Review

Harnessing Stress: Conventional and Unconventional Strategies for Enhancing Microalgal Productivity in Sustainable Biorefineries

Chia-En Chuang [1,†], Yu-Han Chien [1,†], Sheng-Yung Lin [1,†], Adi Kusmayadi [2], Jia-Hui Zhou [1], Chiao Ching Chang [1], Chen Rui Zhang [1] and Yoong Kit Leong [1,3,*]

[1] Department of Chemical and Materials Engineering, Tunghai University, Taichung 407, Taiwan
[2] Department of Mechanical Engineering, Politeknik Negeri Indramayu, Indramayu 45252, Indonesia
[3] Research Center for Smart Sustainable Circular Economy, Tunghai University, Taichung 407, Taiwan
* Correspondence: yoongkit1014@thu.edu.tw
† These authors contributed equally to this work.
Received: 18 January 2025; Revised: 2 March 2025; Accepted: 6 March 2025; Published: 2 April 2025

Abstract: Microalgae are versatile platforms for producing biofuels and high-value metabolites, such as lipids, proteins, and carotenoids. Numerous stress strategies have been adopted to improve microalgal cultivation and biomolecule yield. This review examines how conventional stress factors (light and salinity) and unconventional treatments (electric field treatment) influence microalgal growth and metabolite accumulation. Light intensity, spectrum, and photoperiod significantly affect photosynthesis, biomass yield, and carotenoid biosynthesis, with moderate intensities found to enhance efficiency. However, excessive levels may induce photoinhibition. Salinity stress induces activation of antioxidant systems and lipid accumulation, optimizes biofuel properties. However, excessive high salinity can impair the growth of microalgae. In this review, we focused on the electric field treatment as a potential strategy for enhancing microalgal productivity, representing a major novelty of the study. Unlike traditional stress factors that primarily induce adaptive metabolic shifts, electric field treatment offers a unique and an understudied approach for modulating cellular physiology. Electric treatment technology offers an energy-efficient method for stimulating cell differentiation and enhancing lipid and pigment production while reducing environmental effects. Integrating these stress factors may be an attractive approach for controlling over microalgal metabolism, supporting sustainable and scalable biorefinery applications.

Keywords: light modulation; salinity stress; pulsed electric field; sustainable biofuel; stress-induced pathways; metabolic engineering

1. Introduction

Microalgae are crucial to the Earth's ecosystem, not only serving as oxygen producers, but also major carbon cycling regulators, contributing global climate regulation [1]. As primary producers, microalgae form the foundation of marine and freshwater food chains, providing organic matter essential for other aquatic organisms. Their ability to fix carbon dioxide makes them a potential tool for mitigating climate change. As the global demand for carbon reduction and carbon capture technologies grows, the unique characteristics of microalgae are increasingly studied in environmental science and sustainable development fields.

With the advancement of microalgae biotechnology and the development of large-scale production techniques, the applications of microalgae in energy, environmental protection, pharmaceuticals, and cosmetics continue to grow, making significant contributions to the sustainable development of green economy [2,3]. Compared to traditional crops, microalgae do not require large amounts of land and freshwater, and can be cultivated in closed or non-traditional environments. This makes them ideal sources of sustainable energy. Microalgae can produce valuable biomass such as lipids, proteins, and carbohydrates, which can be used to produce biofuels like biodiesel and bioethanol [4,5]. Furthermore, the bio-oil produced by microalgae is a major ingredient to the future development of renewable energy, capable of meeting energy demands without relying on traditional agricultural land. This efficient biofuel production method makes microalgae an important alterative approach for addressing the global energy shortage [6].

However, the growth of microalgae in extreme environments, particularly under conditions of high salinity, nutrient deficiencies, temperature fluctuations, and pH changes, is limited [7]. These factors can affect their growth and biomass production. Nevertheless, microalgae possess strong adaptive capabilities, enabling them to survive and continue growing under adverse conditions. Microalgae accumulate various energy reserves, such as lipids and starch, which help them maintain their growth under unfavorable environments. In nutrient-deficient or high-salinity environments, microalgae adapt to changes in external conditions by regulating their internal osmotic pressure, and they can convert some of their photosynthetic products into higher-energy substances, such as stored lipids. This is crucial for microalgae growth and biofuel production. Based on these adaptive abilities, microalgae have broad potential applications in environmental protection.

Although extensive research has been conducted on conventional stress factors such as light intensity and salinity, the impact of unconventional stressors like electric field treatment on microalgae metabolism has not been sufficiently investigated. This review provides a comprehensive analysis of conventional and unconventional stress conditions, emphasizing their influence on microalgae growth, biomass composition, and metabolite accumulation.

2. Effect of Light Stress on Microalgal Growth and Metabolites Accumulation

Light is a fundamental driver of microalgal growth and metabolism, influencing photosynthesis, pigment composition, biomass yield, and the accumulation of high-value compounds such as carotenoids and lipids. Numerous studies have demonstrated the significance of fine-tuning light intensity, quality (spectrum), and photoperiod in optimizing the growth and biochemical composition in diverse microalgal species, as shown in Figure 1 [8]. Therefore, understanding how different light regimes affect microalgal physiology is critical for improving commercial cultivation strategies, such as biofuels, nutraceuticals, cosmetics, or other valuable bioproducts.

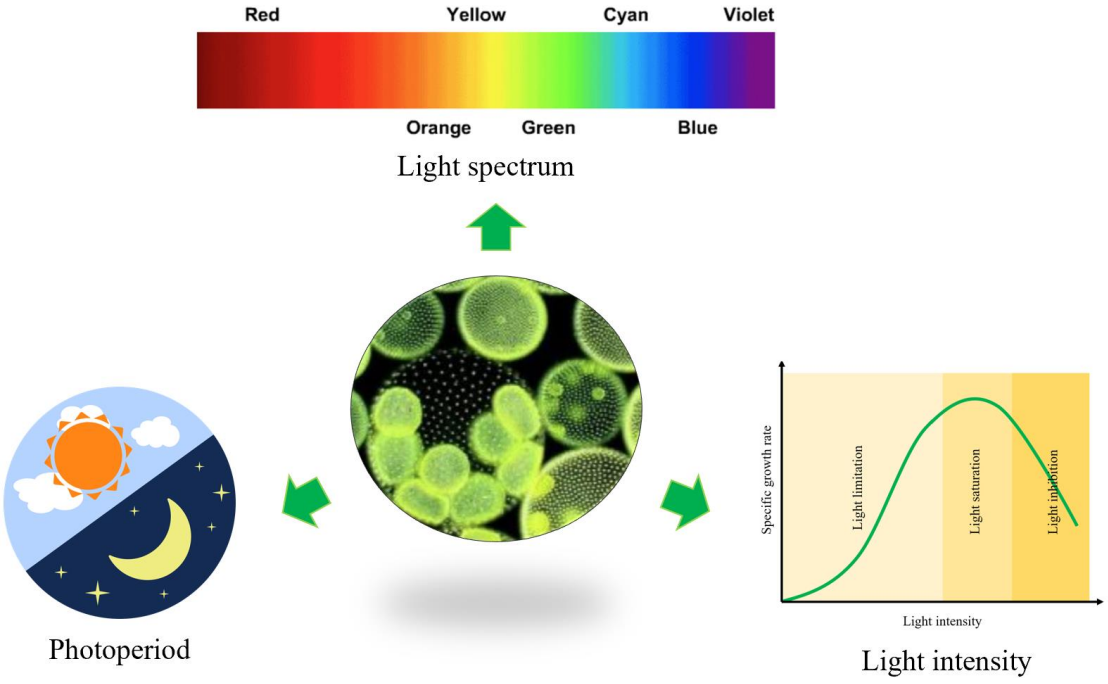

Figure 1. Light factors affecting microalgal growth and metabolite accumulation.

2.1. Influence of Light Stress on Microalgal Growth and Pigment Composition

Microalgae are phototrophic organisms that depend on photosynthetically active radiation (PAR, 400–700 nm) to drive photosynthesis. PAR can be expressed in terms of photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}), which represents the number of photons available for photosynthesis, or as photosynthetic radiant flux density (PAR irradiance, W m^{-2}), which can be more useful when considering energy balances [9,10]. The amount of PAR that reaches the microalgal cells is influenced by multiple factors: the type of light source, the configuration and material of the cultivation vessel, the position of the cultures relative to the light source, and the optical properties of both the growth environment and the cells themselves. In dense cultures, mutual shading reduces the effective amount of light that reaches each cell, influencing pigment accumulation and overall productivity [11].

In photosynthetic organisms like green algae, light-harvesting complexes (LHCs) contain antenna pigments including chlorophyll a, chlorophyll b, and carotenoids. These pigments funnel absorbed light energy to the reaction centers of photosystems I and II, ensuring efficient utilization of available photons. Carotenoids, such as lutein and β-carotene, play critical roles in both light capture (particularly absorption in the blue light region) and photoprotection. They shield the photosynthetic apparatus from oxidative damage induced by high-intensity light, mitigating the formation of reactive oxygen species (ROS) [12,13].

2.2. Photoprotective Mechanisms against Light Stress in Microalgae

The xanthophyll cycle is a critical photoprotective mechanism that modulates carotenoid composition in response to changing light conditions. Under high light intensity condition, violaxanthin can be de-epoxidized to zeaxanthin, which helps dissipate excess energy as heat, reducing photodamage. Enzyme regulation within the cycle (e.g., violaxanthin de-epoxidase (VDE) and zeaxanthin epoxidase (ZEP)) is tightly controlled by light conditions [14,15].

In diatoms like *Phaeodactylum tricornutum*, multiple isoforms of ZEP and VDE are involved. High light intensity (700 μmol photons m^{-2} s^{-1}) early in the growth phase can upregulate VDE, VDL1, and ZEP3 while downregulating ZEP1, leading to the activation of both the diadinoxanthin and violaxanthin cycles and reducing fucoxanthin accumulation—a photoprotective adaptation to intense irradiance [15].

The regulatory effects of light intensity on carotenoid enzymes have been studied extensively in several model microalgal species. In *Haematococcus pluvialis*, high light intensity (150 μmol photons m^{-2} s^{-1}) induces the expression of ipiHp2, which encodes isopentenyl diphosphate isomerase (IDI)—an enzyme that shifts metabolism toward enhanced carotenoid accumulation [16]. Similarly, enzymes like phytoene synthase and phytoene desaturase are upregulated with a maximum increase of 4 to 5 times under high light intensity (200 μmol photons m^{-2} s^{-1}) in *Chlamydomonas reinhardtii*, leading to increased carotenoid content up to 1.3- to 1.5-fold [17]. In *Chromochloris zofingiensis*, elevated light intensity (400 μmol photons m^{-2} s^{-1}) upregulates lycopene beta cyclase (LCYB) while downregulating lycopene epsilon cyclase (LCYE), shifting carotenoid synthesis toward β-carotene at the expense of lutein.

This reprogramming under high light intensity is a photoprotective response aimed at mitigating oxidative stress. By enhancing carotenoid production, cells protect their photosystems against ROS. However, the trade-off is that very high light intensities may reduce overall growth [18]. Thus, commercial cultivation strategies must balance the desire for higher carotenoid yields while maintaining sufficient biomass production. Optimal conditions often involve moderately high but non-inhibitory light intensities.

In *Chromochloris zofingiensis*, increased β-carotene and astaxanthin production under high light intensity (400 μmol photons m^{-2} s^{-1}) is accompanied by alterations in xanthophyll cycle components. β-carotene hydroxylase (BCH) and β-carotene ketolase (BKT) enzymes are upregulated, promoting astaxanthin synthesis. However, the limited zeaxanthin availability caused by increased BKT activity modifies the violaxanthin cycle, upregulating VDE and downregulating ZEP and violaxanthin de-epoxidase–like (VDL), which promotes zeaxanthin conversion into astaxanthin and decreasing violaxanthin and neoxanthin levels [19].

Although not the primary focus here, light interacts with other environmental variables, such as nutrient availability (especially nitrogen), carbon sources, and mixing regimes. Some studies have reported that under mixotrophic conditions—where microalgae can utilize both inorganic (light-driven) and organic carbon sources—moderate increases in light intensity can improve CO_2 fixation. Simultaneously, the TCA cycle might be inhibited to optimize carbon metabolism, ensuring that photosynthesis and heterotrophy complement each other. Such synergy results in a high organic carbon utilization capacity and improved pollutant removal in wastewater treatments. Appropriate light intensities enhance the contribution of photosynthesis to growth and pollutant removal in these systems, underscoring the need for adoption of tailored lighting regimes in environmental biotechnology applications.

2.3. Effect of Light Intensity

Light intensity is a central parameter affecting microalgal growth, photosynthetic capacity, and the regulation of enzymes involved in carotenoid biosynthesis. Moderate increases in light intensity generally enhance photosynthetic efficiency and promote higher growth rates until a species-specific saturation point is reached [20]. Beyond this saturation point, further increases in light intensity can lead to photoinhibition, reducing photosynthetic efficiency, and ultimately lowering biomass yields [18]. Tables 1 and 2 displayed the effect of light quality and photoperiod on lipid and pigment accumulation by microalgae.

Table 1. Effect of light quality and photoperiod on lipid accumulation in microalgae.

Algal Species	Light Color	Light Intensity	Light Duration (Light:Dark)	Biomass Concentration (g/L)	Lipid Production	References
Amphiprora sp.	white	24 μmol/m^2/s	16:8	0.26	135.60 mg/L	[21]
Chlorella pyrenoidosa	white	4000 lux	19:5	0.61	170.00 mg/L	[22]
Chlorella sorokiniana C16	white	10,000 lux	24:0	5.20	27.0 wt%	[23]
Chlorella sorokiniana CY-1	white	8000 lux	24:0	2.12	11.21 wt%	[24]
Chlorella vulgaris	red	-	12:12	3.53	25.50 wt%	[25]
Chlorella vulgaris	-	-	-	3.46	55.2 wt%	[26]

Table 2. Effect of light quality and photoperiod on pigment accumulation in microalgae.

Pigment	Algal Species	Light Color	Light Intensity (μmol/m^2/s)	Light/Dark Period	Biomass Concentration (g/L)	Pigment Production	References
Astaxanthin	*Aurantiochytrium* sp. CJ6	White	250	Continuous (4 days)	9.01	78.3 μg/g	[27]
Fucoxanthin	*Chromochloris zofingiensis*	Blue	—	—	204.5	0.28 g/L	[28]
	Haematococcus pluvialis	White	480	Continuous	0.43	26.77 mg/L/d	[29]
	Haematococcus sp.	Red (5 days) followed by Blue (5 days)	40	Continuous	1.33	3.39 mg/L	[30]
	Oedocladium carolinianum	Red	—	Continuous	0.29	2.89 mg/L/d	[31]
	Chaetoceros calcitrans	Blue	110	12:12	—	3.2 mg/L/d	[32]
	Isochrysis sp.	—	200	12:12	—	6.11 mg/g	[33]
	Odontella aurita	Red/blue light (8:2 ratio)	300	Continuous	0.57	9.41 mg/L/d	[34]
	Pavlova sp.	White	75	Continuous	1.1	7.02 mg/L/d	[35]
	Phaeodactylum tricornutum	White	8.0	Continuous	6.0	26 mg/g	[36]
Lutein	*Chlorella sorokiniana* C16	White	10k lux	Continuous	5.2	17.4 mg/g	[23]
	C. sorokiniana F31	—	211	Continuous	—	15.55 mg/g	[37]
	C. sorokiniana FACHB-275	White	2200 Lux	Continuous	1.14	8.45 mg/g	[38]
	C. sorokiniana MB-1-M12	White	150	Continuous	3.54 (Auto), 2.77 (Mixo)	6.16 mg/g, 4.10 mg/L/d (Auto); 6.48 mg/g, 4.50 mg/L/d (Mixo)	[39]
	Chlorella sp. HS5	Dark	Dark conditions	Continuous	—	3.7 mg/g	[40]
	Chlorophyta MCH-35	Blue	80	Continuous	—	3 mg/g	[41]
	Coccomyxa subellipsoidea	White	240	Continuous	9.40	1.65 mg/g	[42]
	Scenedesmus sp. FSP3	White	1st stage—90, 2nd stage—160	Continuous	1.97	6.34 mg/g, 2.30 mg/L/d	[43]

Bialevich et al. showed that microalgae can increase their growth rates with rising light intensity until saturation. *Desmodesmus quadricauda* and *Parachlorella kessleri* reached saturation at around 250 μmol photons m^{-2} s^{-1}, while *Chlamydomonas reinhardtii* tolerated up to 500 μmol photons m^{-2} s^{-1} [44]. Similarly, Difusa et al. and others found that, although elevated light intensity can enhance lipid content in certain strains (e.g., *Scenedesmus* sp.), it may lower growth rates, suggesting the important of balancing between rapid biomass accumulation and formation of storage compounds like lipids [45].

Gim et al. explored the effects of light intensity (0–200 μmol photons m^{-2} s^{-1}) on three microalgae species—*Isochrysis galbana*, *Nannochloropsis oculata*, and *Dunaliella salina*. At 150 μmol photons m^{-2} s^{-1}, they observed peak fatty acid concentrations [46]. Specifically, *Isochrysis galbana* produced a biomass of 0.89 g/L with a lipid

content of 30.1% (dry weight), *Nannochloropsis oculata* achieved a biomass of 1.69 g/L with a lipid content of 38.5%, and *Dunaliella salina* reached a biomass of 1.17 g/L with a lipid content of 32.4%. They also found that excessive light induced oxidative damage, while low light limited photosynthetic efficiency. Moderate light intensities (80–150 μmol photons m^{-2} s^{-1}) offered the best condition for lipid accumulation, providing a balanced growth environment.

Cheirsilp & Torpee studied marine *Chlorella* sp. and *Nannochloropsis* sp. under mixotrophic conditions and varying light intensities (ranging from 2000 to 10,000 lux, approximately 30–150 μmol photons m^{-2} s^{-1} depending on conversion) [47]. Notably, the biomass production increased as the light intensity was elevated up to a certain point but diminished lipid accumulation. For *Chlorella* sp., the maximum biomass was achieved at 8000 lux, with a dry weight of 3.97 g/L, while its lipid content reached 397.8 mg/L under the same conditions. In contrast, *Nannochloropsis* sp. continued to grow until 10,000 lux, reaching a peak biomass of 5.87 g/L and a lipid yield of 481.0 mg/L. However, at higher light intensities, lipid accumulation in both species decreased, suggesting a trade-off between growth and lipid production.

Under low light, photosynthetic efficiency and growth rates are generally reduced, limiting the large-scale cultivation. However, phytohormones like melatonin (MT) and indole-3-propionic acid (IPA) can redirect carbon flux toward carbohydrates and proteins, enhancing yields even under suboptimal light (as described in the CAMC systems discussion). Although the details are not well understood, this suggests that biochemical interventions or combined strategies may compensate for low-light conditions [48].

2.4. Effect of Light Quality

Besides intensity, light quality (spectral composition) also significantly affects microalgal photosynthesis and metabolism. Light-emitting diode (LED) technology has advanced rapidly, offering precise control over the wavelength distribution. Different spectral regions (e.g., blue: 450–475 nm; red: 630–660 nm) influence photosynthetic pigments and can shift metabolic pathways [49].

Kim et al. demonstrated that combining blue and red LED lights increased the production rate of *Scenedesmus* sp. by ~50% compared to a single-wavelength treatment [20]. The improved photosynthetic efficiency under mixed blue-red illumination was due to the complementarity of absorption peaks of chlorophylls and carotenoids. Blue light is absorbed efficiently by carotenoids and can enhance carotenoid synthesis, while red light tends to stimulate growth and enhance photosynthetic reaction center efficiency.

Similarly, You & Barnett observed that blending blue and red light enhanced the growth rate of *Porphyridium cruentum* by enhancing photosynthetic activity and reached its maximum cell density of 4×10^9 cells L^{-1} [50]. Studies on *C. vulgaris* have also indicated that red light alone or combined with other wavelengths can boost biomass accumulation with biomass increased from 2.07 to 2.64 g/mL [51].

2.5. Effect of Photoperiod and Temporal Light Modulation

The duration of the light period and the ratio of light-to-dark cycles (photoperiod) influence cellular metabolism, growth cycles, and resource allocation. Maltsev et al. found that a 16:8 h light/dark cycle efficiently balanced biomass production, increasing it by 22%, and ellicited a 19% increase in total fatty acid (TFA) content [8]. Light/dark cycles allow microalgae to repair photosynthetic machinery and balance their energy and carbon budgets. On the other hand, Vélez-Landa et al. demonstrated that a balanced light/dark cycle (12:12) achieved the highest biomass density of 6.3×10^6 cells/mL and lipid content of 50.42% in *Verrucodesmus verrucosus* [52]. The study found that continuous light (24:0) led to diminished lipid yields, while shorter dark periods (16:8) resulted in lower growth and lipid accumulation. Therefore, these indicated that some species grow better under continuous light. In contrast, others benefit from dark periods that support respiration, intracellular reorganization, and nighttime metabolic pathways such as carbohydrate catabolism or lipid remodeling.

Flashing light regimes or combined continuous-plus-flashing conditions have proven beneficial in some cases [51]. These light strategies can improve photon utilization efficiency by providing saturating light pulses interspersed with “recovery” intervals. The result can be more efficient use of photons and enhanced growth compared to continuous, non-modulated illumination.

Abu-Ghosh et al. investigated the combination of flashing light with continuous background light in *Dunaliella salina* [51]. The combined light regime significantly enhanced photosynthetic efficiency and growth beyond continuous or flashing light alone. At an intermediate light intensity of 250 μmol photons m^{-2} s^{-1}, the combined regime resulted in an optical density (OD) of 1.4, compared to 0.9 for continuous light and 1.1 for flashing light. Similarly, the dry biomass reached 2.52 g/L under the combined regime, surpassing the 1.87 g/L and 2.01 g/L observed under continuous and flashing light, respectively. Photosynthetic activity, measured as

oxygen production rates, was also significantly higher at 148.9 μmol O_2 (mmol Chl)$^{-1}$ s^{-1}, compared to 31.6 and 47.3 under continuous and flashing light alone.

Under optimal conditions, irradiating *C. vulgaris* with red light and combining this approach with indole-3-acetic acid (IAA) treatment can further enhance biomass production. Studies showed that red light stimulated photosynthetic capacity, respiration, and quantum yield, leading to higher biomass levels (3.19 g L^{-1}) compared to white light conditions (2.78 g L^{-1}) [25]. Combining IAA and red light increased biomass production to 3.53 g L^{-1}, translating to a 27% increase in productivity.

2.6. Commercial and Biotechnological Implications

Optimizing light conditions, intensity, spectrum, and photoperiod, may improve the large-scale cultivation of microalgae at industrial scales. Target compounds, such as lipids for biofuels or high-value pigments like astaxanthin and β-carotene, reach maximum accumulation under stress conditions associated with altered light regimes. For instance, high light stress can trigger carotenoid accumulation as a photoprotective mechanism, providing a valuable product. However, this often comes at the cost of slower growth rates or photoinhibition, which can reduce total volumetric yields [47].

To overcome the above limitations, dynamic lighting strategies and engineering approaches have been proposed. Photobioreactors can be designed to distribute light uniformly and minimize self-shading, or they can use artificial illumination (LEDs) to deliver customized light wavelengths and intensities at different growth stages. Pulsing or flashing lights and meticulously selected photoperiods can further enhance efficiency [46].

An accurate assessment of the available light for microalgae is often challenging. The quantity of PAR available to cells depends on the arrangement and properties of the cultivation system, whether it be photobioreactors, flasks, or open ponds. Mutual shading by cells in dense cultures, light scattering by flask walls, and reflection from surfaces are major factors limiting the measurement. In practice, PPFD is frequently measured outside the culture vessel as a proxy, but this can lead to discrepancies between measured and actual photon availability to the cells [9,10]. Such measurement challenges underscore the need for careful system design and calibration. In particularly, for large-scale applications, ensuring uniform light distribution and avoiding excessive shading are paramount for maximizing productivity.

Commercial microalgal production aims to maximize valuable compounds, such as carotenoids, lipids, and other bioproducts, while maintaining their growth rates. High light intensities and specific spectral qualities can increase carotenoid accumulation, but might simultaneously lower biomass yield due to photoinhibition. Achieving an optimal compromise is critical. Conditions that yield the highest pigment concentrations may not support rapid cell growth, reducing overall productivity on a volumetric basis [18].

One strategy is to modulate light intensities throughout cultivation. Early growth phases might benefit from moderate light to support robust biomass production, while later stages could involve a shift to higher light intensities or different wavelengths to boost carotenoid or lipid synthesis. Such dynamic lighting strategies leverage the physiological plasticity of microalgae [47].

Beyond physical and environmental controls, biochemical strategies can complement light optimization, such as adding phytohormones or manipulating nutrient levels. When tuned appropriately, red and blue light combinations can substantially boost biomass and target compound accumulation. Similarly, altering the nitrogen supply (reducing it at a certain growth phase) while providing optimal light intensities and qualities can force cells into a desired metabolic state, potentially increasing lipid or carotenoid yield without excessively compromising growth.

As the microalgae industry continues to develop, optimizing light conditions will enhance the degree of biomass production and accumulation of high-value compounds. Future research should aim to develop approaches for enhancing light regulation, adjusting light intensity, spectrum combinations, and photoperiods to achieve precise control of microalgal metabolic pathways. For example, moderate light intensities can be used in the early growth phase to promote biomass production, while higher light intensities or spectral shifts in later stages can induce carotenoid or lipid accumulation. Additionally, advancements in LED technology can generate new approaches of spectral control. The combination of blue and red light has been proven to enhance photosynthetic efficiency and product yield, and further refinement of spectral combinations could maximize productivity and energy efficiency.

Furthermore, the interaction between light and other environmental factors, such as nutrient availability and mixing conditions, need to be further investigated to optimize cultivation strategies for various applications. In wastewater treatment, for instance, tailored light conditions can improve microalgal carbon fixation and pollutant removal efficiency. Moreover, integrating biotechnological approaches, such as exogenous phytohormone

regulation or genetic modifications, may be effective in enhancing microalgal adaptability to light conditions and boost the production of target compounds. By combining these strategies, the commercialization of microalgae industry will be significantly improved and expanded to include diverse fields such as biofuels, nutritional supplements, and environmental remediation.

3. Effect of Salt Stress on Microalgal Growth and Metabolite Accumulation

3.1. Effect of Salinity on Biomass Production and Protein Accumulation

Literatures showed that moderate NaCl concentrations support photosynthesis, CO_2 fixation, and osmotic balance [53]. For examples, NaCl concentrations of 5–25 mM promoted growth of *Quadrigula closterioides*, with the highest lipid content of 50.94% dry weight recorded at 5 mM NaCl. However, as salinity exceeds 25 mM, growth and biomass production are inhibited, accompanied by morphological changes such as a shift from normal cell shape to spherical and even brown coloration, indicating an adaptive response to salt stress [54]. Similarly, moderate salinity levels (+100% to +400% NaCl) also enhance microalgal growth and biomass accumulation of *Scenedesmus obliquus*, with the highest biomass productivity of 0.206 g/L/d observed at +400% NaCl [55]. However, further increases in salinity to +600% and +800% NaCl resulted in significant biomass reductions of 32.2% and 50.3%, respectively, due to ROS accumulation and photosynthesis inhibition. Figure 2 depicts the effect of salt stress on microalgal growth and metabolite accumulation.

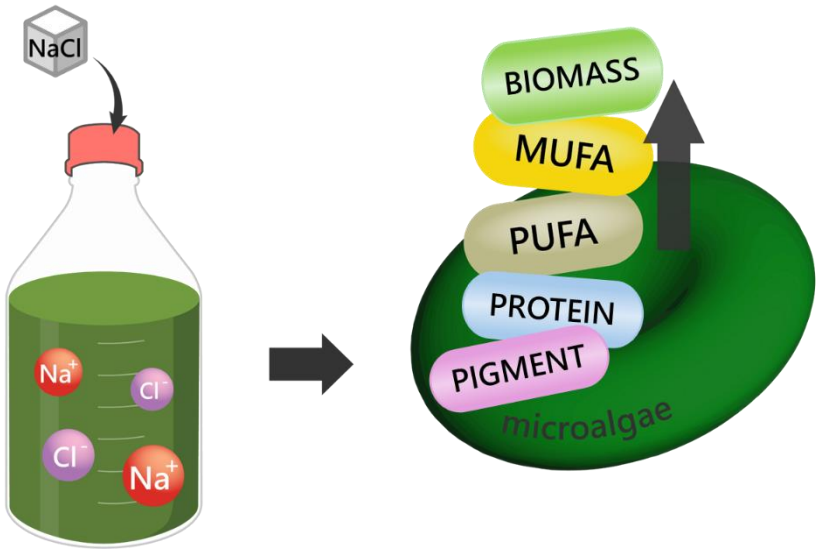

Figure 2. Effect of salt stress on microalgal growth and metabolite accumulation.

There are limited studies of salinity effect on protein accumulation. The *Vischeria punctata* strain IPPAS H-242 exhibited higher protein content of 259 mg/g DW under salt stress of 0.4 mM NaCl, which could be due to the need for cells to synthesize more proteins to cope with environmental stress [56]. The increased protein content may help maintain cellular functions and survival, particularly in response to oxidative stress and changes in osmotic pressure caused by salinity. Despite the overall increase in protein content, the level of D1 protein, which is associated with photosynthesis, significantly decreased under salt stress, suggesting that salt stress may impact the effectiveness of photosynthesis. The increase in total protein content under salt stress may be an adaptive mechanism for cells to withstand environmental pressures, but it also negatively affects certain key photosynthetic proteins. These changes are significant for understanding the physiological responses of microalgae under stress and their potential biotechnological applications.

3.2. Effect of Salinity on Lipid Accumulation

NaCl concentrations can significantly influence microalgal lipid content and quality, fatty acid composition, and eventually the fuel performance of biodiesel. Salinity stress can induce the generation of ROS, activates antioxidant enzyme systems (e.g., superoxide dismutase (SOD) and catalase (CAT), and upregulates lipid synthesis-related gene expression, promoting triacylglycerol (TAG) accumulation and redirecting metabolic carbon flow from starch to lipid synthesis [57]. Additionally, optimal salinity stress was reported to increase the

production of fatty acid methyl ester (FAME) by up to 1.8 times and optimized its composition by increasing the proportions of saturated and monounsaturated fatty acids (e.g., C16:0 and C18:1), significantly improving biodiesel stability and combustion performance. However, excessive salinity can inhibit growth, although it may still induce lipid synthesis through oxidative stress. Therefore, salt stress serves as an effective induction strategy with broad applications in the production of microalgal biofuels and high-value products. Table 3 displayed the effect of salt stress on lipid accumulation by microalgae.

Table 3. Effect of salt stress on lipid accumulation in microalgae.

Algal Species	Conditions	Lipid Accumulation	References
Aurantiochytrium sp.	Natural seawater	62.4%	[58]
Botryococcus sp. NJD-1	10 g/L NaCl	54.5%	[59]
Chlamydomonas sp.	3% & 7% sea salt concentration	31.7–37.2%	[56]
Parachlorella kessleri IC-11	30 g/L NaCl	33.3% and 31.0% in Bold's Basal Medium and wastewater	[60]
Quadrigula closterioides	5 mM NaCl	50.94%	[54]
Scenedesmus quadricauda FACHB-1297	0.88 & 2.63 g/L salt and xylose concentration	39.33%	[57]
Scenedesmus sp.	100 mM NaCl & 10 mM H_2O_2	Increased by 226.4 μg/mg	[61]
Thraustochytrium sp. BM2	20 g/L NaCl	79%	[62,63]

Studies have shown that microalgae exhibit significant metabolic responses and lipid accumulation mechanisms under different salinity stress conditions, demonstrating the significance of salinity as a critical factor influencing lipid production. Most studies agreed that moderate salt concentrations significantly enhance lipid content, with some microalgae achieving lipid content exceeding 50%, such as *Botryococcus* sp. NJD-1 (10 g/L NaCl, lipid content and productivity of 54.5% and 110.5 mg/L/d) [59] and *Quadrigula closterioides* (5 mM NaCl, lipid content of 50.94%) [54]. Liu et al. also reported that under moderate salinity levels (0.88–2.63 g/L), *Scenedesmus quadricauda* FACHB-1297 effectively adapts to salt stress, achieving high biomass and lipid accumulation, with the highest lipid content of 39.33% observed at the optimal salinity of 2.63 g/L [57].

Under moderate salinity level, salt stress-induced ROS triggered antioxidant defenses and increased nicotinamide adenine nucleotide phosphate (NADPH) production through the pentose phosphate pathway and pyruvate-malate cycle, supporting fatty acid synthesis [59]. The generation of ROS activates antioxidant enzyme activity and upregulates lipid synthesis-related genes (e.g., glycerol-3-phosphate acyltransferase (GPAT) and diacylglycerol acyltransferase (DGAT)), promoting neutral lipids accumulation (particularly TAG) and redirecting metabolic flux from carbohydrates to storage lipids [57]. Neutral lipids are synthesized primarily via the acetyl-CoA-dependent Kennedy pathway as a byproduct of ROS detoxification [59]. Excessive salinity, however, results in ROS overaccumulation, inducing cellular damage and inhibiting the growth of microalgae and lipid accumulation [54].

However, Sorokina and colleagues revealed that high salt concentration (30 g/L) significantly enhanced neutral lipid accumulation of *Parachlorella kessleri* IC-11 cultivated in Bold's Basal Medium (BBM) and municipal wastewater (WW), with lipid content reaching 33.3% and 31.0% in BBM and WW, respectively [60]. Salt stress induced a reprogramming of carbon flux and energy storage, reducing adenosine metabolism in BBM and significantly affecting proline metabolism and the citric acid cycle in WW. It also increased the proportion of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in lipid composition, improving biodiesel quality. Nevertheless, high salt levels still causing some negative impact, where the chemical oxygen demand (COD) removal efficiency in WW was reduced, indicating that decreased biomass under salt stress limited wastewater treatment efficiency.

The combined application of gamma-aminobutyric acid (GABA) and salinity has been found to enhance lipid accumulation and biomass productivity in the green microalga *Ankistrodesmus* sp. EHY [64]. Under 2.5 g/L NaCl with 50 μM GABA, lipid content reached 59.42%, and lipid productivity increased to 235.13 mg/L·d, representing 1.36-fold and 1.27-fold increases compared to salinity alone and the control, respectively. Transcriptomic and metabolomic analyses revealed that the combination of GABA and moderate salinity also increased the levels of intermediates in the tricarboxylic acid (TCA) cycle and the GABA shunt, which served as carbon sources and energy for lipid accumulation. Furthermore, the combined treatment improved the fatty acid composition of biodiesel by increasing saturated and monounsaturated fatty acid content, enhancing fuel quality. In 5-L fermenter-scale experiments, the combined strategy significantly improved lipid content and productivity, demonstrating its potential for large-scale microalgal biofuel production.

Under single-stage cultivation conditions, the combined supplementation of glucose and salt significantly enhanced the biomass and lipid production efficiency of *Graesiella emersonii* NC-M1 [65]. Incorporation of 1.75 g/L glucose and 0.3 M NaCl, biomass concentration, lipid concentration, and lipid productivity increased by 3.3-fold, 4.63-fold, and 4.56-fold, respectively, compared to the control. Glucose promoted glycolysis and the pentose phosphate pathway, supporting rapid microalgal growth, while salt stress redirected carbon flux from starch synthesis to lipid synthesis, significantly increasing neutral lipid (particularly TAG) accumulation. Transcriptomic analysis revealed significant upregulation of genes related to cell proliferation, photosynthesis, and NADPH production, while starch synthesis genes were downregulated. Additionally, salt stress elevated intracellular ROS, further activating lipid synthesis-related genes and enhancing lipid accumulation. The combined strategy also optimized FAME profiles, increasing saturated and monounsaturated fatty acid content, while improving biodiesel properties such as cetane number, oxidative stability, and viscosity.

In a two-stage cultivation system, red light and salt stress significantly enhanced the production of high-value compounds in *Chlorella sorokiniana* and improved biodiesel quality [66]. During the second stage, 2-fold salt concentration (0.05 g/L NaCl) increased lipid content from 30.6% to 37.5%, representing a 22.54% improvement. Salt stress also induced the synthesis of polyunsaturated fatty acids (PUFAs) and promoted TAG accumulation by altering cell size and stability, though it caused a reduction in protein content, indicating a metabolic shift towards lipid synthesis. Furthermore, salt stress improved the FAME profile by increasing the proportion of saturated and monounsaturated fatty acids while reducing that of polyunsaturated fatty acids, thereby enhancing biodiesel stability and combustion performance. Integration of salt stress with red light further boosted photosynthetic pigment and lipid production, with carotenoid content increasing by 62.21% and lipid accumulation efficiency significantly enhanced.

Anand and colleagues explored the effects of multi-component stress conditions (salt concentration, nitrate, phosphate, and hydrogen peroxide) on the growth and lipid accumulation of *Scenedesmus* sp., evaluating their potential to enhance biodiesel quality [61]. The results showed that under conditions of 100 mM NaCl, 35.29 mM $NaNO_3$, 5.74 mM K_2HPO_4, and 10 mM H_2O_2, lipid content significantly increased to 226.4 μg/mg, which is 1.3 times higher than without H_2O_2.

Kato and coworkers demonstrated that salt-resistant *Chlamydomonas* sp. JSC4 mutant strains, developed through heavy ion beam mutagenesis and high-salinity adaptation, could grow in 7% seawater, achieving a biomass concentration of 4.08 g/L, which was higher than that of the parental strain [56]. It was further revealed that the expression of key genes related to salt-induced starch-to-lipid biosynthesis switching was suppressed in the salt-resistant strains, which may explain the reduced lipid synthesis. Furthermore, no cellular aggregation or hypertrophy occurred in the salt-resistant strains under salinity stress, indicating their enhanced salt tolerance.

3.3. Effect of Salinity on Pigment Accumulation

Under moderate salinity, photosynthesis in microalgae may be enhanced, thereby promoting the synthesis of pigments. Moderate salt level can induce the generation of appropriate levels of ROS, activating antioxidant enzyme systems such as SOD and CAT, thereby promoting the accumulation of antioxidant pigments like lutein and carotenoids. Conversely, high salt concentrations may suppress the expression of photosynthetic pigments like chlorophyll, causing metabolic shifts toward antioxidant pigment production, reflecting a trend of metabolic redistribution [67].

Patel et al. reported that *Chlorella sorokiniana* C16 demonstrated significant enhancement in lutein production under optimized conditions of light intensity, temperature, salinity, and nutrient levels. Under 10 k lux light intensity, 32 °C temperature, and 25% seawater dilution, lutein content reached a peak of 17.4 mg/g [23]. The two-stage cultivation strategy further increased lutein production to 71.13 mg/L, with the first stage promoting biomass growth and the second stage inducing lutein and lipid accumulation. Excessive light intensity (>15 k lux), salinity (100% seawater), or nutrient concentration (4X BS) inhibited lutein accumulation, highlighting the importance of moderate environmental conditions for optimal production.

Under salt stress conditions, *Golenkinia* sp. SDEC-16 exhibits enhanced pigment metabolism changes, particularly the accumulation of carotenoids [67]. As salt concentration increases to 3%, chlorophyll content decreases about 58.4%, while carotenoid content rises approximately 2.5 times compared to the control group, scavenging the salt-induced ROS. Under high-salinity conditions (3%), ROS production increases by 6.6 times, activating antioxidant enzymes such as SOD. Carotenoids, acting as auxiliary antioxidants, play a crucial role in alleviating oxidative stress. Moreover, salt stress inhibits the activity of Photosystem II (PSII), reducing photosynthetic efficiency and prompting cells to enhance carotenoid synthesis to protect the photosynthetic apparatus from damage.

4. Effect of Electric Field on Microalgal Growth and Metabolite Accumulation

Electric field technology is an innovative tool that relies on electrical energy, and is widely applied in biotechnology, energy conversion, and environmental protection [68]. By applying static or dynamic electric fields, this technology can influence cell membrane permeability, cell metabolism, and the release of intracellular components. In microalgae research, the application of electric fields has garnered significant attention for effectively optimizing growth conditions, enhancing biomass yield, and promoting the accumulation of high-value bioproducts. Table 4 and Figure 3 displayed the effect of electric field treatment on biomass production and metabolite accumulation of microalgae.

Table 4. Effect of electric treatment on biomass production and metabolite accumulation.

Algal Species	Electric Treatment Condition	Results	References
Acutodesmus dimorphus	10 kV LE-PEF, 2 s on, 60 s off	28.8% increase in lipid productivity	[69]
Chlorella vulgaris	nsPEF, 10–50 kV/cm	Significant increase in growth	[70]
Chlorella vulgaris	nsPEF, 100 ns pulse width, 60–100 mA DC	17.5% increase in growth	[71]
Chlorella vulgaris	nsPEF	Increased cultivation efficiency and growth	[72]
Chlorella vulgaris	Medium electric field (2.7 kV/cm), 50 min	51% increase in growth	[73]
Chlorella vulgaris	Post-incubation after PEF	Maximized lipid bioaccessibility post-incubation	[74]
Haematococcus pluvialis	100 mA DC	20% increase in growth	[75]
Pavlova gyrans	Electric field treatment	Increased lipid production	[76]

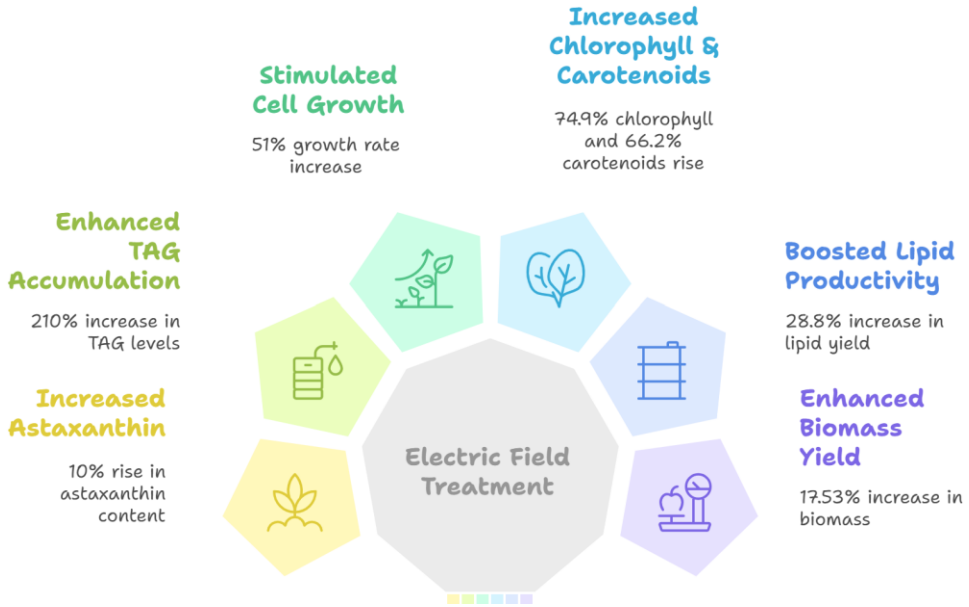

Figure 3. Effect of electric field treatment on microalgal growth and metabolite accumulation.

Periodic electrical treatment (100 mA applied every 4 days) enhanced growth and astaxanthin production of *Haematococcus pluvialis* [75]. Compared to the control, treated cultures exhibited a 20% increase in cell density and a 10% increase in astaxanthin content. This treatment also improved chlorophyll content (24.8 mg/L versus 19.8 mg/L) and accelerated nitrogen uptake, indicating enhanced nutrient utilization. Additionally, treated cells were smaller in size, suggesting active cell division and proliferation.

Mild electric stimulation at 4 V (31 mA cathodic current) for 4 h in a two-chamber electrochemical reactor equipped with carbon-cloth electrodes, using 100 mM sodium phosphate buffer (pH 7.0) and aeration at 100 mL/min, significantly enhanced the accumulation of neutral lipids and essential fatty acids in *Chlorella* sp. KR-1 [77]. Under these conditions, TAG content increased to 2.1 times that of untreated controls, while polyunsaturated fatty acids such as linoleic acid and linolenic acid increased by 36% and 57%, respectively.

The application of medium electric fields (1 to 10 kV/cm) to *Chlorella vulgaris* significantly stimulated cell growth, with a 51% increase in growth rate observed after 50 min of exposure to a moderate static electric field

(2.7 kV/cm) at a concentration of 0.4 g/L [73]. This growth enhancement was attributed to improved cell membrane permeability, facilitating nutrient uptake and promoting cell proliferation. However, prolonged exposure, such as 70 min, led to increased peroxide accumulation, resulting in oxidative stress and a decline in growth due to excessive ROS production, including hydroxyl radicals. At lower cell concentrations (e.g., 0.25 g/L), shorter treatment durations (e.g., 10 min) effectively promoted growth. Conversely, at higher concentrations, the optimal treatment duration increased, with a 70-min exposure found to be most effective for a concentration of 1.0 g/L.

Electric field treatment applied to the single-celled microalga *Pavlova gyrans* demonstrated a significant impact on its biochemical composition, despite not markedly promoting cell growth [76]. Under optimized conditions (0.54 V/cm), applied during the exponential growth phase on day 6 for durations of 6 h and 30 h, chlorophyll a and carotenoid content increased by 74.9% and 66.2%, respectively. Additionally, lipid, carbohydrate, and protein content rose by 4.72%, 18.7%, and 5.41%, respectively. These findings indicate that electric field treatment can effectively modulate the biochemical profile of microalgae, presenting a promising approach for regulating microalgal metabolism and enhancing biorefinery applications.

4.1. Pulsed Electric Field Treatment

Pulsed electric field (PEF) is a technology that applies high-intensity electric fields (10–50 kV/cm) to treat microalgae cells. In principal, PEF uses short-duration electrical pulses (ranging from nanoseconds to milliseconds) to induce electroporation in microalgae cell membranes, offering low energy consumption, lower thermal load, and efficient release of intracellular components [70].

For *Acutodesmus dimorphus*, optimized PEF treatment using a 10 kV low-energy pulse field (pulse cycle of 2 s on and 60 s off, lasting for 15 min, with six cycles per day) resulted in a 28.8% increase in lipid productivity [69]. PEF treatment at an electric field strength of 20 kV/cm, a pulse width of 5 μs, and an energy input of 31.8 kJ/kg, combined with post-PEF incubation at 25 °C or 37 °C for 12 h, or at 4 °C for 48 h, significantly enhanced lipid bioaccessibility in *Chlorella vulgaris*, increasing from 4–7.8% to 18.7–20.9% [74]. Microalgal lipid maintained good oxidative stability after 3 months of storage at 40 °C.

Nanosecond pulsed electric field (nsPEF) treatment significantly enhanced the biomass yield of *C. vulgaris*, achieving an increase of up to 17.53 ± 10.46% [71]. A pulse repetition frequency range of 1 Hz to 3.5 kHz ensured efficient treatment without excessive heat generation. Key parameters, including electric field strength, pulse repetition frequency, and pulse width, with the longest pulse width of 100 ns and electric field strength of 2.7 kV/cm is the most optimal for enhancing biomass yield while maintaining cell viability. The treatment primarily stimulated cell proliferation, suggesting that nsPEF enhances biomass production through mechanisms related to cellular and membrane processes. Furthermore, nsPEF treatment significantly reduced microbial contamination, achieving over a 1 $\log_{10}$ reduction in bacterial colony-forming units, while preserving the viability of *C. vulgaris* cells compared to untreated controls [72].

The PEF technology is a highly efficient, low-energy method for promoting microalgal growth as well as enhancing lipid and metabolite production, particularly in species like *Chlorella*. Optimizing parameters such as electric field strength, pulse frequency, and treatment duration minimizes energy consumption, reduces costs, and increases productivity. Based on these attributes, PEF appear to be economically and environmentally advantageous for sustainable industrial applications. As advancements progress, PEF and similar techniques are poised to further enhance microalgae biorefinery efficiency, supporting sustainable, cost-effective processes and contributing to the development of a circular bioeconomy.

5. Future Perspectives

In the field of microalgal cultivation, research has shown that environmental stress influences various aspects of metabolism, promoting lipid accumulation, protein stability, and pigment synthesis, making it a key strategy for enhancing the production efficiency of biofuels, nutritional supplements, and biopharmaceuticals. Moderate stress can alter carbon metabolism, shifting carbon flow from carbohydrate and protein synthesis toward lipid storage while optimizing fatty acid composition, to improve biodiesel stability and combustion performance [78,79]. In the future, application of genetic engineering and stress-adapted cultivation techniques are advocated to establish more resilient microalgal strains to sustain lipid production under high-stressed conditions, reduce biofuel production costs while integrating seawater cultivation and industrial wastewater utilization to drive sustainable energy development.

Environmental stress factors influence protein metabolism, and studies have identified stress-tolerant microalgae that exhibit high protein stability or even increase protein synthesis under high stress conditions. Therefore, transcriptomic and proteomic approaches should be adopted to analyze stress-induced protein

expression, to identify new bioactive or specialized microalgal proteins which can be applied to improve food nutrition, animal feed, and pharmaceuticals. Such genetic modifications can enhance protein synthesis in microalgae under stress, creating a sustainable protein source.

Given that environmental stress influences pigment metabolism, inhibiting chlorophyll synthesis while promoting the accumulation of carotenoids, lutein, and astaxanthin, future metabolic engineering and fermentation technologies aimed at enhancing pigment production efficiency and screening for stress-tolerant, high-yield microalgal strains, are needed to identify strains that provide sustainable source of natural pigments. Additionally, researchers should identify strategies that can modify environmental stress to improve microalgal pigment composition, antioxidant capacity and cellular protection mechanisms, thereby expand the applications of such microalgae in medicine and functional foods.

In summary, environmental stress not only enhances lipid accumulation but also regulates protein stability and pigment metabolism, demonstrating its potential contribution to the production of biofuels, nutrition, and high-value bioproduct development. In future, advancements in genetic modification, metabolic engineering, and intelligent cultivation technologies are advocated to improve microalgal salt tolerance and metabolic regulation, thereby drive large-scale their applications in renewable energy, environmental sustainability, and high-value industries, offering innovative solutions for global sustainable development.

6. Conclusions

Stress factors, both conventional (light and salinity) and unconventional (electric field treatment), are effective strategies for enhancing microalgal growth and metabolite production. Notably, light intensity, quality, and photoperiod significantly impact photosynthesis, enzymatic activity, and metabolic pathways, with moderate light boosting growth and efficiency. LED spectral control enables precise manipulation of pigment and metabolite synthesis. Salinity stress promotes lipid biosynthesis, antioxidant activity, and valuable metabolites, but it needs to be optimized to balance growth and productivity. Emerging methods like PEF treatment stimulate intracellular compound accumulation with low energy input, offering transformative potential for sustainable biomass processing. Integrating and optimizing these factors in scalable systems is key to producing high-value biomolecules and biofuels. Such advancements will support circular bioeconomy models, addressing global energy and resource sustainability challenges.

Author Contributions: Conceptualization, Y.K.L; Methodology, A.K. and Y.K.L.; Validation, C.-E.C., Y.-H.C., S.-Y.L. and A.K.; Formal Analysis, C.-E.C., Y.-H.C. and S.-Y.L.; Investigation, C.-E.C., Y.-H.C. and S.-Y.L.; Resources, Y.K.L.; Data Curation, C.-E.C., Y.-H.C. and S.-Y.L.; Writing—Original Draft Preparation, C.-E.C., Y.-H.C., S.-Y.L., J.-H.Z., C.C.C. and C.R.Z.; Writing—Review & Editing, A.K. and Y.K.L.; Visualization, A.K. and Y.K.L.; Supervision, Y.K.L.; Project Administration, Y.K.L.; Funding Acquisition, Y.K.L. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully appreciate the financial support from Taiwan's National Science and Technology Council under grant numbers NSTC 111-2221-E-029-001-MY3, 112-2218-E-029-001, and 113-2321-B-029-002.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Data Availability Statement: No underlying data are available for this article, since no datasets were generated or analysed during this study.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Caroppo, C.; Pagliara, P. Microalgae: A Promising Future. *Microorganisms* **2022**, *10*, 1488. https://doi.org/10.3390/microorganisms10081488.
2. Fernández, F.G.A.; Reis, A.; Wijffels, R.H.; et al. The role of microalgae in the bioeconomy. *New Biotechnol.* **2021**, *61*, 99–107. https://doi.org/10.1016/j.nbt.2020.11.011.
3. Viegas, C.; Gouveia, L.; Gonçalves, M. Aquaculture wastewater treatment through microalgal. Biomass potential applications on animal feed, agriculture, and energy. *J. Environ. Manag.* **2021**, *286*, 112187. https://doi.org/10.1016/j.jenvman.2021.112187.
4. Ramandani, A.A.; Lee, S.Y.; Jambrak, A.R.; et al. Synergizing food waste management and microalgae biorefinery for bioenergy production: Recent advance on direct and indirect conversion pathway. *Process Biochem.* **2025**, *151*, 14–26. https://doi.org/10.1016/j.procbio.2025.01.006.
5. Goh, B.H.H.; Ong, H.C.; Cheah, M.Y.; et al. Sustainability of direct biodiesel synthesis from microalgae biomass: A

critical review. *Renew. Sustain. Energy Rev.* **2019**, *107*, 59–74. https://doi.org/10.1016/j.rser.2019.02.012.

6. Ashokkumar, V.; Flora, G.; Kumar, G.; et al. Cutting-edge advances in alga *Botryococcus* for eco-friendly biofuels and high-value bioproducts—A critical review. *Algal Res.* **2024**, *83*, 103676. https://doi.org/10.1016/j.algal.2024.103676.
7. Barera, S.; Forlani, G. The role of proline in the adaptation of eukaryotic microalgae to environmental stress: An underestimated tool for the optimization of algal growth. *J. Appl. Phycol.* **2023**, *35*, 1635–1648. https://doi.org/10.1007/s10811-023-03017-9.
8. Maltsev, Y.; Maltseva, K.; Kulikovskiy, M.; et al. Influence of light conditions on microalgae growth and content of lipids, carotenoids, and fatty acid composition. *Biology* **2021**, *10*, 1060. https://doi.org/10.3390/biology10101060.
9. Begum, H.; Yusoff, F.M.D.; Banerjee, S.; et al. Availability and utilization of pigments from microalgae. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2209–2222. https://doi.org/10.1080/10408398.2013.764841.
10. Singh, S.P.; Singh, P. Effect of temperature and light on the growth of algae species: A review. *Renew. Sustain. Energy Rev.* **2015**, *50*, 431–444. https://doi.org/10.1016/j.rser.2015.05.024.
11. Fu, W.; Guomundsson, Ó.; Paglia, G.; et al. Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2395–2403. https://doi.org/10.1007/s00253-012-4502-5.
12. Leong, Y.K.; Chang, J.S. Lutein biosynthesis from microalgae — Recent advances and circular economy. *Environ. Technol. Innov.* **2023**, *30*, 103097. https://doi.org/10.1016/j.eti.2023.103097.
13. Saha, S.K.; Kazipet, N.; Murray, P. The carotenogenic *Dunaliella salina* CCAP 19/20 produces enhanced levels of carotenoid under specific nutrients limitation. *BioMed Res. Int.* **2018**, *2018*, 7532897. https://doi.org/10.1155/2018/7532897.
14. Jahns, P.; Holzwarth, A.R. The role of the xanthophyll cycle and of lutein in photoprotection of photosystem II. *Biochim. Et Biophys. Acta—Bioenerg.* **2012**, *1817*, 182–193. https://doi.org/10.1016/j.bbabio.2011.04.012.
15. Kuczynska, P.; Jemiola-Rzeminska, M.; Nowicka, B.; et al. The xanthophyll cycle in diatom *Phaeodactylum tricornutum* in response to light stress. *Plant Physiol. Biochem.* **2020**, *152*, 125–137. https://doi.org/10.1016/j.plaphy.2020.04.043.
16. Schulze, P.S.C.; Barreira, L.A.; Pereira, H.G.C.; et al. Light emitting diodes (LEDs) applied to microalgal production. *Trends Biotechnol.* **2014**, *32*, 422–430. https://doi.org/https://doi.org/10.1016/j.tibtech.2014.06.001.
17. Kou, Y.; Liu, M.; Sun, P.; et al. High light boosts salinity stress-induced biosynthesis of astaxanthin and lipids in the green alga *Chromochloris zofingiensis*. *Algal Res.* **2020**, *50*, 101976. https://doi.org/https://doi.org/10.1016/j.algal.2020.101976.
18. Gouveia, L.; Neves, C.; Sebastião, D.; et al. Effect of light on the production of bioelectricity and added-value microalgae biomass in a Photosynthetic Alga Microbial Fuel Cell. *Bioresour. Technol.* **2014**, *154*, 171–177. https://doi.org/10.1016/j.biortech.2013.12.049.
19. Zhang, Y.; Shi, M.; Mao, X.; et al. Time-resolved carotenoid profiling and transcriptomic analysis reveal mechanism of carotenogenesis for astaxanthin synthesis in the oleaginous green alga *Chromochloris zofingiensis*. *Biotechnol. Biofuels* **2019**, *12*, 287. https://doi.org/10.1186/s13068-019-1626-1.
20. Kim, T.H.; Lee, Y.; Han, S.H.; et al. The effects of wavelength and wavelength mixing ratios on microalgae growth and nitrogen, phosphorus removal using *Scenedesmus* sp. for wastewater treatment. *Bioresour. Technol.* **2013**, *130*, 75–80. https://doi.org/10.1016/j.biortech.2012.11.134.
21. Jayakumar, S.; Bhuyar, P.; Pugazhendhi, A.; et al. Effects of light intensity and nutrients on the lipid content of marine microalga (diatom) *Amphiprora* sp. for promising biodiesel production. *Sci. Total Environ.* **2021**, *768*, 145471. https://doi.org/10.1016/j.scitotenv.2021.145471.
22. Han, W.; Jin, W.; Ding, W.; et al. Effects of nutrient composition, lighting conditions, and metal ions on the growth and lipid yield of the high-lipid-yielding microalgae (*Chlorella pyrenoidosa*) cultivated in municipal wastewater. *J. Environ. Chem. Eng.* **2021**, *9*, 106491. https://doi.org/10.1016/j.jece.2021.106491.
23. Patel, A.K.; Vadrale, A.P.; Singhania, R.R.; et al. Enhanced mixotrophic production of lutein and lipid from potential microalgae isolate *Chlorella sorokiniana* C16. *Bioresour. Technol.* **2023**, *386*, 129477. https://doi.org/10.1016/j.biortech.2023.129477.
24. Cheah, W.Y.; Show, P.L.; Juan, J.C.; et al. Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation. *Energy Convers. Manag.* **2018**, *164*, 188–197. https://doi.org/10.1016/j.enconman.2018.02.094.
25. Chang, W.; Li, Y.; Qu, Y.; et al. Mixotrophic cultivation of microalgae to enhance the biomass and lipid production with synergistic effect of red light and phytohormone IAA. *Renew. Energy* **2022**, *187*, 819–828. https://doi.org/10.1016/j.renene.2022.01.108.
26. Felix, C.; Ubando, A.; Madrazo, C.; et al. Non-catalytic in-situ (trans)esterification of lipids in wet microalgae *Chlorella vulgaris* under subcritical conditions for the synthesis of fatty acid methyl esters. *Appl. Energy* **2019**, *248*, 526–537. https://doi.org/10.1016/j.apenergy.2019.04.149.
27. Yen, S.W.; Nagarajan, D.; Chen, W.H.; et al. Fermentative production of astaxanthin from sorghum distillery residue by

an indigenous *Aurantiochytrium* sp. CJ6 strain using a continuous-feeding fed-batch process. *Bioresour. Technol.* **2023**, *376*, 128817. https://doi.org/10.1016/j.biortech.2023.128817.

28. Liu, M.; Chen, Y.; Zhu, C.; et al. Insight into the regulation mechanism of light spectra on astaxanthin biosynthesis in mixotrophic *Chromochloris zofingiensis*. *Algal Res.* **2024**, *82*, 103690. https://doi.org/10.1016/j.algal.2024.103690.
29. Ma, R.; Tao, X.; Wang, B.; et al. Hyper-production of astaxanthin from *Haematococcus pluvialis* by a highly efficient nitrogen feeding strategy accompanied with high light induction. *Algal Res.* **2025**, *85*, 103865. https://doi.org/10.1016/j.algal.2024.103865.
30. Cheirsilp, B.; Wantip, K.; Chai-issarapap, N.; et al. Enhanced production of astaxanthin and co-bioproducts from microalga *Haematococcus* sp. integrated with valorization of industrial wastewater under two-stage LED light illumination strategy. *Environ. Technol. Innov.* **2022**, *28*, 102620. https://doi.org/10.1016/j.eti.2022.102620.
31. Zhang, H.; Tan, Y.; Zhu, R.; et al. Red light trade-off regulating the negative effects of nitrogen concentration to improve lipid and astaxanthin production in the filamentous green microalga *Oedocladium carolinianum*. *Algal Res.* **2024**, *80*, 03510. https://doi.org/10.1016/j.algal.2024.103510.
32. Nur, M.M.A.; Liana, F.M.; Putri, A.N.L.D.; et al. Enhancing fucoxanthin production in Chaetoceros calcitrans under mixotrophic condition and LED wavelength shift strategy. *Biocatal. Agric. Biotechnol.* **2024**, *57*, 103120. https://doi.org/10.1016/j.bcab.2024.103120.
33. Bo, Y.; Wang, S.; Ma, F.; et al. The influence of spermidine on the build-up of fucoxanthin in *Isochrysis* sp. acclimated to varying light intensities. *Bioresour. Technol.* **2023**, *387*, 129688. https://doi.org/10.1016/j.biortech.2023.129688.
34. Zhang, H.; Gong, P.; Cai, Q.; et al. Maximizing fucoxanthin production in *Odontella aurita* by optimizing the ratio of red and blue light-emitting diodes in an auto-controlled internally illuminated photobioreactor. *Bioresour. Technol.* **2022**, *344*, 126260. https://doi.org/10.1016/j.biortech.2021.126260.
35. Chen, C.Y.; Liu, P.Y.; Chang, Y.H.; et al. Optimizing cultivation strategies and scaling up for fucoxanthin production using *Pavlova* sp. *Bioresour. Technol.* **2024**, *399*, 130609. https://doi.org/10.1016/j.biortech.2024.130609.
36. Weickert, S.; Schmid-Staiger, U.; Lewandowski, I. Influence of specific light availability and solvent on process economics—The production of fucoxanthin and eicosapentaenoic acid from *P. tricornutum* using flat-panel airlift photobioreactors with artificial light. *Algal Res.* **2023**, *75*, 103284. https://doi.org/10.1016/j.algal.2023.103284.
37. Zhao, X.; Yan, J.; Yang, T.; et al. Exploring engineering reduced graphene oxide-titanium dioxide (rGO-TiO2) nanoparticles treatment to effectively enhance lutein biosynthesis with *Chlorella sorokiniana* F31 under different light intensity. *Bioresour. Technol.* **2022**, *348*, 126816. https://doi.org/10.1016/j.biortech.2022.126816.
38. Zheng, H.; Wang, Y.; Li, S.; et al. Lutein production by microalgae using corn starch wastewater pretreated with rapid enzymatic hydrolysis. *Bioresour. Technol.* **2022**, *352*, 126940. https://doi.org/10.1016/j.biortech.2022.126940.
39. Chen, J.H.; Kato, Y.; Matsuda, M.; et al. Lutein production with *Chlorella sorokiniana* MB-1-M12 using novel two-stage cultivation strategies—Metabolic analysis and process improvement. *Bioresour. Technol.* **2021**, *334*, 125200. https://doi.org/10.1016/j.biortech.2021.125200.
40. Yu, H.Y.; Cho, D.H.; Seo, D.; et al. Microalga *Chlorella* sp. biomass containing high lutein prevents light-induced photooxidation and retinal degeneration in mice. *Algal Res.* **2024**, *82*, 103620. https://doi.org/10.1016/j.algal.2024.103620.
41. Diaz-MacAdoo, D.; Nagai, S.; Mata, M.T.; et al. Comparative analysis of carotenoid synthesis and transcriptome of a microalga *Chlorophyta* MCH-35, potential lutein producer, in response to different quality light. *Algal Res.* **2023**, *74*, 103206. https://doi.org/10.1016/j.algal.2023.103206.
42. Liu, Y.; Wei, D. Enhancing carbon dioxide fixation and co-production of protein and lutein in oleaginous *Coccomyxa subellipsoidea* by a stepwise light intensity and nutrients feeding strategy. *Bioresour. Technol.* **2023**, *376*, 128885. https://doi.org/10.1016/j.biortech.2023.128885.
43. Li, J.; Zhao, X.; Chang, J.S.; et al. A two-stage culture strategy for *Scenedesmus* sp. FSP3 for CO_2 fixation and the simultaneous production of lutein under light and salt stress. *Molecules* **2022**, *27*, 7497. https://doi.org/10.3390/molecules27217497.
44. Bialevich, V.; Zachleder, V.; Bišová, K. The effect of variable light source and light intensity on the growth of three algal species. *Cells* **2022**, *11*, 1293. https://doi.org/10.3390/cells11081293.
45. Difusa, A.; Talukdar, J.; Kalita, M.C.; et al. Effect of light intensity and pH condition on the growth, biomass and lipid content of microalgae *Scenedesmus* species. *Biofuels* **2015**, *6*, 37–44. https://doi.org/10.1080/17597269.2015.1045274.
46. Gim, G.H.; Ryu, J.; Kim, M.J.; et al. Effects of carbon source and light intensity on the growth and total lipid production of three microalgae under different culture conditions. *J. Ind. Microbiol. Biotechnol.* **2016**, *43*, 605–616. https://doi.org/10.1007/s10295-016-1741-y.
47. Cheirsilp, B.; Torpee, S. Enhanced growth and lipid production of microalgae under mixotrophic culture condition: Effect of light intensity, glucose concentration and fed-batch cultivation. *Bioresour. Technol.* **2012**, *110*, 510–516. https://doi.org/10.1016/j.biortech.2012.01.125.
48. Ho, S.H.; Chen, C.Y.; Chang, J.S. Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate

production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.* **2012**, *113*, 244–252. https://doi.org/10.1016/j.biortech.2011.11.133.

49. Mahari, W.A.W.; Razali, W.A.W.; Khor, W.; et al. Light-emitting diodes (LEDs) for culturing microalgae and cyanobacteria. *Chem. Eng. J.* **2024**, *485*, 149619. https://doi.org/10.1016/j.cej.2024.149619.
50. You, T.; Barnett, S.M. Effect of light quality on production of extracellular polysaccharides and growth rate of *Porphyridium Cruentum*. *Biochem. Eng. J.* **2004**, *19*, 251–258. https://doi.org/https://doi.org/10.1016/j.bej.2004.02.004.
51. Abu-Ghosh, S.; Fixler, D.; Dubinsky, Z.; Iluz, D. Continuous background light significantly increases flashing-light enhancement of photosynthesis and growth of microalgae. *Bioresour. Technol.* **2015**, *187*, 144–148. https://doi.org/10.1016/j.biortech.2015.03.119.
52. Vélez-Landa, L.; Hernández-De León, H.R.; Pérez-Luna, Y.D.C.; et al. Influence of light intensity and photoperiod on the photoautotrophic growth and lipid content of the microalgae *Verrucodesmus verrucosus* in a photobioreactor. *Sustainability* **2021**, *13*, 6606. https://doi.org/10.3390/su13126606.
53. Chakravarty, S.; Mallick, N. Engineering a cultivation strategy for higher lipid accretion and biodiesel production by the marine microalga *Picochlorum soloecismus*. *Sustain. Chem. Pharm.* **2022**, *26*, 100635. https://doi.org/10.1016/j.scp.2022.100635.
54. Anahas AM, P.; Prasannabalaji, N.; Muralitharan, G. Unlocking the potential of coal mine microalgae strains: Enhanced biodiesel production and CO_2 sequestration through cultivation optimization. *Biomass Bioenergy* **2025**, *192*, 107489. https://doi.org/10.1016/j.biombioe.2024.107489.
55. Almutairi, A.W. Harnessing waste glycerol to mitigate salinity constraints in freshwater microalgae cultivation for enhanced biodiesel recovery. *J. King Saud Univ.-Sci.* **2024**, *36*, 103258. https://doi.org/10.1016/j.jksus.2024.103258.
56. Sinetova, M.A.; Sidorov, R.A.; Medvedeva, A.A.; et al. Effect of salt stress on physiological parameters of microalgae *Vischeria punctata* strain IPPAS H-242, a superproducer of eicosapentaenoic acid. *J. Biotechnol.* **2021**, *331*, 63–73. https://doi.org/10.1016/j.jbiotec.2021.03.001.
57. Liu, N.; Mou, Y.; Su, K.; et al. The effect of salinity stress on the growth and lipid accumulation of *Scenedesmus quadricauda* FACHB-1297 under xylose mixotrophic cultivation. *Process Saf. Environ. Prot.* **2022**, *165*, 887–894. https://doi.org/10.1016/j.psep.2022.07.051.
58. Chauhan, A.J.; Patel, A.K.; Chen, C.W.; et al. Enhanced production of high-value polyunsaturated fatty acids (PUFAs) from potential thraustochytrid *Aurantiochytrium* sp. *Bioresour. Technol.* **2023**, *370*, 128536. https://doi.org/10.1016/j.biortech.2022.128536.
59. Yang, Z.; Chen, J.; Tang, B.; et al. Metabolic interpretation of NaCl stress-induced lipid accumulation in microalgae for promising biodiesel production with saline wastewater. *Chem. Eng. Sci.* **2024**, *284*, 119447. https://doi.org/10.1016/j.ces.2023.119447.
60. Sorokina, K.N.; Samoylova, Y.V.; Parmon, V.N. Comparative analysis of microalgae metabolism on BBM and municipal wastewater during salt induced lipid accumulation. *Bioresour. Technol. Rep.* **2020**, *11*, 100548. https://doi.org/10.1016/j.biteb.2020.100548.
61. Anand, V.; Kashyap, M.; Sharma, M.P.; et al. Impact of hydrogen peroxide on microalgae cultivated in varying salt-nitrate-phosphate conditions. *J. Environ. Chem. Eng.* **2021**, *9*, 105814. https://doi.org/10.1016/j.jece.2021.105814.
62. Chen, C.Y.; Lee, M.H.; Dong, C.D.; et al. Enhanced production of microalgal lipids using a heterotrophic marine microalga *Thraustochytrium* sp. BM2. *Biochem. Eng. J.* **2020**, *154*, 107429. https://doi.org/10.1016/j.bej.2019.107429.
63. Chen, C.Y.; Lee, M.H.; Leong, Y.K.; et al. Biodiesel production from heterotrophic oleaginous microalga *Thraustochytrium* sp. BM2 with enhanced lipid accumulation using crude glycerol as alternative carbon source. *Bioresour. Technol.* **2020**, *306*, 123113. https://doi.org/10.1016/j.biortech.2020.123113.
64. Zhao, Y.; Li, Q.; Gu, D.; et al. The synergistic effects of gamma-aminobutyric acid and salinity during the enhancement of microalgal lipid production in photobioreactors. *Energy Convers. Manag.* **2022**, *267*, 115928. https://doi.org/10.1016/j.enconman.2022.115928.
65. Mandal, M.K.; Chaurasia, N. De novo transcriptomic analysis of *Graesiella emersonii* NC-M1 reveals differential genes expression in cell proliferation and lipid production under glucose and salt supplemented condition. *Renew. Energy* **2021**, *179*, 2004–2015. https://doi.org/10.1016/j.renene.2021.07.141.
66. Parveen, A.; Rawat, J.; Bhatnagar, P.; et al. Enhanced production of high-value compounds from *Chlorella sorokiniana* by two-stage cultivation under red light and salinity stress. *Biocatal. Agric. Biotechnol.* **2024**, *60*, 103315. https://doi.org/10.1016/j.bcab.2024.103315.
67. Ma, M.; Jiang, L.; He, Y.; et al. Multi-faceted effects of NaCl on salt-tolerant microalgae *Golenkinia* sp. SDEC-16. *Bioresour. Technol.* **2024**, *406*, 131016. https://doi.org/10.1016/j.biortech.2024.131016.
68. Pereira, R.N.; Jaeschke, D.P.; Mercali, G.D.; et al. Impact of ultrasound and electric fields on microalgae growth: A comprehensive review. *Braz. J. Chem. Eng.* **2023**, *40*, 607–622. https://doi.org/10.1007/s43153-022-00281-z.
69. La, H.J.; Choi, G.G.; Cho, C.; et al. Increased lipid productivity of *Acutodesmus dimorphus* using optimized pulsed

electric field. *J. Appl. Phycol.* **2016**, *28*, 931–938. https://doi.org/10.1007/s10811-015-0674-6.

70. Carullo, D.; Abera, B.D.; Casazza, A.A.; et al. Effect of pulsed electric fields and high pressure homogenization on the aqueous extraction of intracellular compounds from the microalgae *Chlorella vulgaris*. *Algal Res.* **2018**, *31*, 60–69. https://doi.org/10.1016/j.algal.2018.01.017.
71. Haberkorn, I.; Buchmann, L.; Hiestand, M.; et al. Continuous nanosecond pulsed electric field treatments foster the upstream performance of *Chlorella vulgaris*-based biorefinery concepts. *Bioresour. Technol.* **2019**, *293*, 122029. https://doi.org/10.1016/j.biortech.2019.122029.
72. Buchmann, L.; Böcker, L.; Frey, W.; et al. Energy input assessment for nanosecond pulsed electric field processing and its application in a case study with *Chlorella vulgaris*. *Innov. Food Sci. Emerg. Technol.* **2018**, *47*, 445–453. https://doi.org/10.1016/j.ifset.2018.04.013.
73. Nezammahalleh, H.; Ghanati, F.; Adams, T.A.; et al. Effect of moderate static electric field on the growth and metabolism of C*hlorella vulgaris. Bioresour. Technol.* **2016**, *218*, 700–711. https://doi.org/10.1016/j.biortech.2016.07.018.
74. Canelli, G.; Kuster, I.; Jaquenod, L.; et al. Pulsed electric field treatment enhances lipid bioaccessibility while preserving oxidative stability in *Chlorella vulgaris*. *Innov. Food Sci. Emerg. Technol.* **2022**, *75*, 102897. https://doi.org/10.1016/j.ifset.2021.102897.
75. Kim, J.Y.; Lee, C.; Jeon, M.S.; et al. Enhancement of microalga *Haematococcus pluvialis* growth and astaxanthin production by electrical treatment. *Bioresour. Technol.* **2018**, *268*, 815–819. https://doi.org/10.1016/j.biortech.2018.08.014.
76. Barreiros, M.; Maciel, F.; Pereira, R.S.; et al. Electric fields to support microalgae growth with a differentiated biochemical composition. *Innov. Food Sci. Emerg. Technol.* **2024**, *97*, 103829. https://doi.org/10.1016/j.ifset.2024.103829.
77. Choi, S.A.; Lee, S.Y.; Lee, J.; et al. Rapid induction of edible lipids in Chlorella by mild electric stimulation. *Bioresour. Technol.* 2019, 292. https://doi.org/10.1016/j.biortech.2019.121950.
78. Ramos, M.; Dias, A.P.S.; Puna, J.F.; et al. Biodiesel production processes and sustainable raw materials. *Energies* **2019**, *12*, 4408. https://doi.org/10.3390/en12234408.
79. Santos, S.; Puna, J.; Gomes, J. A review on bio-based catalysts (immobilized enzymes) used for biodiesel production. *Energies* **2020**, *13*, 3013. https://doi.org/10.3390/en13113013.