanism of photoactivation and structural location of the cyanobacterial orange carotenoid protein. *Biochemistry* **2014**, *53*, 13–19. [CrossRef] [PubMed]
17. Kirilovsky, D.; Kerfeld, C.A. Cyanobacterial photoprotection by the orange carotenoid protein. *Nat. Plants* **2016**, *2*, 16180. [CrossRef]
18. Wu, Y.P.; Krogmann, D.W. The orange carotenoid protein of *Synechocystis* PCC 6803. *Biochim. Biophys. Acta Bioenerg.* **1997**, *1322*, 1–7. [CrossRef]
19. Kerfeld, C.A.; Kirilovsky, D. Photoprotection in cyanobacteria: The orange carotenoid protein and energy dissipation. In *Bioenergetic Processes of Cyanobacteria: From Evolutionary Singularity to Ecological Diversity*; Peschek, G.A., Obinger, C., Renger, G., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 395–421. [CrossRef]
20. Morone, J.; Alfeus, A.; Vasconcelos, V.; Martins, R. Revealing the potential of cyanobacteria in cosmetics and cosmeceuticals—A new bioactive approach. *Algal Res.* **2019**, *41*, 101541. [CrossRef]
21. Lopes, G.; Clarinha, D.; Vasconcelos, V. Carotenoids from cyanobacteria: A biotechnological approach for the topical treatment of psoriasis. *Microorganisms* **2020**, *8*, 302. [CrossRef]
22. Pagels, F.; Salvaterra, D.; Amaro, H.M.; Lopes, G.; Sousa-Pinto, I.; Vasconcelos, V.; Guedes, A.C. Bioactive potential of *Cyanobium* sp. pigment-rich extracts. *J. Appl. Phycol.* **2020**, *32*, 3031–3040. [CrossRef]
23. Patias, L.D.; Fernandes, A.S.; Petry, F.C.; Mercadante, A.Z.; Jacob-Lopes, E.; Zepka, L.Q. Carotenoid profile of three microalgae/cyanobacteria species with peroxy radical scavenger capacity. *Food Res. Int.* **2017**, *100*, 260–266. [CrossRef] [PubMed]
24. Kelman, D.; Ben-Amotz, A.; Berman-Frank, I. Carotenoids provide the major antioxidant defence in the globally significant N₂-fixing marine cyanobacterium *Trichodesmium*. *Environ. Microbiol.* **2009**, *11*, 1897–1908. [CrossRef]

25. Paliwal, C.; Ghosh, T.; Bhayani, K.; Maurya, R.; Mishra, S. Antioxidant, anti-nephrolithe activities and in vitro digestibility studies of three different cyanobacterial pigment extracts. *Mar. Drugs* **2015**, *13*, 5384–5401. [[CrossRef](#)] [[PubMed](#)]
26. Schwartz, J.L.; Shklar, G. A cyanobacteria extract and β -carotene stimulate an antitumor host response against an oral cancer cell line. *Phyther. Res.* **1989**, *3*, 243–248. [[CrossRef](#)]
27. Belay, A.; Kato, T.; Ota, Y. *Spirulina* (*Arthrospira*): Potential application as an animal feed supplement. *J. Appl. Phycol.* **1996**, *8*, 303–311. [[CrossRef](#)]
28. Liao, W.L.; Nur-E-Borhan, S.A.; Okada, S.; Matsui, T.; Yamaguchi, K. pigmentation of cultured black tiger prawn by feeding with a *Spirulina*-supplemented diet. *Nippon. Suisan Gakkaishi* **1993**, *59*, 165–169. [[CrossRef](#)]
29. Mori, T.; Yamaguchi, K.; Konosu, S.; Muranaka, T.; Miki, W.; Watanabe, T. pigmentation of cultured sweet smelt fed diets supplemented with a blue-green alga *Spirulina maxima*. *Nippon. Suisan Gakkaishi* **1987**, *53*, 433–438. [[CrossRef](#)]
30. Okada, S.; Liao, W.L.; Mori, T.; Yamaguchi, K.; Watanabe, T. pigmentation of cultured striped jack reared on diets supplemented with the blue-green alga *Spirulina maxima*. *Nippon Suisan Gakkaishi* **1991**, *57*, 1403–1406. [[CrossRef](#)]
31. Morone, J.; Lopes, G.; Preto, M.; Vasconcelos, V.; Martins, R. Exploitation of filamentous and picoplanktonic cyanobacteria for cosmetic applications: Potential to improve skin structure and preserve dermal matrix components. *Mar. Drugs* **2020**, *18*, 486. [[CrossRef](#)] [[PubMed](#)]
32. Park, W.S.; Kim, H.J.; Li, M.; Lim, D.H.; Kim, J.; Kwak, S.S.; Kang, C.M.; Ferruzzi, M.G.; Ahn, M.J. Two classes of pigments, carotenoids and c-phycoerythrin, in *Spirulina* powder and their antioxidant activities. *Molecules* **2018**, *23*, 2065. [[CrossRef](#)]
33. Guedes, A.C.; Katkam, N.G.; Varela, J.; Malcata, F.X. Photobioreactors for cyanobacterial culturing. In *Cyanobacteria: An Economic Perspective*; Sharma, N.K., Rai, A.K., Stal, L.J., Eds.; John Wiley & Sons: Hoboken, NJ, USA, 2014; pp. 270–292. [[CrossRef](#)]
34. Saini, D.K.; Pabbi, S.; Shukla, P. Cyanobacterial pigments: Perspectives and biotechnological approaches. *Food Chem. Toxicol.* **2018**, *120*, 616–624. [[CrossRef](#)] [[PubMed](#)]
35. Montgomery, B.L. Seeing new light: Recent insights into the occurrence and regulation of chromatic acclimation in cyanobacteria. *Curr. Opin. Plant Biol.* **2017**, *37*, 18–23. [[CrossRef](#)]
36. Figueroa, F.L. Red, green and blue light photoreceptors controlling chlorophyll *a*, biliprotein and total protein synthesis in the red alga *Chondrus crispus*. *Br. Phycol. J.* **1991**, *26*, 383–393. [[CrossRef](#)]
37. Pagels, F.; Bonomi-Barufi, J.; Vega, J.; Abdala-Díaz, R.; Vasconcelos, V.; Guedes, A.C.; Figueroa, F.L. Light quality triggers biochemical modulation of *Cyanobium* sp.—Photobiology as tool for biotechnological optimization. *J. Appl. Phycol.* **2020**, *32*, 2851–2861. [[CrossRef](#)]
38. Pagels, F.; Lopes, G.; Vasconcelos, V.; Guedes, A.C. White and red LEDs as two-phase batch for cyanobacterial pigments production. *Bioresour. Technol.* **2020**, *307*, 123105. [[CrossRef](#)] [[PubMed](#)]
39. Olaizola, M.; Duerr, E.O. Effects of light intensity and quality on the growth rate and photosynthetic pigment content of *Spirulina platensis*. *J. Appl. Phycol.* **1990**, *2*, 97–104. [[CrossRef](#)]
40. Mishra, S.K.; Shrivastav, A.; Maurya, R.R.; Patidar, S.K.; Haldar, S.; Mishra, S. Effect of light quality on the C-phycoerythrin production in marine cyanobacteria *Pseudanabaena* sp. isolated from Gujarat coast. *India. Protein Expr. Purif.* **2012**, *81*, 5–10. [[CrossRef](#)] [[PubMed](#)]
41. Gutu, A.; Kehoe, D.M. Emerging perspectives on the mechanisms, regulation, and distribution of light color acclimation in cyanobacteria. *Mol. Plant* **2012**, *5*, 1–13. [[CrossRef](#)]
42. Amaro, H.M.; Pagels, F.; Azevedo, I.C.; Azevedo, J.; Sousa Pinto, I.; Malcata, F.X.; Guedes, A.C. Light-emitting diodes—A plus on microalgae biomass and high-value metabolite production. *J. Appl. Phycol.* **2020**, *32*, 3605–3618. [[CrossRef](#)]
43. Schulze, P.S.C.; Barreira, L.A.; Pereira, H.G.C.; Perales, J.A.; Varela, J.C.S. Light emitting diodes (LEDs) applied to microalgal production. *Trends Biotechnol.* **2014**, *32*, 422–430. [[CrossRef](#)]
44. Lima, G.M.; Teixeira, P.C.N.; Teixeira, C.M.L.L.; Filócomo, D.; Lage, C.L.S. Influence of spectral light quality on the pigment concentrations and biomass productivity of *Arthrospira platensis*. *Algal Res.* **2018**, *31*, 157–166. [[CrossRef](#)]
45. Kokabi, M.; Yousefzadi, M.; Soltani, M.; Arman, M. Effects of different UV radiation on photoprotective pigments and antioxidant activity of the hot-spring cyanobacterium *Leptolyngbya* cf. *fragilis*. *Phycol. Res.* **2019**, *67*, 215–220. [[CrossRef](#)]
46. Rath, J.; Adhikary, S.P. Response of the estuarine cyanobacterium *Lyngbya aestuarii* to UV-B radiation. *J. Appl. Phycol.* **2007**, *19*, 529–536. [[CrossRef](#)]
47. Llewellyn, C.A.; Airs, R.L.; Farnham, G.; Greig, C. Synthesis, regulation and degradation of carotenoids under low level UV-B radiation in the filamentous cyanobacterium *Chlorogloeopsis fritschii* PCC 6912. *Front. Microbiol.* **2020**, *11*, 163. [[CrossRef](#)]
48. Ehling-Schulz, M.; Bilger, W.; Scherer, S. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. *J. Bacteriol.* **1997**, *179*, 1940–1945. [[CrossRef](#)] [[PubMed](#)]
49. Huang, L.; McCluskey, M.P.; Ni, H.; LaRossa, R.A. Global gene expression profiles of the cyanobacterium *Synechocystis* sp. strain PCC 6803 in response to irradiation with UV-B and white light. *J. Bacteriol.* **2002**, *184*, 6845–6858. [[CrossRef](#)]
50. Bañares-España, E.; Kromkamp, J.C.; López-Rodas, V.; Costas, E.; Flores-Moya, A. Photoacclimation of cultured strains of the cyanobacterium *Microcystis aeruginosa* to high-light and low-light conditions. *FEMS Microbiol. Ecol.* **2013**, *83*, 700–710. [[CrossRef](#)]
51. Masamoto, K.; Furukawa, K.I. Accumulation of zeaxanthin in cells of the cyanobacterium, *Synechococcus* sp. strain PCC 7942 grown under high irradiance. *J. Plant Physiol.* **1997**, *151*, 257–261. [[CrossRef](#)]
52. Schagerl, M.; Müller, B. Acclimation of chlorophyll *a* and carotenoid levels to different irradiances in four freshwater cyanobacteria. *J. Plant Physiol.* **2006**, *162*, 709–716. [[CrossRef](#)]

53. Pagels, F.; Barufi, J.B.; Vega, J.; Abdala-Díaz, R.; Vasconcelos, V.; Guedes, A.C.; Figueroa, F.L. Light regulating metabolic responses of *Cyanobium* sp. (Cyanobacteria). *Fundam. Appl. Limnol.* **2020**, *193*, 285–297. [[CrossRef](#)]
54. Gris, B.; Sforza, E.; Morosinotto, T.; Bertuccio, A.; la Rocca, N. Influence of light and temperature on growth and high-value molecules productivity from *Cyanobacterium aponinum*. *J. Appl. Phycol.* **2017**, *29*, 1781–1790. [[CrossRef](#)]
55. Walsh, K.; Jones, G.J.; Dunstan, R.H. Effect of irradiance on fatty acid, carotenoid, total protein composition and growth of *Microcystis aeruginosa*. *Phytochemistry* **1997**, *44*, 817–824. [[CrossRef](#)]
56. Juneja, A.; Ceballos, R.M.; Murthy, G.S. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies* **2013**, *6*, 4607–4638. [[CrossRef](#)]
57. Dadheech, P.K.; Glöckner, G.; Casper, P.; Kotut, K.; Mazzoni, C.J.; Mbedi, S.; Krienitz, L. Cyanobacterial diversity in the hot spring, pelagic and benthic habitats of a tropical soda lake. *FEMS Microbiol. Ecol.* **2013**, *85*, 389–401. [[CrossRef](#)]
58. Shukla, S.P.; Kashyap, A.K. An assessment of biopotential of three cyanobacterial isolates from Antarctic for carotenoid production. *Indian J. Biochem. Biophys.* **2003**, *40*, 362–366.
59. Kłodawska, K.; Bujas, A.; Turos-Cabal, M.; Żbik, P.; Fu, P.; Malec, P. Effect of growth temperature on biosynthesis and accumulation of carotenoids in cyanobacterium *Anabaena* sp. PCC 7120 under diazotrophic conditions. *Microbiol. Res.* **2019**, *226*, 34–40. [[CrossRef](#)]
60. Ismaiel, M.M.S.; El-Ayouty, Y.M.; Piercey-Normore, M. Role of pH on antioxidants production by *Spirulina (Arthrospira) platensis*. *Braz. J. Microbiol.* **2016**, *47*, 298–304. [[CrossRef](#)] [[PubMed](#)]
61. Pagels, F.; Salvaterra, D.; Amaro, H.M.; Lopes, G.; Sousa-Pinto, I.; Vasconcelos, V.; Guedes, A.C. Factorial optimization of upstream process for *Cyanobium* sp. pigments production. *J. Appl. Phycol.* **2020**, *32*, 3861–3872. [[CrossRef](#)]
62. Thirumala, M. Optimization of growth of *Spirulina platensis* Ln1 for production of carotenoids. *Int. J. Life Sci. Biotechnol. Pharma Res.* **2012**, *1*, 152–157.
63. El Baky, H.H.A.; El Baroty, G.S.; Mostafa, E.M. Optimization growth of *Spirulina (Arthrospira) platensis* in photobioreactor under varied nitrogen concentration for maximized biomass, carotenoids and lipid contents. *Recent Pat. Food. Nutr. Agric.* **2018**, *11*, 40–48. [[CrossRef](#)]
64. Erdoğan, A.; Demirel, Z.; Eroğlu, A.E.; Dalay, M.C. Carotenoid profile in *Prochlorococcus* sp. and enrichment of lutein using different nitrogen sources. *J. Appl. Phycol.* **2016**, *28*, 3251–3257. [[CrossRef](#)]
65. Tarko, T.; Duda-Chodak, A.; Kobus, M. Influence of growth medium composition on synthesis of bioactive compounds and antioxidant properties of selected strains of *Arthrospira* cyanobacteria. *Czech J. Food Sci.* **2012**, *30*, 258–267. [[CrossRef](#)]
66. D’Alessandro, E.B.; Soares, A.T.; de Oliveira D’Alessandro, N.C.; Antoniosi Filho, N.R. Potential use of a thermal water cyanobacterium as raw material to produce biodiesel and pigments. *Bioprocess Biosyst. Eng.* **2019**, *42*, 2015–2022. [[CrossRef](#)] [[PubMed](#)]
67. Paliwal, C.; Pancha, I.; Ghosh, T.; Maurya, R.; Chokshi, K.; Vamsi Bharadwaj, S.V.; Ram, S.; Mishra, S. Selective carotenoid accumulation by varying nutrient media and salinity in *Synechocystis* sp. CCNM 2501. *Bioresour. Technol.* **2015**, *197*, 363–368. [[CrossRef](#)] [[PubMed](#)]
68. Yang, H.W.; Song, J.Y.; Cho, S.M.; Kwon, H.C.; Pan, C.H.; Park, Y. II Genomic survey of salt acclimation-related genes in the halophilic cyanobacterium *Eubhalothece* sp. Z-M001. *Sci. Rep.* **2020**, *10*, 676. [[CrossRef](#)] [[PubMed](#)]
69. Lagarde, D.; Beuf, L.; Vermaas, W. Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *Synechocystis* sp. strain PCC 6803. *Appl. Environ. Microbiol.* **2000**, *66*, 64–72. [[CrossRef](#)] [[PubMed](#)]
70. Gao, X.; Xu, H.; Zhu, Z.; She, Y.; Ye, S. Improved production of echinenone and canthaxanthin in transgenic *Nostoc* sp. PCC 7120 overexpressing a heterologous crtO gene from *Nostoc flagelliforme*. *Microbiol. Res.* **2020**, *236*, 126455. [[CrossRef](#)]
71. Lin, P.C.; Pakrasi, H.B. Engineering cyanobacteria for production of terpenoids. *Planta* **2019**, *249*, 145–154. [[CrossRef](#)]
72. Panis, G.; Carreon, J.R. Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: A microalgae process model and a techno-economic assessment all through production line. *Algal Res.* **2016**, *18*, 175–190. [[CrossRef](#)]
73. Aguilar-Machado, D.; Morales-Oyervides, L.; Contreras-Esquivel, J.C.; Aguilar, C.; Méndez-Zavala, A.; Raso, J.; Montañez, J. Effect of ohmic heating processing conditions on color stability of fungal pigments. *Food Sci. Technol. Int.* **2017**, *23*, 338–348. [[CrossRef](#)]
74. Kang, C.D.; Sim, S.J. Direct extraction of astaxanthin from *Haematococcus* culture using vegetable oils. *Biotechnol. Lett.* **2008**, *30*, 441–444. [[CrossRef](#)]
75. Amaro, H.M.; Fernandes, F.; Valentão, P.; Andrade, P.B.; Sousa-Pinto, I.; Malcata, F.X.; Guedes, A.C. Effect of solvent system on extractability of lipidic components of *Scenedesmus obliquus* (M2-1) and *Gloeothece* sp. on antioxidant scavenging capacity thereof. *Mar. Drugs* **2015**, *13*, 6453–6471. [[CrossRef](#)]
76. Tavanandi, H.A.; Vanjari, P.; Raghavarao, K.S.M.S. Synergistic method for extraction of high purity allophycocyanin from dry biomass of *Arthrospira platensis* and utilization of spent biomass for recovery of carotenoids. *Sep. Purif. Technol.* **2019**, *225*, 97–111. [[CrossRef](#)]
77. Dorina, S.; Judith, S.; Björn, W.; Julia, S.; Andrea, S.; Sarah, D.N.; Muffler, K.; Roland, U. A new strategy for a combined isolation of EPS and pigments from cyanobacteria. *J. Appl. Phycol.* **2020**, *32*, 1729–1740. [[CrossRef](#)]
78. Assunção, J.; Amaro, H.M.; Lopes, G.; Tavares, T.; Malcata, F.X.; Guedes, A.C. Exploration of marine genus *Chroococcidiopsis* sp.: A valuable source for antioxidant industry? *J. Appl. Phycol.* **2021**. [[CrossRef](#)]

79. Springstein, B.L.; Nürnberg, D.J.; Weiss, G.L.; Pilhofer, M.; Stucken, K. Structural determinants and their role in Cyanobacterial morphogenesis. *Life* **2020**, *10*, 355. [[CrossRef](#)] [[PubMed](#)]
80. Mandal, S.C.; Mandal, V.; Das, A.K. Classification of Extraction Methods. In *Essentials of Botanical Extraction*; Mandal, S.C., Mandal, V., Das, A.K., Eds.; Academic Press: Cambridge, MA, USA, 2015; pp. 83–136. [[CrossRef](#)]
81. Rodríguez-Meizoso, I.; Jaime, L.; Santoyo, S.; Cifuentes, A.; García-Blairsy Reina, G.; Señoráns, F.J.; Ibáñez, E. Pressurized fluid extraction of bioactive compounds from *Phormidium* species. *J. Agric. Food Chem.* **2008**, *56*, 3517–3523. [[CrossRef](#)] [[PubMed](#)]
82. Amaro, H.M.; Catarina Guedes, A.; Preto, M.A.C.; Sousa-Pinto, I.; Xavier Malcata, F. *Gloeothece* sp. as a nutraceutical source—an improved method of extraction of carotenoids and fatty acids. *Mar. Drugs* **2018**, *16*, 327. [[CrossRef](#)] [[PubMed](#)]
83. Dey, S.; Rathod, V.K. Ultrasound assisted extraction of β -carotene from *Spirulina platensis*. *Ultrason. Sonochem.* **2013**, *20*, 271–276. [[CrossRef](#)] [[PubMed](#)]
84. Montero, O.; Macías-Sánchez, M.D.; Lama, C.M.; Lubián, L.M.; Mantell, C.; Rodríguez, M.; De La Ossa, E.M. Supercritical CO₂ extraction of β -carotene from a marine strain of the cyanobacterium *Synechococcus* species. *J. Agric. Food Chem.* **2005**, *53*, 9701–9707. [[CrossRef](#)] [[PubMed](#)]
85. Macías-Sánchez, M.D.; Mantell, C.; Rodríguez, M.; Martínez de la Ossa, E.; Lubián, L.M.; Montero, O. Supercritical fluid extraction of carotenoids and chlorophyll a from *Synechococcus* sp. *J. Supercrit. Fluids* **2007**, *39*, 323–329. [[CrossRef](#)]
86. Sierra, L.S.; Dixon, C.K.; Wilken, L.R. Enzymatic cell disruption of the microalgae *Chlamydomonas reinhardtii* for lipid and protein extraction. *Algal Res.* **2017**, *25*, 149–159. [[CrossRef](#)]
87. Lee, S.Y.; Cho, J.M.; Chang, Y.K.; Oh, Y.K. Cell disruption and lipid extraction for microalgal biorefineries: A review. *Bioresour. Technol.* **2017**, *244*, 1317–1328. [[CrossRef](#)]
88. Juin, C.; Chérouvrier, J.-R.; Thiéry, V.; Gagez, A.-L.; Bérard, J.-B.; Joguet, N.; Kaas, R.; Cadoret, J.-P.; Picot, L. Microwave-assisted extraction of phycobiliproteins from *Porphyridium purpureum*. *Appl. Biochem. Biotechnol.* **2015**, *175*, 1–15. [[CrossRef](#)] [[PubMed](#)]
89. Luengo, E.; Condón-Abanto, S.; Álvarez, I.; Raso, J. Effect of pulsed electric field treatments on permeabilization and extraction of pigments from *Chlorella vulgaris*. *J. Membr. Biol.* **2014**, *247*, 1269–1277. [[CrossRef](#)] [[PubMed](#)]
90. Guedes, A.C.; Gíão, M.S.; Matias, A.A.; Nunes, A.V.; Pintado, M.E.; Duarte, C.M.; Malcata, F.X. Supercritical fluid extraction of carotenoids and chlorophylls a, b and c, from a wild strain of *Scenedesmus obliquus* for use in food processing. *J. Food Eng.* **2013**, *116*, 478–482. [[CrossRef](#)]
91. Khazi, M.I.; Demirel, Z.; Dalay, M.C. Evaluation of growth and phycobiliprotein composition of cyanobacteria isolates cultivated in different nitrogen sources. *J. Appl. Phycol.* **2018**, *30*, 1513–1523. [[CrossRef](#)]
92. Mitra, M.; Mishra, S. Multiproduct biorefinery from *Arthrospira* spp. towards zero waste: Current status and future trends. *Bioresour. Technol.* **2019**, *291*, 121928. [[CrossRef](#)]
93. Bastiaens, L.; van Roy, S.; Thomassen, G.; Elst, K. Biorefinery of algae: Technical and economic considerations. In *Woodhead Publishing Series in Energy*; Gonzalez-Fernandez, C., Muñoz, R., Eds.; Woodhead Publishing: Sawston, UK, 2017; pp. 327–345. [[CrossRef](#)]