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Review

Effects of Environmental Factors and Nutrient Availability on the Biochemical Composition of Algae for Biofuels Production: A Review

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Abstract: Due to significant lipid and carbohydrate production as well as other useful properties such as high production of useful biomolecular substrates (e.g., lipids) and the ability to grow using non-potable water sources, algae are being explored as a potential high-yield feedstock for biofuels production. In both natural and engineered systems, algae can be exposed to a variety of environmental conditions that affect growth rate and cellular composition. With respect to the latter, the amount of carbon fixed in lipids and carbohydrates (e.g., starch) is highly influenced by environmental factors and nutrient availability. Understanding synergistic interactions between multiple environmental variables and nutritional factors is required to develop sustainable high productivity bioalgae systems, which are essential for commercial biofuel production. This article reviews the effects of environmental factors (*i.e.*, temperature, light and pH) and nutrient availability (e.g., carbon, nitrogen, phosphorus, potassium, and trace metals) as well as cross-interactions on the biochemical composition of algae with a special focus on carbon fixation and partitioning of carbon from a biofuels perspective.

Keywords: algae; biochemical composition; environmental effect; biofuel production

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1. Introduction

Increasing demand for energy and global warming are two major challenges facing modern society. Dependence on fossil fuels for meeting increasing energy demands is unsustainable due to increasing levels of consumption and a dearth in discovery of new sources for these non-renewables. This concern has motivated researchers to focus on the development of alternative energy sources including solar, wind, water, and biomass. Biofuels are alternatives to liquid fossil fuels and are produced from sugar, starch, cellulosic or lipid-rich substrates. These substrates can be derived from feedstocks such as cereal crops, including corn and wheat [1]; sugar crops, including sorghum and sugarcane [2]; energy crops, such as switchgrass [3,4]; agricultural wastes, including straws and corn stover [5–8]; municipal wastes; and, several aquatic species. Currently, ethanol is produced from corn and sugarcane in significant volumes as a supplemental fuel and as a partial substitute for gasoline. Production and use of ethanol as a transportation fuel results in a net reduction of greenhouse gas (GHG) emissions. This claim is based on the idea that carbon dioxide emissions produced during the processing of biomass and from the use of biofuels is readily sequestered.

Biofuels produced from cereal crops and sugarcane are commonly called first-generation biofuels (FGB). The large-scale production (and use) of FGB products as competitive substitutes for fossil fuels is hindered by several limitations including: intensive agricultural inputs, land requirements, and trade-offs between food crop and fuel crop production (*i.e.*, the food *vs.* fuel debate) [9]. Therefore, although they are renewable, FGBs alone are not a viable solution for solving global liquid fuel demands. Second-generation biofuels (SGB) are fuels derived from lignocellulosic biomass. Production of SGBs circumvents several of the negative outcomes associated with FGBs [10]. At present, the development of competitive SGB products is at various stages of research and pilot demonstrations. A few products have been commercialized. However, SGB feedstock production still requires agricultural inputs, land, and freshwater that could be used for food crops. Thus, SGBs are also subject to the food-versus-fuel dilemma in the long term. Producing liquid fuels from aquatic organisms, such as algae, is considered to be the third-generation in biofuels (TGB). The use of TGB feedstocks, which contain significant amounts of lipids and carbohydrates, from which biodiesel and bioethanol products may be produced, avoids most of the limitations noted for FGBs and SGBs. Arguably, this includes overcoming the food *vs.* fuel dilemma. Most aquatic plant feedstocks are championed as a viable source of lipids for the production of bio-oil [11]. Specifically, via thermochemical conversions or biochemical conversions, algae can be used to produce: biofuel oil and gas; or, bioethanol, biodiesel, and biohydrogen, respectively [12].

A recent resurgence of interest in algal-based TGBs is attributed to significant benefits associated with TGB production. These benefits include: year-round production; higher productivity compared to terrestrial crops; the potential for off-shore production (and, thus, non-competition with food crops); reduced need for arable land; reduced need for water treatment; and potential advantages in nutrient cycling [13–15].

Earlier research demonstrated that under select conditions, algae have the potential to produce 40 times the amount of oil for biodiesel production compared to oilseed crops (*i.e.*, soy and canola) per unit land area [16]. However, such advantages over FGBs and SGBs have only been established in academic/research facilities. Presently, in the absence of publicly available data, it is unknown whether

such gains can be realized on a commercial scale. Therefore, the economic potential of algal-based biofuels to significantly impact current and future fuels needs remains in question. Nonetheless, algal-based TGBs are an intense focus within the alternative fuels research community and production companies are paying close attention to advances. Of particular interest are microalgae-based TGB products. Technical and economic aspects of high-yield production of biodiesel from microalgae are currently being studied by many groups. A few review papers have discussed the processes and challenges in detail [17–20]. The potential of ethanol production from algae has also been investigated [21–24]. In both cases, it is generally agreed that there is significant potential for algal-based products; however, progress toward commercial-scale biofuels production from algae has been slow due to multiple challenges in production and processing.

Whether in open ponds or in closed photobioreactors, culturing algae requires consideration of numerous environmental conditions. Environmental factors such as temperature, light, pH, and nutrients not only affect photosynthesis and growth rate of the algae, but also influence the activity of cellular metabolism and composition. These effects have been identified individually by researchers [25–31], however, no consolidated review on the effect of these factors on algae is available. Most of the recent reviews have focused on production and processing techniques [11,16,32] with few data on the impacts of environmental and nutritional factors on algal growth rates. One recent, highly informative review, does consider microalgae and multiple environmental factors including temperature, light, pH, and salinity [33–35], however, it is almost exclusively focused on impacts of environmental factors on lipid production. Understanding how these factors influence algal growth and broader metabolic functions (not only lipid induction) is critical for successful scale-up of algae cultures in commercial systems for algal biofuels and bioproducts production. This review directly addresses this issue by focusing on the impacts of environmental and nutritional factors on algae biochemistry specifically related to biofuels production processes. The paper is organized as follows: This introductory section is followed by a brief overview of different production and processing technologies. The subsequent section provides a detailed review of the environmental and nutritional factors that affect algal growth. The third section addresses interaction effects between environmental factors and nutrient availability. This is followed by a discussion of sustainable production of algal biofuels. Finally, we offer a discussion of future outlook and some concluding remarks.

1.1. Algae Production, Processing and Use

Algal production processes can be categorized into three general classes of growth regimes based on the energy source and mode of utilization; these include: photoautotrophic, heterotrophic and mixotrophic. These growth processes can occur in open raceway ponds or closed bioreactor systems [36]. Different production schemes involving combinations of different growth regimes in various reactor configurations have been proposed in an effort to maximize biofuel productivity. Substrates for algal feedstock production can range from industrial effluents and municipal waste water to synthetic media consisting of sugars from molasses, starch or lignocellulosic feedstocks depending on the growth regime and bioreactor configuration. Although open raceway ponds require low energy inputs and lower capital costs, several issues such as contamination (*i.e.*, by unwanted algal species as well as viral, bacterial and fungal pathogens) and low final biomass concentrations (often less than 1.0 g/L)

increase production costs to levels that are still economically unviable for large-scale production of biofuels [32]. Closed photobioreactors (CPBR) in different configurations have been proposed to address the contamination issues associated with open raceway systems. CPBRs also permit more stringent control of growth conditions and harvesting [13]. However, CPBRs often require extensive upfront capital investments compared to open raceway ponds and therefore face similar commercialization challenges.

In addition to system setup, there are challenges associated with specific stages within the production process. Lipid and starch fractions of algae can be processed into renewable fuel by several different processing methods, including: pyrolysis [37], thermochemical liquefaction [38], fermentation [39], and transesterification [40]. Most thermochemical conversion methods such as direct combustion, gasification, and pyrolysis require low-moisture-content biomass. This poses a challenge due to high energy requirements for drying algal feedstock. However, hydrothermal liquefaction process can use wet slurry for algal-oil production [41] thus reducing costs associated with drying. Biochemical conversion processes such as anaerobic digestion produce methane (60%–70%) and carbon dioxide (30%–40%) [42]. Fermentation of sugars produced from the starch fraction can be used to produce ethanol from algal biomass [39]. Advantages and limitations of these processes are tabulated in Table 1.

Table 1. Advantages and limitations of biofuel production processes from microalgae.

Process	Advantages	Limitations
Pyrolysis	High bio-oil yields possible(up to 57.5% *w/w* for fast and flash pyrolysis [43])	Low-moisture-content biomass required High-energy-content required to dry feedstock
Thermochemical liquefaction	Algal wet slurry can be used Energy (and cost) reduction High yields possible (up to 60% *w/w* [44])	Reactors are complex and expensive
Fermentation	Co-products can be utilized Conversion of sugar to bioethanol possible	Long processing times required Biomass has to be preprocessed to be converted to sugars
Transesterification	Enhanced physical properties of renewable fuels Biodiesel has a current market that simplifies commercialization	Limited to conversion of lipids and does not utilize carbohydrate and protein fractions of feedstock

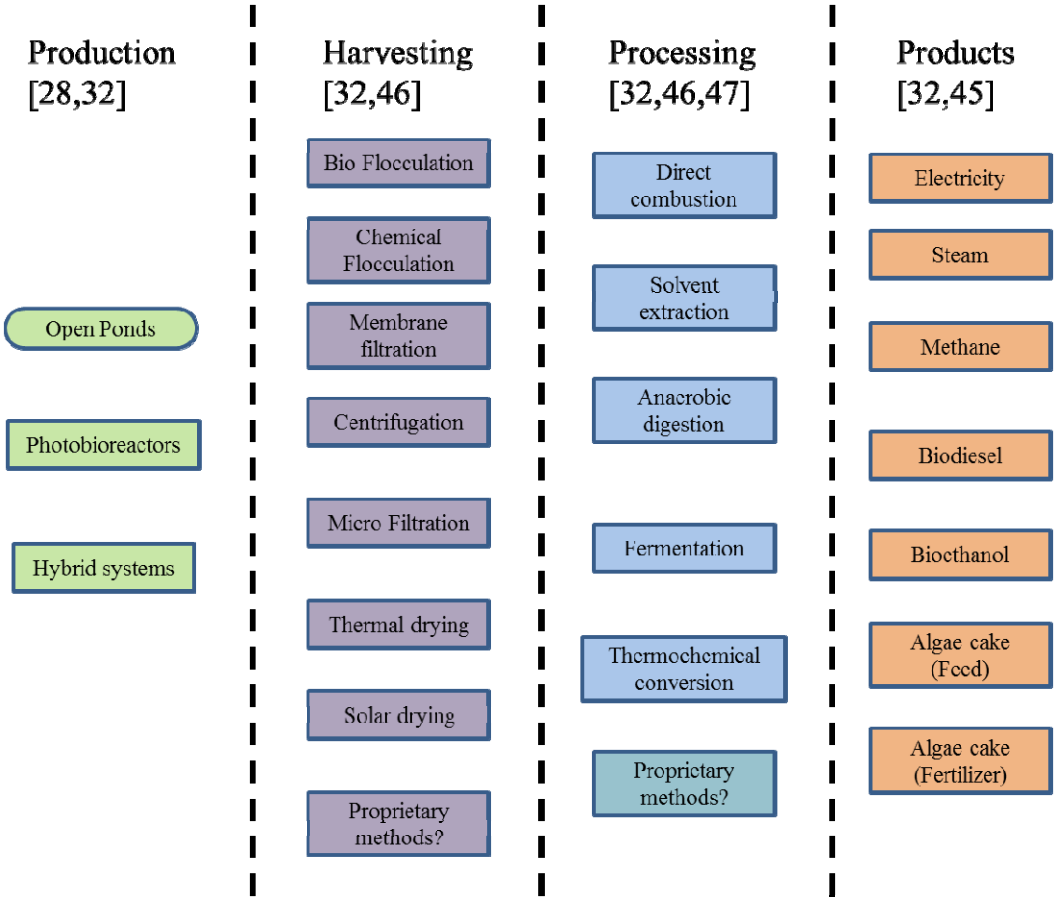
Details concerning algae biomass production processes, different processing technologies for conversion into biofuels and bioproducts, and challenges associated with commercialization of algal biofuels have been discussed thoroughly in recent reviews [11,32,35,45]. A summary of various algae production, harvesting and processing alternatives are presented in Figure 1 [36,46,47].

1.2. Relevance of Individual Algal Cellular Components for Biofuels Production

The exploitation of microalgae as a protein source has led to increased interest in the use of microalgae (e.g., *Spirulina*, *Chlorella* and *Scenedesmus*) in health food production [48]. Algae such as *Dunaliella* and *Spirulina* are also used for pigment production [49,50]. However, it is the high density lipid content that renders algae (e.g., *Chlamydomonas*, *Nannochloropsis*, *Schizochytrium*, *Chlorella*, *Nitzschia*) attractive for biofuels production [51]. Lipid content in algae can be as high as 50% (dry-weight) with higher percentages of total dry weight (~60%) found as protein and starch [11]. These fractions of

microalgae can be used to produce biodiesel [20,52], bioethanol [22,23], biohydrogen [53,54], bioplastics [55], and other products, while simultaneously contributing to CO_2 mitigation [56,57]. Still, it is the lipid fraction that has been the major focus for biodiesel production and renewable jet fuel. Depending on the species and growth conditions, dry algae weight can contain 20%–50% lipid [52], which is the major substrate for biofuels production. Total lipid composition includes (but is not limited to) neutral lipids, polar lipids, wax esters, sterols, hydrocarbons, and prenyl derivatives such as tocopherols, carotenoids, terpenes, quinones and phytylated pyrrole derivatives, including chlorophylls [51].

Figure 1. Production, harvesting and processing alternatives for algae.



Carbohydrates (primarily starch) are another valuable component of the algal cell. Typical dry weight content of carbohydrates in algae range from 20% to 40% of total cell mass [58]. Certain components, such as starch, can be easily converted to ethanol by hydrolysis and fermentation. Currently, the starch used for ethanol production is obtained from food crops such as corn, wheat, sorghum, and others [1]. For algae, high starch strains including *Chlorella vulgaris* (with 37% dry weight starch) [59], are being studied for potential use has high-yield feedstocks. Algal starch is known to be readily fermentable by yeast [60] and, therefore, is being intensely studied for use in ethanol production [22,23].

Upon utilization of lipids and starch fractions for bio-oil production, the residual algae cake, which is rich in proteins, is important for producing valuable co-products. Protein (and starch) content can constitute up to 60% of dry weight of algae [48]. This residual protein from the biomass can be used for livestock, poultry, and fish feed additives [11]. It has been reported that algae can replace about

5%–10% of conventional protein sources in poultry feed [61]. Recently residual algae cake after lipid extraction has been used in large animal feeding trials [62]. However high concentrations of nucleic acids in algae can pose challenges for the utilization in animal feed applications [63].

2. Effect of Environmental Factors

During photosynthesis, using only light and nutrients, algae produce lipids, proteins, and carbohydrates. The relative amounts of these metabolic products are tightly linked to environmental and nutrient conditions including: the amount and intensity of sunlight; CO_2 levels; pH; temperature; available nutrients; and, the presence (or absence) of other organisms. Carbon, hydrogen, and oxygen are required non-mineral nutrients for algal growth. Macronutrients include nitrogen, phosphorus, sulfur, potassium and magnesium. Micronutrients such as iron and manganese are also required in small amounts (30–2.5 ppm) while other elements such as cobalt, zinc, boron, copper and molybdenum are essential trace elements (4.5–2.5 ppm) [64]. Collectively, environmental conditions (especially light and temperature) and the availability of non-mineral nutrients, macronutrients, and micronutrients, greatly influence the biochemical composition of microalgae [25,30,65–67]. Other factors such as pH and the present toxic metals are also important factors impacting algal growth and metabolism. In general, all of these factors can affect photosynthesis, thus altering carbon fixation and the allocation of carbon into different types of macromolecules. In turn, the cell's macromolecular composition determines its usefulness in biofuels production.

2.1. Temperature

Temperature is perhaps one of the most important environmental factors that influences algal growth rate, cell size, biochemical composition and nutrient requirements. In the United States, algae grow under a broad range of temperatures (from 15 to 40 °C), depending upon strain, region, and season. Below optimal growth temperatures, growth rate (μ) increases with increasing temperature but declines markedly above the species- or strain- specific optimum [68]. Growth at temperature optima results in minimal cell size [69,70] and the efficiency of carbon and nitrogen utilization decreases at non-optimal temperatures [71]. It has been suggested that changes in cytoplasmic viscosity under sub-optimal temperature conditions is responsible for less efficient carbon and nitrogen utilization [72,73]. Temperature may also play a key role in photoinhibition, which is known to impact algal growth rate. Several mechanisms of temperature-dependent photoinhibition have been postulated. These include mechanisms under which: (i) low temperature results in reduced electron transport at a given photon flux rate due to slower rate of CO_2 fixation; (ii) low temperature inhibits the active oxygen species, which results in reducing photoinhibition by protecting PSII; and (iii) low temperature inhibits the synthesis of the D1 protein degraded during photoinhibition, consequently impeding the PSII repair cycle [74].

One of the most commonly observed changes with temperature shift is the alteration in the level of unsaturation of fatty acids in the lipid membrane [75–77]. In a study on eight marine plankton species, fatty acids (14:0) increased from ~4% at 10 °C to >20% at 25 °C while PUFA (polyunsaturated fatty acids) were consistently higher at lower temperature (10 °C) [27]. *Dunaliella salina* has shown a considerable increase in fatty acid unsaturation in response to decrease in temperature from 30 to

12 °C [78]. Lower temperatures decrease the fluidity in the cell membrane. Cells then compensate by increasing levels of unsaturated fatty acids to increase fluidity. However, it also makes the membranes more susceptible to damage by free radicals [73,79]. Along with greater fluidity, increased levels of unsaturated fatty acids tend to enhance the stability of the cellular membranes (particularly the thylakoid membrane). This, in turn, protects the photosynthetic machinery from photoinhibition at low temperatures [79]. For example, in a study involving *Botryococcus braunii*, a green alga that secretes extracellular lipids, differences in lipid composition were observed at three different growth temperatures (18 °C, 25 °C, and 32 °C). Intracellular lipid synthesis was found to be inhibited at supra-optimal temperature (32 °C); consequently, lipid content decreased to 5% dry weight at 32 °C in comparison with 22% at 25 °C. The decrease in lipid content led to an accumulation of polysaccharides. However, temperature did not affect the secretion of extracellular lipids [66]. Similar effects were observed in *Nannochloropsis oculata* and *Chlorella vulgaris*, both of which have an optimum growth temperature of 25 °C. Increasing the growth temperature from 20 to 25 °C doubled the lipid content (from 7.90% to 14.92%) in *N. oculata*. Increase of temperature from 25 to 30 °C decreased the lipid content in *C. vulgaris* from 14.71% to 5.90% [80].

Increasing temperature beyond the optimum reduces protein synthesis and consequently results in decreased growth rates [81]. Morris *et al.* [25] studied the growth of alga, *Phaeodactylum tricornutum*, a marine diatom, and reported a considerable increase in protein synthesis rates at night with lower the temperatures, presumably due to the fact that protein synthesis is a significant component of nighttime algal metabolism [82]. Similarly, Rhee and Gotham [83] observed an increase in protein concentration in *Scenedesmus* sp. with decreasing temperature. However, in this study, the efficiency of protein synthesis (in terms of rate of protein synthesis per unit RNA) was reduced upon increasing temperatures beyond the optimum. An increase in temperature from 20 to 30 °C in cultures of *Ulva pertusa* resulted in higher intercellular free amino acid concentrations from approximately 840 to 1810 mg/100 g dry weight [84]. An increase in free amino acid concentration is an indicator of lower protein content.

Temperature is also reported to impact starch content in the algal cell. Starches are synthesized by phosphorylated metabolites in the dark reactions of the photosynthesis cycle using energy-rich phosphate bonds (*i.e.*, ATP) formed in the light reactions [85]. Increased temperature leads to degradation of the starch produced [65,86]. Enzymes that have been suggested to play a critical role in the temperature dependent degradation of starch are α-amylase and α-glucan phosphorylase [87]. Nakamura and Miyachi studied the effect of temperature on the starch degradation in *Chlorella vulgaris* grown autotrophically at 20 °C [65]. This study reported a significant reduction in starch (17%) with concomitant increase in sucrose (57%), when culture was exposed to 38 °C for 10 min after 30 min at 20 °C. However, another study with *Chlorella vulgaris* grown autotrophically at 38 °C, reported a reduced degradation of starch at 38 °C [88]. This effect could be explained by noting that the starch produced at 38 °C was not subjected to any degradation since there was no change in temperature, but the cultures grown at 20 °C degraded the starch into sugars in response to increasing culture temperatures. Consistent with this reasoning, Nakamura and Imamura observed a reversible transformation of high-molecular-weight L starch (amylopectin-like molecule fraction with MW $> 2 \times 10^6$) to low-molecular-weight S starch (amylose-like molecule fraction with MW $<10^4$) at high temperatures (38 °C) [89].

Temperature has a significant effect on the formation of carotenoids. Carotenoids absorb light energy for use in photosynthesis. They also protect chlorophyll from photodamage [90]. Furthermore, they play a vital role in the photosynthetic reaction center by either participating in the energy-transfer process or protecting the reaction center from auto-oxidation. Carotenoid accumulation in algal species increases with temperature because of the increased oxidative and photodamaging effects noted at elevated temperatures [91–93]. Tjahjono *et al.* [91] reported a three-fold increase in astaxanthin formation in the green alga *Haematococcus pluvialis* with an increase in cultivation temperature from 20 to 30 °C. These results were confirmed by another study on a different green alga, *Chlorococcum* sp., in which a two fold increase in total carotenoid content was observed by raising the temperature from 20 to 35 °C under conditions of nitrogen deprivation [92]. Increase in carotenoid formation with increasing temperature is generally attributed to cellular response to enhanced active free oxygen radical formation [91] or increased biosynthetic enzyme activity [92].

2.2. Light

Light is the energy source during photoautotrophic growth phase and organisms use light energy to convert carbon dioxide to organic compounds—especially, sugars. The range of light intensity in USA varies from 1500 to 8500 $W \cdot h/m^2/day$ with strong regional and seasonal dependence [94]. Light intensity effects growth of algae through its impact on photosynthesis [95]. Although rate of growth under increasing light intensity is a function of strain and culture temperature, the growth rate of algae is maximal at *saturation intensity* and decreases with both increase or decrease in light intensity [96]. The photoadaptation/photoacclimation process in algae leads to changes in cell properties according to the availability of light and an increase in photosynthetic efficiency [97]. Adaptation can occur through multiple mechanisms such as changes in types and quantities of pigments, growth rate, dark respiration rate or the availability of essential fatty acids [67]. Morphological photoacclimation is accompanied by changes in cell volume and the number and density of thylakoid membranes [98]. Algae overcome light limitation by desaturation of chloroplast membranes [99]. Light intensity increase above saturating limits causes photoinhibition [30,100]. This is due to the disruption of the chloroplast lamellae caused by high light intensity [101] and inactivation of enzymes involved in carbon dioxide fixation [102]. For example, growth rate of *Dunaliella viridis* decreased to 63% with increase in light intensity from 700 to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [30].

Light intensity also affects the cellular composition of algae. *Dunaliela tertiolecta* exhibits a decrease in protein content and an increase in the lipid fraction with increasing light intensities up to saturation [82]. Similar results were reported by Morris *et al.* [25], in a study on the marine diatom, *Phaeodactylum tricornutum*, in which low light (400 lux at the culture surface) led to an increase in the rate of protein synthesis. Low light intensity has been observed to result in higher protein content while high photon flux density (PFD) results in increased extracellular polysaccharide content [102]. Absence of light was observed to increase the total lipid content of the *D. virdis* but reduce triglycerides, free fatty acids, free alcohols and sterols [103]. In *Nannochloropsis* sp., grown under low light conditions (35 $\mu E \cdot m^{-2} \cdot s^{-1}$), 40% of the total lipids were found to be galactolipids and 26% were found to be triacylglycerols. In the same system, high light (550 $\mu E \cdot m^{-2} \cdot s^{-1}$) conditions resulted in an increased synthesis of triacylglycerol with a reduction in galactolipid synthesis [26]. High light,

in general, leads to oxidative damage of PUFA. Numerous studies have suggested that the cellular lipid content and PUFA decrease with increase in light intensity [104–106]. Conversely, *Nannochloropsis* cells under low light conditions were characterized by high lipid content and high proportions of eicosapentaenoic acid (EPA; 5,8,11,14,17-icosapentanoic acid) [26]. Confirming this observed trend, another study on the same species reported an increase in unsaturated fatty acids mainly due to an increase in EPA (from 44.3% to 60.7% of the organic content) and a decrease in protein content, with decreasing irradiance [67]. Increase in PUFA under light-limited growth conditions are coupled with an increase in total thylakoid membrane in the cell [98]. However, there are some contradictory studies in which PUFA levels were observed to be increasing with higher light intensity [107]. This difference in response to environmental conditions by different alga may be related to difference in their metabolic pathways. Increase in oxygen-mediated lipid desaturation could be one potential reason for the observed increase in PUFA levels under conditions of higher light intensity [107].

In addition to total light intensity, light cycles and the spectral composition of incident light impact algae. For example, Wu and Merchuk [108] investigated the effect of light and dark cycles on the growth of algae and observed that with increasing photon flux density (PFD), specific growth rate increases up to a certain threshold PFD value after which a decline in growth rate was observed. Sustained high light intensities have also been reported to cause photoinhibition and reduce light utilization efficiency. Light utilization efficiency may be optimized by prolonging the dark period under conditions of high light intensity. This allows the photosynthesis machinery in the cell to fully utilize captured photons and convert them into chemical energy thus avoiding the effects of photoinhibiton [109].

Since the energy content of near-ultraviolet (300–400 nm) and blue light (400–480 nm) is greater than that of red light (620–750 nm), fewer photons of blue light are required to achieve an equivalent magnitude of energy intensity using red light. In addition to differences in the energy intensity, specific components of light are known to impact the cellular regulatory processes including: chlorophyll synthesis, photodamage repair, and cell division. For example, blue light was shown to be essential for the division of *Chlamydomonas reinhardtii* cells [110]. Evidence for the influence of blue light on short-term growth rate is equivocal [111]. It has been observed that blue and red light can help to increase growth and polysaccharide production [100]. Emerson and Lewis also reported blue and red light to be the most effective for photosynthesis of *Chlorella* [112]. Miyachi and Kamiya studied the starch formation in *Chlorella vulgaris* under blue (456 nm) and red (660 nm) light. They reported that the carbon pathway in photosynthesis is regulated by short wavelength light (blue), even under low intensity [113]. Red light of high intensity was observed to incorporate carbon from CO_2 into sucrose and starch synthesis pathways. However, superimposition of monochromatic blue light even at low intensities resulted in a significant decrease in sucrose and starch formation along with increasing levels of alanine, aspartate, glutamate, glutamine, malate, citrate, lipids and the alcohol-water-insoluble non-carbohydrate fraction [113].

Ultraviolet light (UV; 215–400 nm) adversely affects the algal primarily due to the damage to the photosynthetic machinery in the cells. UV-B (215–380 nm) causes more damage to the cells compared to UV-A radiation (380–400 nm) even at similar intensities [114]. The UV-B radiation causes direct damage to cellular DNA, UV-A damage is limited to indirect damage through enhance production of

reactive oxygen and hydroxyl radicals. At moderate levels, UV-A may stimulate photosynthesis while UV-B has a negative effect of photosynthesis irrespective of the intensity. Some of the response of the algae to minimize the damage caused by UV radiation includes migration, development of protective cell walls, increased synthesis of carotenoids and other pigments [114–116].

2.3. pH

One of the most important factors in algal cultivation is pH since it determines the solubility and availability of CO_2 and essential nutrients, and because it can have a significant impact on algal metabolism [28,117]. Due to uptake of inorganic carbon by algae, pH can rise significantly in algal cultures [118]. Maximum algal growth occurs around neutral pH, although optimum pH is the initial culture pH at which an alga is adapted to grow. Changing pH in media may limit algal growth via metabolic inhibition [119]. Pruder and Bolton observed that *T. pseudonana* cells adapted to low pH (6.5) had lower growth rate at sub-optimal pH (8.8) [120]. Normal growth rate was restored after the pH was lowered by addition of HCl. Similar results were reported by Chen and Durbin, where photosynthetic rate and algal growth was minimal at pH 9.0, but carbon uptake rates were enhanced when the pH was lowered to 8.3 [28].

Notably, pH is the major determining factor of relative concentrations of the carbonaceous species in water [121]. Higher pH limits the availability of carbon from CO_2, which, in turn, suppresses algal growth [28,121]. At higher pH, the carbon for algae is available in form of carbonates [122]. Higher pH also lowers the affinity of algae to free CO_2 [121,123]. In photoautotrophic cultures, replacement of CO_2 taken up for photosynthesis is slower resulting in a decrease of CO_2 partial pressure and thus leading to an increase in pH [120]. Alkaline pH increases the flexibility of the cell wall of mother cells, which prevents its rupture and inhibits autospore release, thus increasing the time for cell cycle completion [124]. Alkaline pH indirectly results in an increase in triglyceride accumulation but a decrease in membrane-associated polar lipids because of cell cycle inhibition. Membrane lipids in *Chlorella* were observed to be less unsaturated under conditions of alkaline pH [124].

Similar to alkaline pH, acidic conditions can alter nutrient uptake [125] or induce metal toxicity [126,127] and thus affect algal growth. As previously stated, most species of algae grow maximally around neutral pH (7.0–7.6). This has been observed in studies of *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum* [118] and *Chlamydomonas applanata* [31]. Visviki and Santikul [31] studied the growth of *Chlamydomonas applanata* within a pH range 1.4 to 8.4 with 1 point increments. No growth was observed from pH 1.4 to 3.4, above which tolerance of pH in *C. applanata* was observed (with optimum growth observed at 7.4). Exponential growth was observed for up to five days at pH 5.4 to 8.4, but maximum growth was achieved at pH 7.4. In a study on *Chlamydomonas acidophila* at pH 4.4, it was observed that hydrogen ions denature V-lysin, a proteolytic enzyme that facilitates releasing of daughter cells from within the parental wall [128]. Hargreaves and Whitton [129] studied the effects of low pH on the morphology of five algal species. Acidic conditions (pH 1.3–1.5) were observed to limit the motility of cells in *Chlamydomonas applanata* var. *acidophila* and *Euglena mutabilis*. Coleman and Colman studied the effect of external pH on photosynthesis of *Coccochloris peniocystis* and found a significant decrease in total accumulated carbon and oxygen evolution at pH 5.0 and 6.0, which suggested the reduction in photosynthesis in this cyanobacteria

(blue-green alga) at these pH ranges [130]. Maintenance of neutral intracellular pH in an acidic pH external environment would require an expenditure of energy to pump protons out of the cell [131]. On the other hand, acid-tolerant algae such as *Chlorella saccharophila* [132] and *Euglena mutabilis* [133] can change intracellular pH in response to changing external pH. In *Chlorella saccharophila*, an internal pH of 7.3 was maintained for an external pH range of 5.0–7.5; however, decreasing the pH further to 3.0 caused a decrease in cellular pH to 6.4 [132]. Similarly, *Euglena mutabilis* exhibited an internal pH range from 5.0 (at low external pH < 3.0) to 8.0 (at high external pH > 9.0) [133]. The energy required to maintain internal pH in these acid-tolerant algae is conserved as the internal pH goes down. This may be a mechanism for maintaining cellular metabolism such that algal growth is not drastically affected under acidic conditions [132]. Such a mechanism would endow acid-tolerant algae with the ability to adjust internal pH in response to external pH fluctuations, thereby, maintaining an energy advantage over acid-intolerant species at low external pH.

Some algae such as *Dunaleilla acidophila* adapt to acidic conditions in growth media by accumulating glycerol to prevent the osmotic imbalance caused by high concentrations of H_2SO_4 [134] while other species such as *Chlamydomonas* sp. and *Pinnilaria braunii* var. *amplicephala* (an acidophilic diatom) accumulate storage lipids such as triacylglycerides under highly acidic conditions (pH 1) [135]. Another adaptation observed under acidic conditions is an increase in saturated fatty acid content, which reduces membrane fluidity and inhibits high proton concentrations [135]. Such adaptation was reported in a *Chlamydomonas* sp., in which total fatty acid content increased from 2% at pH 7 to 2.4% at pH 2.7, a modest but statistically significant increase [136].

Under alkaline conditions whereby the extracellular pH is higher than intracellular pH, the cell must rely on active transport of HCO_3^- and not on passive flux of CO_2^- for inorganic carbon accumulation [121,137]. Affinity of algae for CO_2 increases at lower pH [121,123]. Moroney and Tolbert studied the effects of pH on carbon uptake of *Chlamydomonas reinhardtii* [138]. They reported an efficient utilization of CO_2 for photosynthesis at lower pH (<6.95). However, at high external pH (6.95–9.5), where HCO_3^- dominates, algae cannot efficiently accumulate carbon and require high supply of carbonates for maintaining photosynthetic activity. In more acidic environments, where the internal pH exceeds that of the surrounding medium and where CO_2 comprises a major portion of the total external inorganic carbon, carbon accumulation is thought to be accomplished by the passive movement of CO_2 along a pH gradient into the cell or chloroplasts [139,140].

2.4. Salinity

Salinity is another important factor that alters the biochemical composition of algal cells (salinity refers primarily to sodium chloride concentration unless otherwise specified). Exposing algae to lower or higher salinity levels than their natural (or adapted) levels can change growth rate and alter composition. For example, higher salinity increases the algae lipid content [141–144]. *Dunaliella*, a marine alga, exhibited an increase in saturated and monounsaturated fatty acids with an increase in NaCl concentration from 0.4 to 4 M [145]. In another study with *Dunaliella tertiolecta*, an increase in intracellular lipids (60% to 67%) and triglyceride concentration (40% to 56%) with an increase in NaCl concentration from 0.5 (freshwater concentration) to 1.0 M was observed [146]. Increasing the NaCl level in cultures of *Botryococcus braunii*, a fresh water alga, showed an increase in growth rate,

carbohydrate content, and lipid content; however, the greatest biomass concentration was achieved at the lowest salinity level [147]. These results are supported by another study in which lipid content of *Botryococcus braunii* grown in 0.50 M NaCl was higher compared to media without NaCl addition, but protein, carbohydrates, and pigments levels were lower [143]. Another study with the same alga reported a decrease in protein content with unchanged carbohydrate and lipid content with an increase in salinity [148]. This study also reported reduced growth at higher salinities, which may have been due to an inability of the alga to adapt to high salinity. A study with *Tetraselmis suecica* also reported reduction in protein content per cell of up to 20% with increasing salinity [144].

2.5. Nutrients

Considerable variation in the biochemical composition under conditions of nutrient limitation can be observed in algae depending upon which nutrient is limited and to what degree. In general, the growth rate of algae is proportional to the uptake rate of the most limiting nutrient under optimal conditions of temperature and pH and is generally described by Michaelis-Menten equation [149].

Nitrogen and phosphate are two important macronutrients for growth and metabolism of algal cells. Nitrogen is a fundamental element for the formation of proteins and nucleic acids. Being an integral part of essential molecules such as ATP, the energy carrier in cells, phosphate is another very important nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids. It is not unusual for algae to become nutrient-limited (*i.e.*, nitrogen- and phosphorus-limited) in the natural environment [70]. Limitation of these key nutrients shifts the metabolic pathway of the organism. For example, nitrogen and phosphorus starvation shifts the lipid metabolism from membrane lipid synthesis to neutral lipid storage. This, in turn, increases the total lipid content of green algae [58]. Specific effects of major nutrients are discussed below.

Carbon. Carbon, hydrogen, and oxygen are three essential non-mineral nutrients. Abundance of hydrogen and oxygen in the media for algae cultures means that their availability is not a challenge to cellular growth or metabolism. Carbon is one of the other major nutrients that must be supplied. It is essential for photosynthesis and hence algal growth and reproduction. Carbon fixed by the algae can end up in three destinations; it will either be used: (a) for respiration; (b) as an energy source; or, (c) as a raw material in the formation of additional cells [150]. Reduced carbon fixation rate implies a reduction in algal growth rate. Algae require an inorganic carbon source to perform photosynthesis. Carbon can be utilized in the form of CO_2, carbonate, or bicarbonate for autotrophic growth and in form of acetate or glucose for heterotrophic growth. CO_2 in water may be present in any of these forms depending upon pH, temperature and nutrient content:

$$CO_2 + H_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad (1)$$

With an increase in pH, carbonate increases while molecular CO_2 and bicarbonate decrease [28]. At the average pH of seawater (8.2), 90% of the total CO_2 is present in the form of HCO_3^-; only 1% exists as molecular CO_2 and the rest is bicarbonate [122,151].

Riebesell *et al.* [152] studied the effect of CO_2 concentration on lipid distribution in *Emiliania huxleyi*. A significant effect of CO_2 on the composition of the polyunsaturated fatty acids and alkenones was

reported. Specifically, lower CO_2 concentrations led to an increase in 22:6 (n−3) PUFA, whereas 14:0 fatty acids were found to be predominant at higher CO_2 concentrations. Along with the change in composition, increased CO_2 was also observed to increase the amount of fatty acid accumulation in *Dunaliella salina* [153]. Similar increase in fatty acid content and unsaturation with increase in CO_2 concentrations were reported by Tsuzuki *et al.* [154]. Another study with the cyanobacterium *Spirulina platensis* reported that elevated CO_2 concentrations decrease relative concentrations of proteins and pigments in the cells but increase carbohydrate content. This change in the cell composition was accompanied by reduction in the maximum biomass yield [155].

Nitrogen. Nitrogen is an essential constituent of all structural and functional proteins in the algal cells and accounts for 7%–20% of cell dry weight [58]. Inorganic nitrogen taken up by algae is rapidly assimilated into biochemically active compounds and recycled within cells to meet changing physiological needs [156,157]. Major effects of nitrogen deficiency in algal culture include the enhanced biosynthesis and accumulation of lipids [75,80,158–160] and triglycerides [161,162] with a concomitant reduction in protein content [25,29,163–166]. This, in turn, results in a higher lipid/protein ratio [80] at the expense of growth rate [167]. Thus, attempts to increase lipid concentration via nitrogen limitation must be carefully evaluated to ensure high lipid productivity [168]. Algae grown in nitrogen-depleted cultures also tend to divert their photosynthetically fixed carbon to carbohydrate synthesis [58]; however, the physical significance of this is not clear. Other effects of nitrogen reduction include decrease in oxygen evolution, carbon dioxide fixation, chlorophyll content, and tissue production [169,170]. Holm-Hansen *et al.* [164] reported an increase in amino acid content of *Chlorella pyrenoidosa* at the expense of sugar phosphates (such as glucose-6-phosphate, fructose-6-phosphate) with addition of ammonium (nitrogen source) to the growing culture.

Degradation of phycolbillisomes with nitrogen limitation has been demonstrated in the case of cyanobacteria and red alga [171]. Phycolbillisomes are the light harvesting antennae of photosystem II in these algae. Photosynthesis continues at a reduced rate, until cell nitrogen falls below a particular species-dependent threshold value. Under nitrogen deficient conditions, *Spiriluna platensis* cells exhibit reduced carbon fixation capacity even under normal to high available CO_2 concentrations [155]. Nitrogen starvation also alters the enzyme balance of cells, resulting in the synthesis of lipids and a decrease in chlorophyll synthesis leading to excess carotenoids in the cells [172]. *Dunaliella* sp. and *Haematococcus pluvialis* are observed to accumulate high amounts of carotenoids, astaxanthin and its acylesters (up to 13% *w/w*), when grown under nitrogen-depleting conditions [173–175]. Zhekisheva *et al.* [176] reported that under nitrogen depleting conditions, *Haematococcus pluvialis* produced fatty acids and astaxanthin in a 5:1 ratio. It was suggested that the production of the oleic acid-rich triacyl-glycerols and the esterification of the astaxanthin, maintain a high content of astaxanthin esters by enabling the oil globules.

Phosphorus. Phosphorus is an important component required for normal growth and development of algal cells [58]. It has been shown that phosphorus, rather than nitrogen, is the primary limiting nutrient for microalgae in many natural environments [177]. Phosphorus typically constitutes 1% of dry weight of algae [178], but it may be required in significant excess since not all added phosphate is bioavailable due to formation of complexes with metal ions [52]. Immediate effects of phosphorus limitation include a reduction in the synthesis and regeneration of substrates in the Calvin-Benson cycle and a consequential reduction in the rate of light utilization required for carbon fixation [179].

Phosphorus limitation also leads to accumulation of lipids. Total lipid content in *Scenedesmus* sp. was observed to increase from 23% to 53% with a reduction in initial total phosphorus (as phosphate) concentration of 0.1 from 2.0 mg L^{-1} [180]. Phosphatidylglycerol (PG), which is one of four major glycerolipids constituting membrane lipids in chloroplasts, was observed to decrease with phosphorus limitation in *Chlamydomonas reinhartdtii* [181]. PG is essential for cell growth, the maintenance of chlorophyll-protein complex levels, and normal structure-function of the PSII complex. Total acidic lipid (such as sulphoquinovosyldiacylglycerol and PG) content of the chloroplast did not change significantly since a decrease in one acidic lipid was accompanied by an increase in another acidic lipid [181]. Phosphate limitation also reduces the synthesis of n-3 PUFA [182].

Similar to the effects of nitrogen deficiency, phosphorus starvation reduces chlorophyll *a* and protein content thereby increasing the relative carbohydrate content in algal cells [29,183,184]. Phosphate deficiency has been demonstrated to result in accumulation of astaxanthin and an overall reduction in cell growth [185]. A decrease in cellular phycobilisome under conditions of phosphorus deficiency (due to cell division and the cessation of phycobilisomes synthesis) has also be shown [171]. Theodorou *et al.* [186] observed that phosphorus starvation in *Selenastrum minutum* reduces respiration rate.

Trace Metals. Trace metals are metals present in algal cells in extremely small quantities (<4 ppm) but that are an essential component of phycophysiology. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu) and nickel (Ni) are the six most important trace metals required by algae for various metabolic functions [187]. As the aqueous concentration of trace metals is not an indicator of the bioavailability of metals, trace metal availability to algae is highly dependent on speciation (free ion concentration) [188]. Deficiencies in trace metals can limit algal growth, whereas excesses or high metal concentrations (above the toxicity threshold) may inhibit growth, impair photosynthesis, deplete antioxidants, and damage the cell membrane.

Iron is an important trace metal for normal growth and functioning of photosynthesis and respiration in algae. It acts as redox catalyst in photosynthesis and nitrogen assimilation and mediates electron transport reactions in photosynthetic organisms [131]. Iron limitation significantly depresses photosynthetic electron transfer, resulting in a reduction in NADPH formation. Reduction in iron decreases the cellular abundance of ferredoxin, which contains Fe, and forces the substitution of flavodoxin, a non-iron functional equivalent, in the cell [189–192]. Since the catalytic capacity of ferredoxin is much higher than flavodoxin, this can be problematic [193]. Iron limitation also reduces cellular chlorophyll concentration [194]. High concentrations of iron in cultures of *Chlorella vulgaris* were observed to increase the lipid content [195]. Decrease in iron content reduces carotenoid composition [185,196].

While there are some non-essential metals (e.g., Cd, Pb and Cr), which can inhibit many metabolic processes even in small quantities [197], there are some essential elements (e.g., Zn and Cu) which when in excess can cause toxicity [198]. The cell surfaces of algae contain a number of functional groups with high affinity for metal ions and that carry a net negative charge mainly due to carboxylic, sulfhydryl, and phosphatic groups [199]. These groups are binding sites that transport metal ions across the cell membrane and into the cell. Cu, Ni and Fe are metals that are commonly observed to be toxic to the algae, if present in supra-threshold concentrations. Cu is one of the most toxic of these metals. Toxic metals can inhibit carbon fixation and delay nutrient uptake [200]. Copper ions were observed to inhibit both cell division and photosynthesis in *Asgerionella glacialis* (marine diatom) and

Chlorella pyrenoidosa (freshwater alga) [201]. Metal toxicity in algae is observed to be a pH-dependent effect, possibly due to the "competition between H^+ and free metal cations for cellular binding sites". For example, copper toxicity was observed to increase 76-fold from pH 5.0–6.5 for the green alga *Scenedesmus quadricauda* [202]. Cadmium (Cd) is particularly toxic to algal cells. Although Cd has no biological significance in a living cell, it is taken up by marine cells in the form of complexes with organic matter and is absorbed onto organic matter and inorganic matter in ionic form [203]. Cd is inhibits phosphorus uptake, which is also a pH-dependent phenomena. This toxic effect increases in the pH range of 5.5–8.5 [202]. Zinc is also a toxic metal which is rapidly taken up by the algae and is incorporated primarily into polysaccharide and nucleic acid fractions [204].

3. Interaction among Environmental Factors

Environmental factors may influence other systems factors related to algal growth or cellular composition. For example, increase in temperature can lead to reductions in nutrient availability [205]. Such interaction effects have been observed in many studies and could underlie contradictory results between some studies. For example, in a study on phytoplankton in Antarctic Ocean, Smith and Morris [206] reported that at higher temperatures phytoplankton incorporate more carbon into the protein fraction with a concomitant reduction in lipids. To the contrary, Morris *et al.* [25] reported an increase in the protein fraction of *P. tricornutum* at low temperatures. This variability could be due to the species-specific effects, differences in light intensity, and/or differences in the primary growth conditions.

In another study, Kudo *et al.* [207] observed the effect of iron stress on growth rate and cellular composition of the marine diatom *Phaeodactylum tricornutum*, over the temperature range of 5–30 °C. (Note that the optimum temperature for growth of *P. tricornutum* was 20 °C). The growth rate of Fe-stressed cells was 50% of Fe-replete cell growth rate at the optimum growth temperature. Differences in growth rates diverged significantly at suboptimum temperatures. It was also reported that at optimal temperature, the C:N ratio in the cell decreased by about 5% in cells induced with an iron stress (2 μM to 2 nM Fe). However, an increase of about 4% was demonstrated with the same transition in iron concentration but at a lower temperature (10 °C).

Algal growth rates are also affected by light-by-nutrient interactions. Cloern *et al.* [208] developed a model of phytoplankton chlorophyll—specifically, carbon ratio as a function of light and nutrients. The model suggests an increase in growth efficiency with nutrient availability under low light conditions. Morgan and Kalff [209] studied the interaction of temperature and light on the growth of *Cryptomonas erosa* under nutrient saturated conditions. Results indicated that algal carbon uptake capacity is reduced by 85% with a 90% reduction in light at 23.5 °C compared to 71% with light reduction of 82% at 4 °C. At 4 °C and 1.792 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity, or 1 °C and 0.32 $\mu E \cdot m^{-2} \cdot s^{-1}$, cell division was strongly inhibited. Another study on the interaction effects of light and temperature using *Chlorella pyrenoidosa* showed an increased saturation light intensity for algal growth at higher temperatures compared to lower temperatures. For higher temperatures, this alga exhibited a higher growth rate even at higher light intensities. The limitation on growth of algae under low temperature and high light intensity conditions is through effects on photosynthesis [210].

Converti *et al.* [80] studied the effects of temperature and nitrogen concentration on cell growth and lipid content in two strains of algae—*Chlorella vulgaris* and *Nannochloropsis oculata*. Reducing the nitrate concentrations in the growth media by 75% (1.5 to 0.375 g L^{-1} for *Chlorella vulgaris* and 0.3 to 0.075 L^{-1} for *Nannochloropsis oculata*), lipid accumulation tripled and doubled respectively, with only a small reduction in growth rate at optimal growth temperature. This result indicates that it may be possible to achieve higher lipid productivity for biofuels production by employing nitrogen limitation with fine temperature control. Interactions between salinity and nutrient concentrations (*i.e.*, $NaNO_3$) for a salt-tolerant alga, *Tetraselmis suecica*, were also reported [144]. Optimum growth conditions were found at high salinity (25%–35%) and low nutrient concentrations (2, 4, and 8 mM $NaNO_3$). It was also reported that the total protein and protein content per cell increased with increase in salinity at constant nutrient concentration. Furthermore, a decrease in protein content was observed with increase in nitrate concentration at constant salinity. The transformation of nitrate to protein increased with the salinity and decreased with increasing nitrate concentrations.

High light (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) with warm temperatures (22 °C) was observed to promote carbon fixation since this pathway acts as a sink for energy in conjunction with NO_3^- reduction pathways, which dissipate excess light energy. Confirming this hypothesis, a change in nitrogen source from NO_3^- to NH_4^+ controlling for light and temperature resulted in an increase of photorespiration, which is also a sink for excess energy. On the contrary, high light intensity (300 $\mu E \cdot m^{-2} \cdot s^{-1}$) and lower temperatures (12 °C) with NO_3^- as the nitrogen source resulted in increased energy dissipation by means of photorespiration and NO_3^- reduction (rather than via diversion to cellular carbohydrates). It was postulated that this result is due to lower carbon flows through the Calvin cycle at lower temperatures; in turn, this necessitates dissipation of the excess energy via photorespiration and NO_3^- reduction. Synergistic interaction effects of light, temperature, and nitrogen source on the transcription of five genes that are central to the carbon and nitrogen metabolism pathways in *Thalassiosira pseudonana* were also demonstrated [211].

The effects of environmental factors on biochemical composition of algae are summarized in Table 2. These results suggest that past growth conditions, interaction effects of various nutrients and environmental factors are all important to attain a thorough understanding of the behavior of algal cells in large-scale systems.

Table 2. Summary of general impact of environmental factors on biochemical composition of algae.

Factor	Organism	Conditions	Biochemical changes observed	References
Temperature	*Botryccoccus braunii*	Increased from 25 to 32 °C	Decrease in intracellular lipid content from 22% to 5% wt. Accumulation of polysaccharides	[66]
	Chlorella vulgaris	Increased from 20 to 38 °C	Decrease in starch resulting in increase in sucrose	[65]
		Increased from 10 to 38 °C	Transformation of L starch (high molecular weight) to S starch (low molecular weight) Reversible with temperature	[89]
	Haematococcus pluvialis	Increased from 20 to 30 °C	3-fold increase in astaxanthin formation	[91]
	Chlorococcum sp.	Temperature increase from 20 to 35 °C under nitrogen deprivation	Two fold increase in total carotenoid content	[92]
	Nitella mucronata Miquel	Increased from 5 to 20 °C	Increase in velocity of cytoplasmic streaming	[73]
Light	*Dunaliella virdis*	Darkness (No light)	Increase in total lipid content Decrease in free fatty acids, alcohol, sterol	[30]
	Nannochloropsis sp.	Light limited conditions	Increase in lipid content Increase in EPA * proportions	[26]
	Porphyridium cruentum	Red light	Enhanced Photosystem II relative to Photosystem I and phycobilisome	[212]
	Chlorella vulgaris	Red light	Increase in sucrose and starch formation	[113]
		Blue light	Increase in lipid fraction and alcohol-water insoluble non carbohydrate fraction	
pH	*Chlamydomonas acidophila*	pH 4.4	Denaturation of V-lysin	[128]
	Coccochloris peniocystis	pH decreased from 7.0 to 5.0 and 6.0	Decrease in total accumulated carbon and oxygen evolution	[130]

Table 2. *Cont.*

Factor	Organism	Conditions	Biochemical changes observed	References
Nitrogen	*Nannochloropsis oculata*	75% decrease in Nitrogen	Increase in lipid synthesis from 7.90% to 15.31%	[80]
	Phaeodactylum tricornutum	Nitrogen limitation	Increase in lipid synthesis; Decrease in protein content	[25]
	Chlorella vulgaris	75% decrease in Nitrogen	Increase in lipid synthesis from 5.90% to 16.41%	[80]
	Haematococcus pluvialis	Nitrogen limitation	Increase in carotenoid formation (13% *w/w*)	[174]
Phosphorus	*Chlamydomonas reinhardtii*	Limitation	Decrease in phosphatidylglycerol	[181]
	Ankistrodesmus falcatus	Limitation	Decrease in chl *a* and protein; Increase in carbohydrate and lipids	[29,183,184]
	Selenastrum minutum	Starvation	Reduced rate of respiration; Decreased photosynthetic CO_2 fixation	[186]
Iron	*Dunaliella tertiolecta*	Limitation	Decrease in cellular chlorophyll concentration	[194]
	Chlorella vulgaris	High concentration of iron	Increase in lipid content	[195]
	Haematococcus pluvialis	High concentration of iron	Increase in carotenoid formation	[185]
Carbon	*Chlamydomonas reinhardtii*	pH exceeding 9.0	Inefficient accumulation of carbon High supply of carbonates required to maintain photosynthetic activity	[138]
	Dunaliella salina	CO_2 concentration increased from 2% to 10% for 1 day	30% increase in amount of fatty acid (dry weight basis)	[153]
		CO_2 concentration increased from 2% to 10% for 7 days	2.7 fold increase in fatty acid	
	Spirulina platensis	Elevated CO_2 concentrations	Increase in carbohydrate content; Decrease in proteins and pigments	[155]

Note: * EPA: Eicosapentanoic acid, 5,7,11,14,17-icosapentanoic acid.

4. Conclusions

4.1. Algae as a Sustainable Biofuel Source

For the success of any sustainable biofuel, there are three principal considerations: *technical feasibility*; *economic viability*; and, *resource sustainability*. Algal-based biofuel is technically feasible. However, to date, economic viability has not been achieved. Furthermore, resource sustainability, in terms of land, water, nutrient and energy utilization, must be meticulously quantified for each type of production system in order for the feedstock to be considered truly "sustainable". With large-scale biofuel production processes, this water-energy-nutrient nexus is the subject of significant consideration and debate.

Sustainable use of wastewater and seawater resources poses one of the most significant challenges to large-scale production of algal-based biofuels. Algal biofuel production using waste water, brackish water, or sea water in open ponds or using closed photobioreactors, in which the evaporative water losses are negligible, are both potential solutions to this challenge.

Sustainable use of nutrients, such as nitrogen and phosphorous, also poses a serious challenge to large-scale production of algal-based biofuel. Energy intensive production methods for nitrogenous fertilizers and concerns about long-term availability of phosphorous accentuate these concerns. Phosphate is typically obtained by mining and some recent studies estimate that peak phosphorous could occur as early as 2030 [213]. Recycling nitrogen, phosphorous and other nutrients is a strategy to address some of these challenges while addressing other ecological issues such as eutrophication. Algae are capable of utilizing nutrients (including, nitrogen and phosphorus) from wastewater and thus could play a key role in nutrient recovery from waste waters [42,51,214–221]. Maximizing algae production and minimizing costs associated with harvesting are critical to cost-effective nutrient removal system development [222].

4.2. Future Outlook

In this review, we discussed the effects of various nutritional factors (*i.e.*, carbon, nitrogen, phosphorous, iron and trace metals) environmental factors (*i.e.*, light, temperature and pH) and their interactions on algae growth and composition. During the last four decades, significant progress has been made toward a better understanding of algae growth. This includes an enhanced understanding of the effects of the nutrient availability and environmental factors on algae cell division and composition. However, interaction effects induced by multiple factors acting in concert have been generally underexplored. With advances in biotechnology and bioinformatics, a large volume of genetic information is now available rendering studies of interaction effects tractable. For example, full genomes for *Chalmydomonas reinhardtii* [223], *Chlorella vulgaris* [224] and *Dunaleilla salina* [225] have been sequenced. Many genetic manipulation tools have been developed for *Chlamydomonas reinhardtii* and others are being developed for other algal species. Continued research will not only lead to an enhanced understanding of basic algal cell biology but it will also aid in development of more accurate predictive models for algae growth [226]. Predictive models, in turn, can be used for development of automated optimal control systems for managing algae growth in large-scale production systems.

This knowledge will be critical for successful scale-up of algae production systems for sustainable production of biofuels and other algae-based bioproducts.

Conflicts of Interest

The authors declare no conflict of interest.

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