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Review

Bioprocess Strategy of *Haematococcus lacustris* for Biomass and Astaxanthin Production Keys to Commercialization: Perspective and Future Direction

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Abstract: *Haematococcus lacustris* (formerly called *Haematococcus pluvialis*) is regarded as the most promising microalgae for the production of natural astaxanthin, which is secondary metabolism used as a dietary supplement, also for cosmetic applications, due to its high anti-oxidant activity. Astaxanthin has a wide range of biological activities and high economic potential, and currently dominates the market in its synthetic form. Furthermore, because of the difficulty of bioprocess and the high cost of cultivation, astaxanthin extracted from this microalga is still expensive due to its low biomass and pigment productivities. Large-scale biomass production in biotechnological production necessitates the processing of a large number of cultures as well as the use of both indoor and outdoor systems, such as open pond raceway systems and photo-bioreactors (PBR). The photo-bioreactors systems are suitable for mass production because growth conditions can be controlled, and the risk of contamination can be reduced to a certain extent and under specific culture parameters. This review discusses current technologies being developed to improve cultivation and operation efficiency and profitability, as well as the effect of parameter factors associated with *H. lacustris* cultivation on biomass and astaxanthin bioproduction, and even strategies for increasing bioproduction and market potential for *H. lacustris* astaxanthin.

Keywords: *Haematococcus lacustris*; astaxanthin; secondary metabolism; bioprocess

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1. Introduction

Astaxanthin is a red ketocarotenoid with a high anti-oxidant capacity that is widely used in a variety of industries due to its potential in the nutraceutical and pharmaceutical industries [1]. Astaxanthin can be synthesized chemically or naturally produced [2]. Furthermore, natural astaxanthin has a 20-fold higher anti-oxidant potency than synthetic astaxanthin and about 100–1000-fold higher than coenzyme Q10 or vitamin E [3,4]. The microalgae *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) has become a significant commercial source of natural astaxanthin since it was shown to be reliable and approved

for human consumption as a dietary supplement [5]. Furthermore, no proven adverse reactions have occurred during its 20-year distribution as a food supplement for humans and clinical study [6]. The high demand for natural astaxanthin from *H. lacustris* is a result of the fact that retail prices for nutraceutical-grade astaxanthin have reached as high as USD 100,000 per kg [7]. For commercial purposes, *H. lacustris* production efficiency has become a primary concern in the industrial production of natural astaxanthin; biofertilization can also be obtained from residual *H. lacustris* biomass [8].

H. lacustris is currently produced in two distinct cultivation systems: open and closed [9]. The open system makes use of both natural and man-made ponds (raceway ponds), whereas the closed system makes use of closed systems such as photo-bioreactors (PBR) [10]. In microalgae cultivation, optimization can be accomplished by exploiting key process parameters that affect biomass growth and bioactive compound accumulation [11,12]. The interaction of these factors may have a significant impact on biomass production and intracellular culture composition [13]. *H. lacustris* is a freshwater strain of green microalgae with the life cycle of *H. lacustris* cells, from green to red cyst cells, which synthesize and accumulate astaxanthin [14]. The cultivation strategy for astaxanthin-rich *Haematococcus*, such as phototrophic, heterotrophic, or mixotrophic cultivation, or methods that overcome the disadvantages of heterotrophic–phototrophic and mixotrophic–mixotrophic cultivation, has the potential to improve the process economics of astaxanthin production from *H. lacustris* [2]. The comparative effects of various cultivation strategies on growth and astaxanthin accumulation result in an increase in dry biomass weight, an increase in astaxanthin yield, and a decrease in cultivation costs and energy consumption [15].

The goal of this review is to provide current research and knowledge dealing with a cultivation system using a green microalgae *H. lacustris* and the influence of parameter factors related to *H. lacustris* cultivation in producing biomass and astaxanthin bioproduction. This review also discusses future directions for improving bioproduction and expanding the market potential of astaxanthin derived from *H. lacustris*.

2. Composition Biochemical of Microalgae *Haematococcus lacustris*

2.1. The Astaxanthin Molecules

Astaxanthin is as well known as (3,3′-dihydroxy-,-carotene−4,4′-dione). Figure 1 shows the structure of astaxanthin, which belongs to xanthophyll ketocarotenoid, which contains carbon, hydrogen, and oxygen [1]. Additionally, it contains 13 conjugated double bonds (which generate the red pigment), as well as hydroxyl and keto groups [16]. The molecular formula of astaxanthin is $C_{40}H_{52}O_4$, and its molar mass is 596.84 g/mol. It is fat-soluble and solid at room temperature, with a melting point of 182.5 °C and a log P (octanol/water partition) of 13.27 [16]. Currently, both synthetic and natural supplies meet the high demand for astaxanthin. The synthetic form has been approved as a food colorant for salmon, trout, and ornamental fish, as well as for fish feed, by the US Food and Drug Administration (USFDA) [1]. Astaxanthin was created by combining other carotenoids, multivitamins, and omega−3, 6 fatty acids [17]. In *H. lacustris*, the natural astaxanthin esters are predominantly composed of oleic acid (C18:1, n9), followed by palmitic (C16:0) and linoleic (C18:2, n6) acids [1]. Because dietary components like lipids promote carotenoid absorption, the time of natural astaxanthin intake affects its bioavailability for human intake. For instance, in broilers fed with *Phaffia rhodozyma*, the small intestine had the highest concentration of astaxanthin, followed by adipose tissues, spleen, liver, heart, kidneys, skin, and ultimately, muscles [16]. Surprisingly, only natural astaxanthin is used in the pharmaceutical, cosmetic, and food industries. Antioxidant-rich natural astaxanthin has recently been shown to provide therapeutic benefits in the treatment of human and animal disorders, including diabetes and inflammation, as well as neurological, cardiovascular, ophthalmic, and skin ailments. This means it has excellent potential for commercialization [17].

An efficient extraction technique is required in cell recovery to extract the highest percentage of astaxanthin produced by the cells of *H. lacustris*. Thus, by employing solvent

extraction or other technologies such as ultrasound and supercritical CO_2 (SC-CO_2) during cell disruption, astaxanthin extraction can be performed in a single step and yield a high-purity product [18]. Recently, the combination of liquid-liquid chromatography, such as high-speed counter-current chromatography (HSCCC), and liquid-solid chromatography has resulted in high productivity and astaxanthin purity of up to 99%. Furthermore, SC-CO_2 and two-dimensional chromatography show great promise [15]. Moreover, when compared to synthetic astaxanthin, natural astaxanthin has stronger pigmentation and a much higher oxygen radical absorbance capacity (ORAC). In terms of anti-oxidant properties, there are notable differences between natural and synthetic astaxanthin [1].

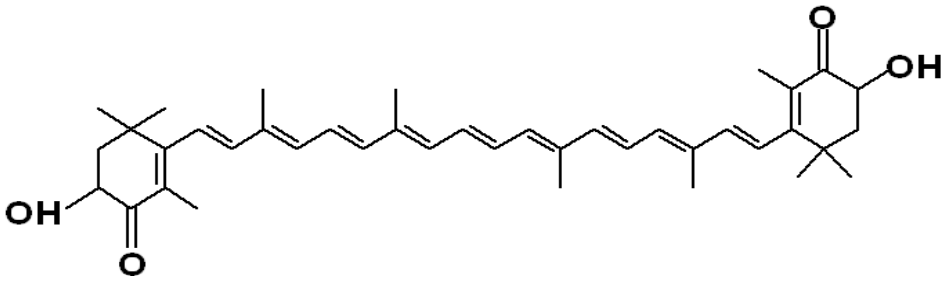

Figure 1. Structure of astaxanthin.

2.2. *Haematococcus lacustris*

H. lacustris is a green microalgae found in a variety of habitats around the world [18]. In nature, *H. lacustris* belongs to the Volvocales; it is mainly found in temporary water points and natural or artificial freshwater pools [19]. The unique life cycle of *H. lacustris* can be divided into a two-stage process. In the first phase, favorable growth conditions for *H. lacustris* strains are exposed in the green stage. *H. lacustris* accumulates for astaxanthin occurs in the second phase, which is in the red stage under stress conditions (e.g., nutrient depletion, light stress, high acidity, temperature fluctuations) [1,20,21].

In general, the metabolic regulation of astaxanthin biosynthesis in *H. lacustris* is a complex process that is highly up-regulated in response to stress conditions, which coincides with the accumulation of triacylglycerols (TAGs) [10]. During the "red" or encysted aplanospore stage of *H. lacustris* culture, the compounds are deposited in cytosolic lipid bodies. β-carotenoid is formed when red lycopene undergoes cyclization following isopentenyl pyrophosphate (IPP) conversion, condensation into colorless phytoene, and dehydrogenation [18]. Astaxanthin formation begins with β-carotene, a byproduct of photosynthesis in the microalgae *H. lacustris* that serves as a precursor for keto-carotenoids in the chloroplast and cytosol during the green stage. Echinenone and canthaxanthin are produced when β-carotene is oxygenated by -carotene ketolase (BKT). Multiple BKT genes are up-regulated to a certain threshold in the red stage under stress conditions. Astaxanthin is produced in *H. lacustris* through the hydroxylation of canthaxanthin catalyzed by CrtR-b. *H. lacustris* then begins to synthesize astaxanthin [1].

The primary morphotypes of the green *H. lacustris* cycle are vegetative cells containing motile cells (Figure 2a), aplanospore-accumulating astaxanthin (Figure 2b), and a red cyst cell (Figure 2c) when under unfavorable stress conditions containing immobile cells [1]. During the green stage, a nutrient-dense medium is required, as well as moderate light intensity, temperature, and pH [9]. Recent research has concentrated on astaxanthin production via vegetative cells in *H. lacustris* in first stage cultivation, which include a mixture of motile and non-motile cells [8,19,21]. The second stage is a red, non-motile resting stage during which green vegetative cells transform into red cysts with a thick cell wall, where cell division ceases, and chlorophyll levels remain constant, resulting in a continuous increase in astaxanthin content and cellular dry weight. Under high-stress conditions, astaxanthin can reach 5% of DBW (dry weight) and can reach up to 7.72% DBW when 5% of CO_2 is supplied [12].

When microalgal cells are deprived of nutrients, the inhibition of cell propagation results, and the accumulation of astaxanthin is triggered. Light intensity or nutrient deple-

tion are the primary factors stimulating the synthesis of astaxanthin in *H. lacustris* [8]. High temperatures and high salt concentrations also have been reported to enhance astaxanthin accumulation. Nevertheless, these stress factors may cause cell death, leading to decreased astaxanthin efficiency in *H. lacustris s* cultures [22]. Furthermore, Gu et al. [23] revealed that when cell density decreased by 41%, the intent to (photo-bleach) mass cells owing to die-off was detected during the process's transition between the green and red stages. The mechanisms by which cells die during the transition from green to red stages of cultivation are critical for minimizing productivity losses [24].

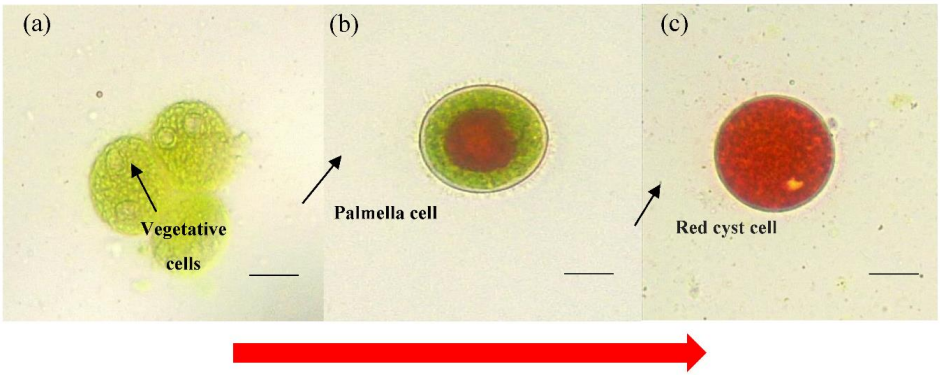

Figure 2. Light microscopic images of cells *H. lacustris* morphology to observe the astaxanthin accumulation (**a**) vegetative cells, (**b**) astaxanthin-accumulating, (**c**) red cyst cell. Scale bar: 10 µm [1].

3. Microalgae Cultivation Strategies for Production of *H. lacustris*

Microalgae have a rapid growth rate. Although microalgae are easily grown, maintaining high productivity in large-scale manufacturing is difficult. It should have adequate lighting, good material transfer across the liquid-gas barrier, be simple and cheap, have a low contamination rate, have a low total building and production cost, and be area efficient. Open cultivation systems, such as open ponds, tanks, and raceway ponds, and controlled closed cultivation systems using various types of bioreactors are the two most common microalgae culture methods [25]. Each strategy has benefits and drawbacks. *H. lacustris*, a commercially viable microalgae, is presently cultivated in open and closed systems. In this context, it is critical to develop microalgae for mass production at a low cost and on a huge scale [26].

3.1. Open Cultivation Systems

Of the raceway pond cultivation designs available, a single paddlewheel is adequate to appropriately agitate a 5-hectare open pond cultivation, making it one of the most energy-efficient [25]. The open pond system is the most cost-effective cultivation technique due to its low construction, maintenance, and operation costs. Open pond systems also have low energy requirements and are easily scaled up [27]. Despite the vast production area, open-system microalgae cultivation has lower biomass productivities than closed-system microalgae cultivation, necessitating a more effective harvesting approach due to more diluted cultures [28,29]. Furthermore, bad weather conditions and precipitation runoff can negatively impact microalgae development conditions such as salinity and pH, while the erosion of banks can lead to leaks and increased water turbidity [10,25]. Moreover, the open system makes it difficult to control temperature and light intensity, which can influence microalgae growth [25].

In the cultivation of *H. lacustris*, open pond cultivation is typically used only during the stressed phase and for a short period of time (4 to 6 days) during the cultivation cycle [30]. In the study by Zhang et al. [31], the changes in cell density and medium pH were closely monitored, and the astaxanthin content and yield of the four *Haematococcus* strains were measured after 12 days of cultivation. The strains *H. lacustris* 26 and *H. lacustris* WZ produced astaxanthin yields of 51.06 mg L^{-1} and 40.25 mg L^{-1}, respectively, which are equivalent to 2.79 and 2.50% of their dry biomass, respectively. The two-stage growth one-

step process of *H. lacustris* WZ cultures produced astaxanthin in two 100 m^2 open race-way ponds and content from 1.61 to 2.48 g/100 g of dry weight, with an average of 2.10 g/100 g dry weight. The cultivation has been conducted in outdoor two-stage *H. lacustris* production processes. The researcher has reported that astaxanthin yields can reach 8–10 mg L^{-1} day^{-1} over a 10-day cycle (4 days green, 6 days red), with astaxanthin accounting for up to 4% DW under high light and nitrate depleted conditions during the red stage in two-stage *H. lacustris* outdoor cultivation [32]. Technically, open-system *H. lacustris* cultivation is preferred over closed-system cultivation due to its simplicity and low cost. However, the pond's limitations should be addressed, such as contamination, low growth rate, and inefficient light and CO_2 mass transfer [12,31]. Bacterial contamination is a major issue when growing microalgae in open cultivation [1]. Large-scale open-pond farming systems commonly suffer from pollution, bacterial loads, and grazers [10]. Bacterial contamination is generally increased when organic carbon is present in the medium, and bacterial growth more accurately correlates with the nutrients in the culture medium [33,34]. Wen et al. [14] recently reported that adding acetic acid to the culture increased astaxanthin production by more than 20% under nitrogen-depleted conditions, and bacteria reproduction was limited.

3.2. Closed Cultivation Systems

The other method of microalgal cultivation is the use of closed control systems, also referred to as closed photo-bioreactors, which do not make a direct exchange of material between the culture and the environment but do ensure homogeneous CO_2 mass transfer and light intensity distribution [19,25]. The photo-bioreactor can overcome numerous limits that are often encountered in open pond culture design. Because they can be operated under precisely controlled circumstances, closed photo-bioreactors are more efficient in terms of quality. This corresponds to improved nutritional and metabolic efficiency, which results in more biomass production per unit of substrate [10,28]. As a consequence, cultivation in closed systems can overcome the drawbacks of open cultivation by producing a contamination-free, single-strain microalgae culture [35]. Closed systems, however, have higher capital and running expenses [28]. Photo-bioreactors are usually tanks of various sizes and shapes that have been specially designed for the purpose and are automatically controlled for optimal conditions using process monitoring equipment [10].

Moreover, several studies indicated that *H. lacustris* could be cultured using an immobilized biofilm technique utilizing a multilayer photo-bioreactor. This technique enabled researchers to achieve astaxanthin productivities of approximately 390 mg m^{-2}·day^{-1} in a one-phase culture [36] and 160 mg m^{-2}·day^{-1} in a two-phase culture [37]. Wan et al. [26] reported indoor culture utilizing 1 L column bioreactors with a light intensity of around 250 µmol photons m^{-2} s^{-1}, a 12 h:12 h light–dark cycle, and a temperature range of 8 °C to 33 °C during the day and 28 °C at night. Astaxanthin accumulation in *H. lacustris* was shown to be optimum between 20 and 28 °C, with the highest biomass at 28 °C and the lowest at 23 °C. The cell slowly dissolved and died at 33 °C. At the temperature of 28 °C, the maximum net biomass and astaxanthin productivities were 0.12 g L^{-1} day^{-1} and 5.4 mg L^{-1} day^{-1}, respectively. Recently, Do et al. [30] reported biomass productivity of *H. lacustris* was achieved at 6.5 mg L^{-1} day^{-1} under conditions of 80 µmol photons m^{-2} s^{-1} and a closed system by using a photo-bioreactor. Current findings on the type cultivation of *H. lacustris* for astaxanthin production in open and closed system strategies are summarized in Table 1. Furthermore, to induce astaxanthin in the red stage, microalgae cultivation processes must be carried out under environmental stresses to induce astaxanthin. With current cultivation strategies, biomass and astaxanthin production from *H. lacustris* could be sustained [18]. At the green stage, the optimum temperature and pH ranges for *H. lacustris* are 20 to 25 °C and pH 6 to 8 [3]. In addition, cultivation conditions with low light intensity, a white plasma light source, and urea as the nitrogen source under a photoperiod of 12:12 h light/dark cycle are the best for cell growth with the suitable for cultivating green microalgae starting with an initial biomass of 0.4 to 0.5 g L^{-1} [1].

Table 1. Summarized recent research of type cultivation *H. lacustris* for astaxanthin production in open and close system strategy.

System	Medium	Temperature (°C)	Light Intensities (μmol photon m^{-2} s^{-1})	Productivity	References
Closed Column Photobioreactors	BG−11	25	50, 100, 200, 400	The highest level of astaxanthin production was achieved in nitrogen starvation and under light intensity at 200 μmol photons m^{-2} s^{-1}, which productivity obtained 1.64 times higher than the control group under the photoautotrophic mode.	[3]
Closed air-lift photo-bioreactors	Bold's Basal Medium	23–25	190	The system comprised of 90 dm^3 volume photo-bioreactors was obtained biomass concentration in the range 1.40–1.99 g dm^{-3} on the eighth day of conducting experiments	[11]
Vertical bubble column photo-bioreactor (VBC-PBR)	BG−11	28	55–280	High-intensity light stress increased total carotenoids (5.21 mg g^{-1}) and FA production (19.62 mg g^{-1}), while low light intensity stress decreased total carotenoids (5.21 mg g^{-1}) under the different photoautotrophic conditions.	[12]
Outdoor raceway pond	Modified BG11	Not Control	300, 800, 1800	The mixotrophic cultivation in an outdoor raceway pond of *H. lacustris* reached a maximum biomass productivity of 6.75 g m^{-2}·day^{-1}, the astaxanthin productivity reached 140 mg m^{-2}·day^{-1} and about 1.2 times that of the simple phototrophic cultivation.	[14]
Twin-Layer porous substrate photo-bioreactor (TL-PSBR)	Modified Blue Green 11 (BG11)	26	300–1000	The TL-PSBR produced the most biomass and astaxanthin, at 8.7 g m^{-2} day^{-1} and 170 mg m^{-2} day^{-1}, respectively. The longer storage times reduced productivity, but the most efficient use occurred between 300–500 μmol photon m^{-2}·s^{-1}. The experiment has been cultivated in the photoautotrophic mode.	[30]
Twin-Layer porous substrate photo-bioreactor (TL-PSBR)	Modified BG11	26	300	In the 0.05 m^2 and 2 m^2 systems, dry biomass productivity reached 12 g m^{-2} day^{-1} (3% astaxanthin content in the dry biomass) and 11.25 g m^{-2} day^{-1} (2.8% astaxanthin) after 10 days of cultivation. The cultivation was conducted under photoautotrophic mode.	[38]
Small-scale angled (TL-PSBR)	Modified BG11	26	20–80	The cultivation under photoautotrophic with initial biomass at 6.5 g·m^{-2} (inoculum) density of the biofilm was 6.5 g·m^{-2}·day^{-1} dry biomass yield under 80 mol photon m^{-2}·s^{-1} light intensity.	[39]

3.3. Cultivation of H. lacustris

Generally, *H. lacustris* can grow under phototrophic, heterotrophic, or mixotrophic conditions. When microalgae are grown phototrophically, sunlight is used as an energy source and CO_2 as a carbon source [1]. Microalgae grow heterotrophically when they consume organic molecules such as glucose or acetate as their primary source of nutrition [40], and no light is used during cultivation [34]. Additionally, heterotrophic cultivation has received much interest recently as a result of its high cell density and high rate of biomass production [41]. Furthermore, in comparison to photoautotrophic systems, heterotrophic cultivation can achieve more consistent and predictable biomass productivity due to the consistent availability of the energy source in the form of organic carbon uptake [42]. In addition, microalgae cultivation can be carried out under mixotrophy conditions by using alternative organic carbon sources, such as acetate organic acid and light, in which organic carbon and CO_2 are assimilated concurrently, and respiratory and photosynthetic metabolisms coexist [43]. Cell growth in mixotrophic conditions is able to promote the growth of microalgae using a variety of nitrogen sources and is not dependent on the process of photosynthesis [8]. Thus, the cultivation method of *H. lacustris* using heterotrophic and mixotrophic is effective for increasing astaxanthin productivity, as indicated by increased biomass concentration and growth rate [44].

Recently, Wen et al. [14] reported that when *H. lacustris* cells were grown initially in the dark and then accompanied with acetate/acetic acid when the culture media became depleted of nitrate, the productivity of astaxanthin can be reached 140 mg $m^{-2}{\cdot}day^{-1}$, which is higher than phototrophic cultivation. Thus, using a two-stage cultivation method that included perfusion culture during the vegetative stage and stepwise light irradiation during the induction stage, the mixotrophic culture system produced high-density biomass (2.47 g L^{-1}), which was 3.09 and 1.67 times greater than batch and fed-batch processes, respectively [42]. Moreover, Sipaúba-Tavaresa et al. [41] reported that high cell density increased from 0.4×10^5 cells mL^{-1} to 1.7×10^5 cells mL^{-1} during exponential growth from day 1 to day 10 under mixotrophic cultivation in *H. lacustris* when using *E. crassipes* culture medium.

4. Influence of Stress Factor on Biomass Growth and Astaxanthin Accumulation of *H. lacustris*

Microalgae cultivation is influenced by a variety of factors, including light intensity, temperature, nutrients such as carbon and nitrogen source, and initial biomass before starting the cultivation of microalgae. The interaction of these factors has the potential to have a significant effect on biomass production, intracellular composition, and chlorophyll content [13].

In terms of influence stress factors on biomass growth of *H. lacustris*, Saha et al. [45] investigated the effects of photosynthetically active radiation (PAR), nitrate and/or phosphate restrictions, light intensity, and quality. PAR without P and low N produced high biomass production. In addition, perfusion culture and sequential light irradiation were used in *H. lacustris* cultures to improve immature cell stress tolerance while enhancing biomass production. Moreover, the use of additional carbon sources and subsequent heterotrophic-photoautotrophic growth could boost biomass yield. Integrating waste carbon and nutrients in a biorefinery setup could help to reduce cultivation costs [18].

4.1. Light Intensity

Light is a critical factor in microalgal cultivation because it provides the energy required for photosynthesis [11]. When *H. lacustris* cells are exposed to bright light, the phytoene synthase gene (psy) expression is increased, and the cells transition from the green to the red stage [12]. Additionally, it was suggested that high-intensity light could be used to create an electrochemical in trans membranes such as proton gradient, resulting in the photooxidation of the PS II reaction centers [46]. Recently, Do et al. [39] demonstrated that high light intensities, up to 2000 µmol photons $m^{-2}{\cdot}s^{-1}$ sunlight, are the most impor-

tant single factor in the induction of astaxanthin accumulation; in contrast to low light intensities, less than 100 µmol photons $m^{-2} \cdot s^{-1}$, which are frequently used to maintain and increase the density of microalgae suspended in a suspension in the green phase. Additionally, blue light increases cell size, while red light increases cell division rate. When 2500 lux and 500 lux LED blue light were used, the cell size accelerated by 4.1 and 2.5 times, respectively, in the red stage [12]. There are a few lighting colors: sunlight, blue, deep red, green, warm-white, orange-red, and yellow, which have always been used to optimize microalgae cultivation productivity [11]. Since green algae contain chlorophyll a and b, which are major light-harvesting pigments, they are sensitive to blue and red light. However, green and white light had a detrimental effect on red phase induction and growth, resulting in a chlorophyll-a deficiency of approximately 26% [47]. Mehareya et al. [12] recently demonstrated the interaction between light intensity and nutrient concentration, demonstrating that the growth medium contained more nutrients when the light intensity was (280 µmol photons $m^{-2} \cdot s^{-1}$), as opposed to (55 µmol photons $m^{-2} \cdot s^{-1}$). The results indicated that growth under conditions of reduced nutrient and low light intensity resulted in a greater biomass concentration. Growth cultivation of *H. lacustris* under the optimum concentration of nitrate, sulfate, phosphate, and magnesium with high light intensity resulted in an 85% increase in total carotenoids and a 58% increase in fatty acids, respectively. Additionally, Kiperstok et al. [36] demonstrated that the total biomass content was 130 g m^2 at 1000 µmol photons $m^{-2} \cdot s^{-1}$, which was related to the higher CO_2 concentrations used during cultivation.

4.2. Temperature

Temperature has a direct impact on the rate of growth of microalgae and has a major influence on the cellular chemical composition, nutrient absorption, and CO_2 production in all green algae species. Temperature is a significant and fundamental element determining biomass concentration and astaxanthin content in *H. lacustris* production [26]. According to Shah et al. [18], increased temperature affects astaxanthin synthesis by stimulating the formation of oxygen radicals. Temperatures above 30 °C cause a transition from the green vegetative stage to the red stage, and astaxanthin accumulation is 2–3 times higher than at 20 °C. In batch culture, the growth rate, cell cycle, and astaxanthin accumulation of *H. lacustris* Flotow were strongly affected by growth conditions. The optimal temperature for *H. lacustris* growth was found to be between 25 and 28 °C, with a specific growth rate of 0.054 h^{-1}. Furthermore, higher light intensities (e.g., 400 µmol photons $m^{-2} \cdot s^{-1}$) stimulated astaxanthin accumulation. In addition, other researchers also stated that an optimum temperature range of 20 to 30 °C was discovered for the growth of various algae species such as *Chlorella*, *Spirogyra*, and *Chlamydomonas* (Chlorophyta) [47].

4.3. Different Types of Nutrients

Apart from light absorption and CO_2 supply, the growth medium must contain an adequate supply of macro- and micronutrients for microalgal growth. Nutrient concentration and consumption are critical for cell growth and metabolic activity in *H. lacustris*, particularly nitrogen and phosphorus [12]. For example, phosphate plays a critical function in cellular developments such as energy transfer and signal transduction and causes significant degradation of chlorophyll under starvation of phosphorus [18,48]. It was discovered that inorganic nitrogen sources such as sodium nitrate and urea could be used at the optimal concentration. Urea is composed of approximately 46% nitrogen and 20% carbon by elemental composition. Furthermore, both nitrate and ammonium ions are present in the urea-based medium under both light and dark conditions, indicating that the nitrogen source could increase biomass accumulation [1]. When a culture is starved of nutrients, astaxanthin accumulates within the cell [18]. However, using excessive ammonium hydrogen carbonate (NH_4HCO_3) as a nitrogen source in *H. lacustris* cultivation could result in medium acidification and cell death [1].

Furthermore, Li et al. [7] recently studied the impacts of phosphorus deficiency on the structure of non-motile cells in *H. lacustris* and discovered that after 3 days of treatment with 0.1% NaCl in the presence of phosphorus deficiency, more than 80% of motile cells had transformed into non-motile cells. The green phase cultivation was carried out under mixotrophic conditions on EG:JM media with optimal growth conditions, followed by resuspension in medium without nitrate, leading to accumulation of astaxanthin and carotenoids content, which is 2.74% and 78.4%. respectively [24]. Numerous nutrient mediums have been used for the cultivation of microalgae, of which the Bold Basal Medium (BBM) enriched in nitrogen or a modified BBM, the Blue Green medium (BG−11), the National Institute for Environmental Studies medium (NIES), and the Optimal *Haematococcus* Medium (OHM) are currently used to increase biomass productivity of *H. lacustris* [16]. Recently, it was demonstrated in two-step cultivation that BBM had higher biomass productivity than BG−11 during the first vegetative growth stage, whereas the nitrogen-starved BG−11 medium had a higher astaxanthin content [49].

To increase biomass production during the green phase of *H. lacustris* cultivation, carbon dioxide (CO_2) was supplied as a carbon source [50]. Microalgae can primarily capture CO_2 from three sources: (1) CO_2 in the atmosphere; (2) CO_2 in industrial process gas emissions (e.g., flue- and flaring gases); and (3) fixed CO_2 in the form of soluble carbonates (e.g., $NaHCO_3$ and Na_2CO_3) [9]. Furthermore, the hydrodynamic performance of CO_2 from flue gas is critical during *H. lacustris* cultivation in the PBRs system [46]. To maintain agitation in PBR, air and CO_2 were alternately administered. The administrated air and carbon dioxide flow rates were kept constant at 0.5 dm^3 gas per minute [11]. A CO_2 concentration greater than 2.2 mg L^{-1} is required for high biomass productivity [9]. *H. lacustris* cultivation under conditions of high CO_2 (15%) may indeed increase the productivity of astaxanthin [51]. Furthermore, CO_2 is also important in closely monitored culture pH; whenever the culture pH reaches high at 10, the CO_2 bubbling system is able to reduce the culture pH back to 6–7 by supplementing CO_2. Additionally, glucose containing approximately 2.8 KJ mol^{-1} and acetate contains only 0.8 KJ mol^{-1} when used as a carbon source in the cultivation of microalgae, whereas glucose contains more energy than any other carbon source [19,52]. For example, the low-cost carbon source sugarcane molasses can be utilized to grow microalgae [41]. Molasses comprises roughly 50% total sugars, with sucrose making up the vast bulk [53].

4.4. Initial Biomass Density

Additionally, the initial biomass density of the red stage is critical for optimizing astaxanthin productivity [16]. The volume of biomass that was utilized significantly ranged between 0.42 and 0.78 g dm^{-3} [11]. However, when cell density is high in culture and cells have limited access to light, photoinhibition of photosynthesis is disrupted as a result of photooxidative damage to the cells [1]. As a consequence, it is critical to optimize the initial cell density when switching to a new culture system [39]. The cultivation of *H. lacustris* in an outdoor photo-bioreactor with varying initial biomass densities resulted in astaxanthin productivity of 17.1 mg L^{-1} day^{-1} at an initial biomass density of 0.8 g L^{-1} [54]. However, during the winter season, photoinhibition of photosynthesis was observed at low initial biomass densities, such as 0.1 g L^{-1} cultures. Recently, Li et al. [21] reported that the optimal initial biomass density is 0.5, and the maximum astaxanthin content is 38.02 ± 2.40 mg g^{-1}. The cell density should be greater than 10 million cells per milliliter to avoid a long lag period following inoculation, which increases costs and introduces the risk of contamination [55]. When scaling up microalgae growth, inoculation levels of 1–10% of the tank volume used for the next stage are recommended. Scaling up cultivation in stages is necessary, beginning with a small volume of initial production and ending with commercial-scale production [11]. However, Hanan et al. [56] suggested that the inoculum size range is from 5% to 40% of total volume, with 40% being the best inoculum size to maximize culture growth and being directly proportional to the increase in cell density in the culture. Additionally, the influence of the inoculum demonstrates that reusing the

inoculum has no detrimental effect on *H. lacustris* cultivation. Pham et al. [57] have studied the influence of inoculum size on the cultivation of *H. lacustris*, resulting in finding that inoculum size from 3×10^4 cells.ml^{-1} was suitable for the growth. Furthermore, numerous studies have demonstrated the beneficial effect of reusing inoculum [58,59]. Sun et al. [58] reported a replacement inoculum increased biomass and astaxanthin accumulation in batch (1.97 ± 0.08 g/L) and fed batch (89.17 ± 4.07 mg L^{-1}) cultures. Thus, the experiment strategy resulted in a 1.16-fold increase in biomass concentration when *H. lacustris* was cultivated in the green growth phase.

5. Perspective and Future Direction

At the present time, natural astaxanthin contributes a negligible portion of the market, whereas synthetic astaxanthin monopolizes the market. Although many organisms can produce astaxanthin, only a few are widely produced. Based on an initial economic assessment of the cost of producing natural astaxanthin from *Haematococcus*, Li et al. [60] from China predicted that natural astaxanthin can be produced more cheaply than synthetic astaxanthin using currently available technologies, including photo-bioreactors and raceway ponds in low-cost areas. A comprehensive cost analysis of *Haematococcus* astaxanthin and microalgae biomass production shows that the costs can be as low as USD 718/kg and USD 18/kg, respectively. In terms of economics and the environment, using wastewater as an alternative growth medium allows for the recovery of essential nutrients needed for microalgae growth [61] as well as increased biomass and astaxanthin yield. However, high organic matter content may have a negative impact on biomass growth and metabolite accumulation. As a result, pre-treatment of wastewater should be used to address these issues. Consequently, wastewater undergoes phyco-remediation, promoting the circular economy concept and increasing the process's sustainable development [62]. It is also possible to use tropical organic fruit waste medium as a nutrient supplement during *H. lacustris* cultivation instead of an inorganic medium. This has the potential to be a successful cost-cutting strategy. In this context, wastewater and fruit waste, which both contain essential nutrients for microalgae growth, could be used as a substitute culture medium for *H. lacustris*. To meet the current bio-requirement economy's goal of producing "high-value products first," newly integrated processes in biorefinery research can be applied, and they can be found in a variety of industrial sectors, such as for fertilizer commercialization [63].

Furthermore, to our knowledge, there have been limited reports of *Haematococcus* isolation in Malaysia. Malaysia is home to a diverse array of tropical algae that have yet to be fully exploited for commercial purposes. As a result, isolating *Haematococcus* strains to overcome these issues is a critical area of research in the production of natural astaxanthin. The demand for astaxanthin as a functional ingredient will grow at the fastest rate in both the domestic and international markets. Large-scale biomass production requires the processing of a large number of cultures and the use of both indoor and outdoor systems with varying engineering principles and variable ambient conditions. With laboratory results in mind, most large-scale cultivation systems produced lower yields than expected. In terms of salinity and temperature variation, the cultivation of microalgae in closed systems provided a number of advantages over the tanks placed in open system cultivation. In terms of appearance, it enabled cultures to reach higher temperatures (about 4 °C increased), particularly during autumn and winter for indoor treatment. Hence, the closed system produced more biomass, particularly during the winter experiment, when the water temperature was at its lowest. However, no differences were observed in warmer seasons, as clearly demonstrated in the spring and autumn experiments. Similarly, during the rainy season, the closed system avoided a decrease in salinity caused by rainwater input and, as a result, a decrease in productivity caused by cell dilution [64].

New fermentation strategies must be investigated in order to increase the productivity of natural astaxanthin without increasing cultivation costs. The two most important criteria on a laboratory scale are minimizing required time and costs. Large-scale processes require the development of appropriate processes and strategies. Due to the obvious high down-

stream processing cost, novel fermentation strategies (cell recovery, cell disruption, and cell drying) must be investigated. Since astaxanthin is an intracellular product, obtaining it from *H. lacustris* is challenging because microalgae have a thick cell wall; cell disruption and extraction are the most important steps in the overall recovery process. Following harvest from cultivation systems, the current industrial processes for extracting astaxanthin from biomass, such as ultrasonication, solvent, acid, edible oils, and supercritical CO_2 (SC-CO_2) [18,65], have been investigated for industrial microalgae. As a consequence, the other challenge is to encapsulate astaxanthin for the functional ingredients in industry. To date, in order to achieve a good balance of astaxanthin extraction efficiency, environmental compatibility, economics, astaxanthin quality, and safety must be considered. On the other hand, employing these techniques may significantly reduce sample processing time and entail the use of environmentally friendly technology.

6. Conclusions

Globally, natural astaxanthin bioprocessing using *H. lacustris* cultivation is now being explored and appears to be economically viable. High anti-oxidant characteristic makes *H. lacustris* among the most promising microalgae, and it has a huge amount of potential for producing products that have high market value in a sustainable manner, as well. Astaxanthin's limited utilization is hampered by the expensive cost of its manufacture. A better understanding of microalgal physiology and cultivation strategy, as well as process innovation, are all necessary for increasing the biomass and astaxanthin productivity of *H. lacustris*. The production of biomass and astaxanthin is influenced by innovative techniques and an understanding of the interplay between stress factors. Open pond raceway systems and photo-bioreactors, for instance, are utilized in biotechnological manufacturing for large-scale biomass production. Discovering more sustainable and energy-efficient inputs and processes can reduce the environmental impact of this industry.

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References

1. Oslan, S.N.H.; Shoparwe, N.F.; Yusoff, A.H.; Rahim, A.A.; Chang, C.S.; Tan, J.S.; Oslan, S.N.; Arumugam, K.; Ariff, A.B.; Sulaiman, A.Z.; et al. A Review on *Haematococcus pluvialis* Bioprocess Optimization of Green and Red Stage Culture Conditions for the Production of Natural Astaxanthin. *Biomolecules* **2021**, *11*, 256. [CrossRef] [PubMed]
2. Khazi, M.I.; Shi, L.; Liaqat, F.; Yang, Y.; Li, X.; Yang, D.; Li, J. Sequential Continuous Mixotrophic and Phototrophic Cultivation Might Be a Cost-Effective Strategy for Astaxanthin Production from the Microalga *Haematococcus lacustris*. *Front. Bioeng. Biotechnol.* **2021**, *5*, 740533. [CrossRef] [PubMed]
3. Zhang, W.; Zhou, X.; Zhang, Y.; Cheng, P.; Ma, R.; Cheng, W.; Chu, H. Enhancing Astaxanthin Accumulation in *Haematococcus pluvialis* by Coupled Light Intensity and Nitrogen Starvation in Column Photo-bioreactors. *J. Microbiol. Biotechnol.* **2018**, *28*, 2019–2028. [CrossRef] [PubMed]
4. Zhang, C.; Chen, X.; Too, H.P. Microbial astaxanthin biosynthesis: Recent achievements, challenges, and commercialization outlook. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 5725–5737. [CrossRef]
5. Zhuge, F.; Ni, Y.; Wan, C.; Liu, F.; Fu, Z. Anti-diabetic effects of astaxanthin on an STZ-induced diabetic model in rats. *Endocr. J.* **2020**, *68*, 451–459. [CrossRef] [PubMed]
6. Capelli, B.; Talbott, S.; Ding, L. Astaxanthin sources: Suitability for human health and nutrition. *Funct. Foods Health Dis.* **2019**, *9*, 430. [CrossRef]

7. Li, F.; Zhang, N.; Zhang, Y.; Lian, Q.; Qin, C.; Qian, Z.; Wu, Y.; Yang, Z.; Li, C.; Huang, X.; et al. NaCl Promotes the Efficient Formation of *Haematococcus pluvialis* Nonmotile Cells under Phosphorus Deficiency. *Mar. Drugs* **2021**, *19*, 337. [CrossRef]
8. Li, F.; Cai, M.; Lin, M.; Huang, X.; Wang, J.; Zheng, X.; Wu, S.; An, Y. Accumulation of Astaxanthin Was Improved by the Nonmotile Cells of *Haematococcus pluvialis*. *BioMed Res. Int.* **2019**, *2019*, 8101762. [CrossRef]
9. 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]
10. Narala, R.R.; Garg, S.; Sharma, K.K.; Thomas-Hall, S.R.; Deme, M.; Li, Y.; Schenk, P.M. Comparison of Microalgae Cultivation in Photobioreactor, Open Raceway Pond, and a Two-Stage Hybrid System. *Front. Energy Res.* **2016**, *4*, 29. [CrossRef]
11. Borowiak, D.; Pstrowska, K.; Wiśniewski, M.; Grzebyk, M. Propagation of Inoculum for *Haematococcus pluvialis* Microalgae Scale-Up Photobioreactor Cultivation System. *Appl. Sci.* **2020**, *10*, 6283. [CrossRef]
12. Mehariya, S.; Sharma, N.; Iovine, A.; Casella, P.; Marino, T.; Larocca, V.; Molino, A.; Musmarra, D. An Integrated Strategy for Nutraceuticals from *Haematoccus pluvialis*: From Cultivation to Extraction. *Antioxidants* **2020**, *9*, 825. [CrossRef] [PubMed]
13. Ferreira, D.S.V.; Sant'Anna, C. Impact of culture conditions on the chlorophyll content of microalgae for biotechnological applications. *World J. Microbiol. Biotechnol.* **2016**, *33*, 20. [CrossRef] [PubMed]
14. Wen, X.; Wang, Z.; Ding, Y.; Geng, Y.; Li, Y. Enhancing the production of astaxanthin by mixotrophic cultivation of *Haematococcus pluvialis* in open raceway ponds. *Aquacult. Int.* **2020**, *28*, 625–638. [CrossRef]
15. Rodríguez-Sifuentes, L.; Marszalek, J.E.; Hernández-Carbajal, G.; Chuck-Hernández, C. Importance of Downstream Processing of Natural Astaxanthin for Pharmaceutical Application. *Front. Chem. Eng.* **2021**, *2*, 601483. [CrossRef]
16. Jannel, S.; Caro, Y.; Bermudes, M.; Petit, T. Novel Insights into the Biotechnological Production of *Haematococcus pluvialis*-Derived Astaxanthin: Advances and Key Challenges to Allow Its Industrial Use as Novel Food Ingredient. *J. Mar. Sci. Eng.* **2020**, *8*, 789. [CrossRef]
17. Oslan, S.N.H.; Tan, J.S.; Oslan, S.N.; Matanjun, P.; Mokhtar, R.A.M.; Shapawi, R.; Huda, N. *Haematococcus pluvialis* as a Potential Source of Astaxanthin with Diverse Applications in Industrial Sectors: Current Research and Future Directions. *Molecules* **2021**, *26*, 6470. [CrossRef]
18. Shah, M.M.R.; Liang, Y.; Cheng, J.J.; Daroch, M. Astaxanthin-producing green microalga *Haematococcus pluvialis*: From singlecell to high value commercial products. *Front. Plant Sci.* **2016**, *7*, 531. [CrossRef]
19. Bauer, A.; Minceva, M. Examination of Photo-, Mixo-, and Heterotrophic Cultivation Conditions on *Haematococcus pluvialis* Cyst Cell Germination. *Appl. Sci.* **2021**, *11*, 7201. [CrossRef]
20. Xi, T.; Kim, D.G.; Roh, S.W.; Choi, J.-S.; Choi, Y.-E. Enhancement of astaxanthin production using *Haematococcus pluvialis* with novel LED wavelength shift strategy. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 6231–6238. [CrossRef]
21. Li, F.; Cai, M.; Lin, M.; Huang, X.; Wang, J.; Ke, H.; Wang, C.; Zheng, X.; Chen, D.; Yang, S. Enhanced Biomass and Astaxanthin Production of *Haematococcus pluvialis* by a Cell Transformation Strategy with Optimized Initial Biomass Density. *Mar. Drugs* **2020**, *18*, 341. [CrossRef] [PubMed]
22. Hong, M.-E.; Hwang, S.K.; Chang, W.S.; Kim, B.W.; Lee, J.; Sim, S.J. Enhanced autotrophic astaxanthin production from *Haematococcus pluvialis* under high temperature via heat stress-driven Haber–Weiss reaction. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 5203–5215. [CrossRef] [PubMed]
23. Gu, W.; Li, H.; Zhao, P.; Yu, R.; Pan, G.; Gao, S.; Xie, X.; Huang, A.; He, L.; Wang, G. Quantitative proteomic analysis of thylakoid from two microalgae (*Haematococcus pluvialis* and *Dunaliella salina*) reveals two different high light-responsive strategies. *Sci. Rep.* **2014**, *4*, 6661. [CrossRef] [PubMed]
24. Butler, T.O.; McDougall, G.J.; Campbell, R.; Stanley, M.S.; Day, J.G. Media Screening for Obtaining *Haematococcus pluvialis* Red Motile Macrozooids Rich in Astaxanthin and Fatty Acids. *Biology* **2017**, *7*, 2. [CrossRef] [PubMed]
25. Tan, C.H.; Tan, X.; Ho, S.-H.; Lam, S.S.; Show, P.L.; Nguyen, T.H.P. Conceptual design of a hybrid thin layer cascade photobioreactor for microalgal biodiesel synthesis. *Int. J.* **2020**, *44*, 9757–9771.
26. Wan, M.; Zhang, J.; Hou, D.; Fan, J.; Li, Y.; Huang, J.; Wang, J. The effect of temperature on cell growth and astaxanthin accumulation of *Haematococcus pluvialis* during a light–dark cyclic cultivation. *Bioresour. Technol.* **2014**, *167*, 276–283. [CrossRef]
27. Costa, J.A.V.; de Morais, M.G. An open pond system for microalgal cultivation. In *Biofuels from Algae*; Pandey, A., Lee, D.-J., Chisti, Y., Soccol, C.R., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; pp. 1–22.
28. Liyanaarachchi, V.C.; Premaratne, M.; Ariyadasa, T.U.; Nimarshana, P.H.V.; Malik, A. Two-stage cultivation of microalgae for production of high-value compounds and biofuels: A review. *Algal Res.* **2021**, *57*, 102353. [CrossRef]
29. Fasaei, F.; Bitter, J.H.; Slegers, P.M.; van Boxtel, A.J.B. Techno-economic evaluation of microalgae harvesting and dewatering systems. *Algal Res.* **2018**, *31*, 347–362. [CrossRef]
30. Do, T.-T.; Ong, B.-N.; Nguyen Tran, M.-L.; Nguyen, D.; Melkonian, M.; Tran, H.-D. Biomass and Astaxanthin Productivities of *Haematococcus pluvialis* in an Angled Twin-Layer Porous Substrate Photobioreactor: Effect of Inoculum Density and Storage Time. *Biology* **2019**, *8*, 68. [CrossRef] [PubMed]
31. Zhang, B.Y.; Geng, Y.H.; Li, Z.K.; Hu, H.J.; Li, Y.G. Production of astaxanthin from Haematococcus in open pond by two-stage growth one-step process. *Aquaculture* **2009**, *295*, 275–281. [CrossRef]
32. Aflalo, C.; Meshulam, Y.; Zarka, A.; Boussiba, S. On the relative efficiency of two- vs. one-stage production of astaxanthin by the green alga *Haematococcus pluvialis*. *Biotechnol. Bioeng.* **2007**, *98*, 300–305. [CrossRef] [PubMed]

33. Deschênes, J.-S.; Boudreau, A.; Tremblay, R. Mixotrophic production of microalgae in pilot-scale photobioreactors: Practicability and process considerations. *Algal Res.* **2015**, *10*, 80–86. [CrossRef]
34. Hu, J.; Nagarajan, D.; Zhang, Q.; Chang, J.-S.; Lee, D.-J. Heterotrophic cultivation of microalgae for pigment production: A review. *Biotechnol. Adv.* **2018**, *36*, 54–67. [CrossRef] [PubMed]
35. Posten, C. Design principles of photo-bioreactors for cultivation of microalgae. *Eng. Life Sci.* **2009**, *9*, 165–177. [CrossRef]
36. Kiperstok, A.C.; Sebestyén, P.; Podola, B.; Melkonian, M. Biofilm cultivation of *Haematococcus pluvialis* enables a highly productive one-phase process for astaxanthin production using high light intensities. *Algal Res.* **2017**, *21*, 213–222. [CrossRef]
37. Zhang, W.; Wang, J.; Wang, J.; Liu, T. Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. *Bioresour. Technol.* **2014**, *158*, 329–335. [CrossRef]
38. Tran, H.D.; Do, T.T.; Le, T.L.; Tran-Nguyen, M.L.; Pham, C.H.; Melkonian, M. Cultivation of *Haematococcus pluvialis* for astaxanthin production on angled bench-scale and large-scale biofilm-based photo-bioreactors. *Vietnam J. Sci. Technol. Eng.* **2019**, *61*, 60–69. [CrossRef]
39. Do, T.-T.; Ong, B.-N.; Le, T.-L.; Nguyen, T.-C.; Tran-Thi, B.-H.; Thu Hien, B.T.; Melkonian, M.; Tran, H.-D. Growth of *Haematococcus pluvialis* on a Small-Scale Angled Porous Substrate Photobioreactor for Green Stage Biomass. *Appl. Sci.* **2021**, *11*, 1788. [CrossRef]
40. Wan, M.; Zhang, Z.; Wang, J.; Huang, J.; Fan, J.; Yu, A.; Wang, W.; Li, Y. Sequential Heterotrophy–Dilution–Photoinduction Cultivation of *Haematococcus pluvialis* for efficient production of astaxanthin. *Bioresour. Technol.* **2015**, *198*, 557–563. [CrossRef]
41. Sipaúba-Tavaresa, L.H.; Tedesquea, M.G.; Scardoeli-Truzzi, B. Evaluation of the effects of sugarcane molasses as a carbon source for Ankistrodesmus gracilis and *Haematococcus pluvialis* (Chlorophyceae). *Braz. J. Biol.* **2020**, *80*, 594–600. [CrossRef]
42. Park, J.C.; Choi, S.P.; Hong, M.E.; Sim, S.J. Enhanced astaxanthin production from microalga, *Haematococcus pluvialis* by two-stage perfusion culture with stepwise light irradiation. *Bioprocess Biosyst. Eng.* **2014**, *37*, 2039–2047. [CrossRef] [PubMed]
43. Wang, H.; Zhou, W.; Shao, H.; Liu, T. A comparative analysis of biomass and lipid content in five Tribonema sp. strains at autotrophic, heterotrophic and mixotrophic cultivation. *Algal Res.* **2017**, *24*, 284–289. [CrossRef]
44. Wang, S.K.; Wang, X.; Tao, H.H.; Sun, X.S.; Tian, Y.T. Heterotrophic culture of Chlorella pyrenoidosa using sucrose as the sole carbon source by co-culture with immobilized yeast. *Bioresour. Technol.* **2018**, *249*, 425–430. [CrossRef]
45. Saha, S.K.; McHugh, E.; Hayes, J.; Moane, S.; Walsh, D.; Murray, P. Effect of various stress-regulatory factors on biomass and lipid production in microalga *Haematococcus pluvialis*. *Bioresour. Technol.* **2013**, *128*, 118–124. [CrossRef] [PubMed]
46. Choi, Y.Y.; Hong, M.E.; Jin, E.S.; Woo, H.M.; Sim, S.J. Improvement in modular scalability of polymeric thin-film photo-bioreactor for autotrophic culturing of *Haematococcus pluvialis* using industrial flue gas. *Bioresour. Technol.* **2018**, *249*, 519–526. [CrossRef]
47. Singh, S.P.; Singh, P. Effect of temperature and light on the growth of algae species: A review. *Renew. Sust. Energy Rev.* **2015**, *50*, 431–444. [CrossRef]
48. Choi, Y.Y.; Joun, J.M.; Lee, J.; Hong, M.E.; Pham, H.-M.; Chang, W.S.; Sim, S.J. Development of large-scale and economic pH control system for outdoor cultivation of microalgae *Haematococcus pluvialis* using industrial flue gas. *Bioresour. Technol.* **2017**, *244*, 1235–1244. [CrossRef]
49. Zhao, Y.; Yue, C.; Geng, S.; Ning, D.; Ma, T.; Yu, X. Role of media composition in biomass and astaxanthin production of *Haematococcus pluvialis* under two-stage cultivation. *Bioprocess Biosyst. Eng.* **2019**, *42*, 593–602. [CrossRef]
50. Cheng, J.; Li, K.; Yang, Z.; Lu, H.; Zhou, J.; Cen, K. Gradient domestication of *Haematococcus pluvialis* mutant with 15% CO_2 to promote biomass growth and astaxanthin yield. *Bioresour. Technol.* **2016**, *216*, 340–344. [CrossRef]
51. Cheng, J.; Li, K.; Zhu, Y.; Yang, W.; Zhou, J.; Cen, K. Transcriptome sequencing and metabolic pathways of astaxanthin accumulated in *Haematococcus pluvialis* mutant under 15% CO_2. *Bioresour. Technol.* **2017**, *228*, 99–105. [CrossRef]
52. Cheah, W.Y.; Show, P.L.; Juan, J.C.; Chang, J.S.; Ling, T.C. Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation. *Energy Convers. Manag.* **2018**, *164*, 188–197. [CrossRef]
53. Piasecka, A.; Krzeminska, I.; Tys, J. Enrichment of *Parachlorella kessleri* biomass with bio-products: Oil and protein by utilization of beet molasses. *J. Appl. Phycol.* **2017**, *29*, 1735–1743. [CrossRef] [PubMed]
54. Wang, J.; Han, D.; Sommerfeld, M.R.; Lu, C.; Hu, Q. Effect of initial biomass density on growth and astaxanthin production of *Haematococcus pluvialis* in an outdoor photo-bioreactor. *J. Appl. Phycol.* **2013**, *25*, 253–260. [CrossRef]
55. Das, D. (Ed.) *Algal Biorefinery: An Integrated Approach*; Springer: Berlin, Germany, 2015.
56. Hanan, N.A.; Al-Shorgani, N.K.; Shukor, H.; Abd. Rahman, N.; Kalil, M.S. Pre-Optimization Conditions for *Haematococcus pluvialis* Growth. *Int. J. Adv. Sci. Eng. Inf. Technol.* **2013**, *3*, 70–73. [CrossRef]
57. Pham, K.T.; Nguyen, T.C.; Luong, T.H.; Dang, P.H.; Vu, D.C.; Do, T.N.; Phi, T.C.M.; Nguyen, D.B. Influence of inoculum size, CO_2 concentration and LEDs on the growth of green microalgae *Haematococcus pluvialis* Flotow. *Vietnam J. Sci. Technol. Eng.* **2018**, *60*, 59–65. [CrossRef]
58. Sun, H.; Liu, B.; Lu, X.; Cheng, K.W.; Chen, F. Staged cultivation enhances biomass accumulation in the green growth phase of *Haematococcus pluvialis*. *Bioresour. Technol.* **2017**, *233*, 326–331. [CrossRef]
59. Witono, J.R.; Gunadi, A.; Santoso, H.; Miryanti, A.; Kumalaputri, A.J. The optimal condition on the growth of green *Haematococcus pluvialis* as one of the future natural resources. In Proceedings of the 26th Regional Symposium on Chemical Engineering (RSCE 2019), Kuala Lumpur, Malaysia, 30–31 October 2019.
60. Li, J.; Zhu, D.; Niu, J.; Shen, S.; Wang, G. An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. *Biotechnol. Adv.* **2011**, *29*, 568–574. [CrossRef]

61. Chandra, R.; Iqbal, H.M.N.; Vishal, G.; Hyung-Sool, L.; Sunil, N. Algal Biorefinery: A Sustainable Approach to Valorize Algal-based Biomass towards Multiple Product Recovery. *Bioresour. Technol.* **2019**, *278*, 346–359. [CrossRef]
62. Nishshanka, G.K.S.H.; Liyanaarachchi, V.C.; Premaratne, M.; Nimarshana, P.H.V.; Ariyadasa, T.U.; Kornaros, M. Wastewater-based microalgal biorefineries for the production of astaxanthin and co-products: Current status, challenges and future perspectives. *Bioresour Technol.* **2021**, *342*, 126018. [CrossRef]
63. Chew, K.W.; Yap, J.Y.; Show, P.L.; Suan, N.H.; Juan, J.C.; Ling, T.C.; Lee, D.; Chang, J. Microalgae biorefinery: High value products perspectives. *Bioresour. Technol.* **2017**, *229*, 53–62. [CrossRef]
64. Fabio, R.; Paula, M.; Tatiana, M.; Paulo, C.A. Comparison of open-air and semi-enclosed cultivation system for massive microalgae production in sub-tropical and temperate latitudes. *Biomass Bioenergy* **2013**, *59*, 418–424.
65. Gulzar, S.; Benjakul, S. Impact of pre-treatment and atmosphere on quality of lipids extracted from cephalothorax of Pacific white shrimp by ultrasonic assisted process. *Food Chem.* **2019**, *309*, 125732. [CrossRef] [PubMed]