Fuel 284 (2021) 119058

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Full Length Article

Impact of cultivation conditions on the biomass and lipid in microalgae with an emphasis on biodiesel

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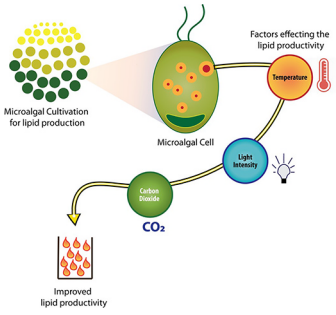
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Biodiesel
Environmental factors
Light intensity
Lipid
Microalgae
Temperature

ABSTRACT

This paper reviews the different cultivation conditions such as temperature, light intensity, and pH for enhancing lipid accumulation for green fuel production. The various levels of physical factors on the lipid yield and growth of algae with respect to biofuel production have been discussed in this review. Initially, the bottlenecks in microalgal biodiesel production have been highlighted and the need for optimal conditions for lipid accumulation have been analyzed. Further, the impact of the cultivation conditions such as light intensity/color, temperature, and CO_2 concentration were comprehensively interpreted to understand how lipid accumulation in microalgae can be enhanced in the perspective of biofuel production. Besides, all the recent advancements and experimental works on biomass and lipid production in microalgae concerning the effect of different light intensities and color, temperature regimes, CO_2 concentration and pH ranges are discussed and summarized.

1. Introduction

In the present scenario, the transportation sector plays a major role in human beings life. The requirement of energy for transportation and industrial applications is usually fulfilled through fossil fuel and it contributes to ~85% of the total energy produced [48,58]. Generally,

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https://doi.org/10.1016/j.fuel.2020.119058
Received 13 April 2020; Received in revised form 2 August 2020; Accepted 20 August 2020
Available online 09 October 2020
0016-2361/

fossil fuels are non-renewable and expensive; besides, they also contribute to air/water pollution and global warming [39]. Apart from this, many issues are being raised in utilizing fossil-based fuel for various applications. The rapid depletion of fossil-based fuels is increasing the cost of the fuels at an alarming rate. High-energy import and hike in fuel prices and unsustainable supply of petroleum, environmental concerns are increasing an interest towards the production of renewable fuels [47,62]. The major environmental concern with the fossil fuels is emission of greenhouse gases [57] and it creates enormous damage to humanity and the environment [14]. Therefore, the exploration of alternative, renewable energy to substitute conventional fossil fuel is need of the hour [43].

At present, microalgae have gained prominent attention as an alternative source of energy [27]. Scientists, researchers and entrepreneurs of the biofuel sector are considering microalgae as a potential biofuel feedstock owing to their superior biomass productivity, versatility to grow in all types of habitats, higher oil or lipid content compared to terrestrial sources [38,62]. A key constraint in cost-effective microalgal biodiesel production is the operation and capital investment owing to intricate bioreactor design and expensive oleaginous materials [25]. To obtain higher lipid content from microalgae either grown in lab scale or at the outdoor scale, lipid triggering conditions need to be optimized and maintained.

At this juncture, physical and chemical factors such as light intensity, culturing temperature, CO_2 concentration, pH, salinity, mixing/aeration, are being experimented to improve the algal growth [66]. Further, lipid content or yield could be improved by various factors such as light intensity, temperature, pH and CO_2 concentration [34]. The physical parameters viz., light, temperature, and the nutrient supplements of the medium not only influences the metabolic machinery of the strain, but they also regulate the composition and yield of microalgal lipid [39]. In-depth experiments on the alteration of the physical factors for accelerating the lipid productivity in microalgae are the present necessity. In the nature, microalgae generally respond to the change in environmental or cultivation conditions to which they are grown and therefore, considering these phenomena, researchers are targeting the non-biotic factors for enhancing microalgae quality [35]. This review article is currently the need of hour giving a clear insight on improving lipid production for algal biodiesel by unraveling the effect of various cultivation conditions on the microalgal lipid content. In addition, extensive discussion on the bottlenecks in microalgal lipid production and effect of various physical factors on the growth and lipid production of microalgae, i.e. with an emphasis on biodiesel, makes this review article different from other published literatures. Apart from these extensive ideas on cultivating microalgae under different temperature regimes, different photoperiods or light: dark condition will also be discussed. The multiple applications of microalgae like CO_2 sequestration or tolerance capability of microalgae to CO_2 and subsequent biodiesel production will also be reviewed.

2. Effect of environmental factors on the lipid content in microalgae

Lipid composition of a particular algal species is altered by its life cycle due to physical factors in addition to the medium composition [46]. During photosynthesis, sunlight driven cell factories (algae) produce carbohydrates, lipids, proteins and it depends on various factors, [28], e.g. light intensity, temperature, CO_2 and pH levels (Fig. 1).

2.1. Effect of temperature

Temperature is an important physical factor or environmental condition that affects the lipid content in microalgae [54]. Temperature alters the physiological processes of the strain by increasing or decreasing the biochemical reaction rate in cellular compartments [73]. Though lipid induction techniques involve temperature, reports on portraying the effect of different cultivation temperature on the growth and lipid content of microalgae were found to be contradictory (Table 1). The lipid content of *Ochromonas danica* (chrysophytan) and *N. salina* (eustigmatophyte) increased correspondingly with increasing cultivation temperature and contradictorily, *Chlorella sorokiniana* did not show considerable alteration in lipid content [17].

Another study was undertaken by Kalacheva et al. [29] using *Botryococcus braunii*, wherein temperatures of 18 °C, 25 °C and 32 °C showed differences in lipid composition. Intracellular lipid content decreased to 5% under supra-optimal temperature (32 °C) in comparison with 22% at 25 °C and correspondingly, polysaccharides were accumulated and extracellular lipids synthesis was unaltered [29]. Noticeably, the optimum growth temperature for microalgae *Nannochloropsis oculata* and *Chlorella vulgaris* is 25 °C, and a temperature shift from 20 to 25 °C showed a two-fold increase in lipid content from 7.90% to 14.92% in *N. oculata*, and in contrast, 25 to 30 °C shift decreased the lipid composition (14.71% to 5.90%) in *C. vulgaris* [10]. The change in the lipid content and TAG (triglyceride) of *Nannochloropsis salina* under the influence of varying conditions of temperature and nitrogen was carried out by Fakhry and El Maghraby [17]. The authors reported that the lipid and triglyceride content increased when the temperature was raised from 15 to 35 °C.

Further, it has been reported that both nitrogen and temperature's synergistic effect on lipid accumulation was not observed to be as fundamental as for nitrogen depletion. Similarly, *Thalassiosira pseudonana, Odontella aurita, Nannochloropsis oculata, Isochrysis galbana, Chromulina ochromonoides,* and *Dunaliella tertiolecta*, tested under 10 and 20 °C and dual nutrient conditions (sufficient and deficient) apparently revealed that multiple stressors (interactive conditions) did not have an additive effect on the lipid content and the lipid content improved only due to nutrient deficiency rather than temperature [54,65]. Concerning the effect of temperature on the fatty acid content, microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* were grown under different temperature ranges, i.e. 15, 20, 25, 30, and 35 °C [77]. At supra optimal temperature (high), neutral lipid and PUFAs were decreased coupled with an increase in MUFAs [77]. Similarly, the marine microalga *Dunaliella salina* was comprehensively studied for the analysis of lipid profile under low temperature conditions. Shifting the temperature from high to low values (30 °C to 12 °C) increased the unsaturated fatty acids content by 20% [63]. Irrespective of a taxonomic group, prokaryotic alga *Spirulina platensis* and eukaryotic algae *C. vulgaris* and *Botryococcus braunii*, showed alteration in their fatty acid profile under different temperature regime, i.e., the relative percentage or level of UFAs decreased over SFAs with increasing temperature [70]. Increase in UFA's concentration with decreasing temperature and high saturated fatty acid content with reduction in temperature was generally noticed in several algal strains, and high unsaturated fatty acid content under low temperature enhances the membrane fluidity and provides an adaptation to stressful environments [8,38].

Literature data inferring the effect of temperature on microalgae does not suggest a similar trend, as different experiments have been performed with different temperature ranges. It is unclear to explain the exact phenomenon behind the lipid accumulation in microalgae when exposed to different temperature regimes. Few proposals have been suggested pertaining to the lipid changes during temperature exposure. Increased lipid yield of microalgae under high temperatures is owing to maximal utilization of lipids as a storage product [59]. In disparity, it is speculated that inferior biomass production and low lipid yield of microalgae was presumably due to the stress of photosystem II activity when it is cultured beyond the optimal temperature [64]. Further, the reduced microalgal growth under extreme cultivation temperature is due to the obstruction of metabolic processes of the microalgal cell and termination of cell multiplication by irreversible enzyme destruction [52].

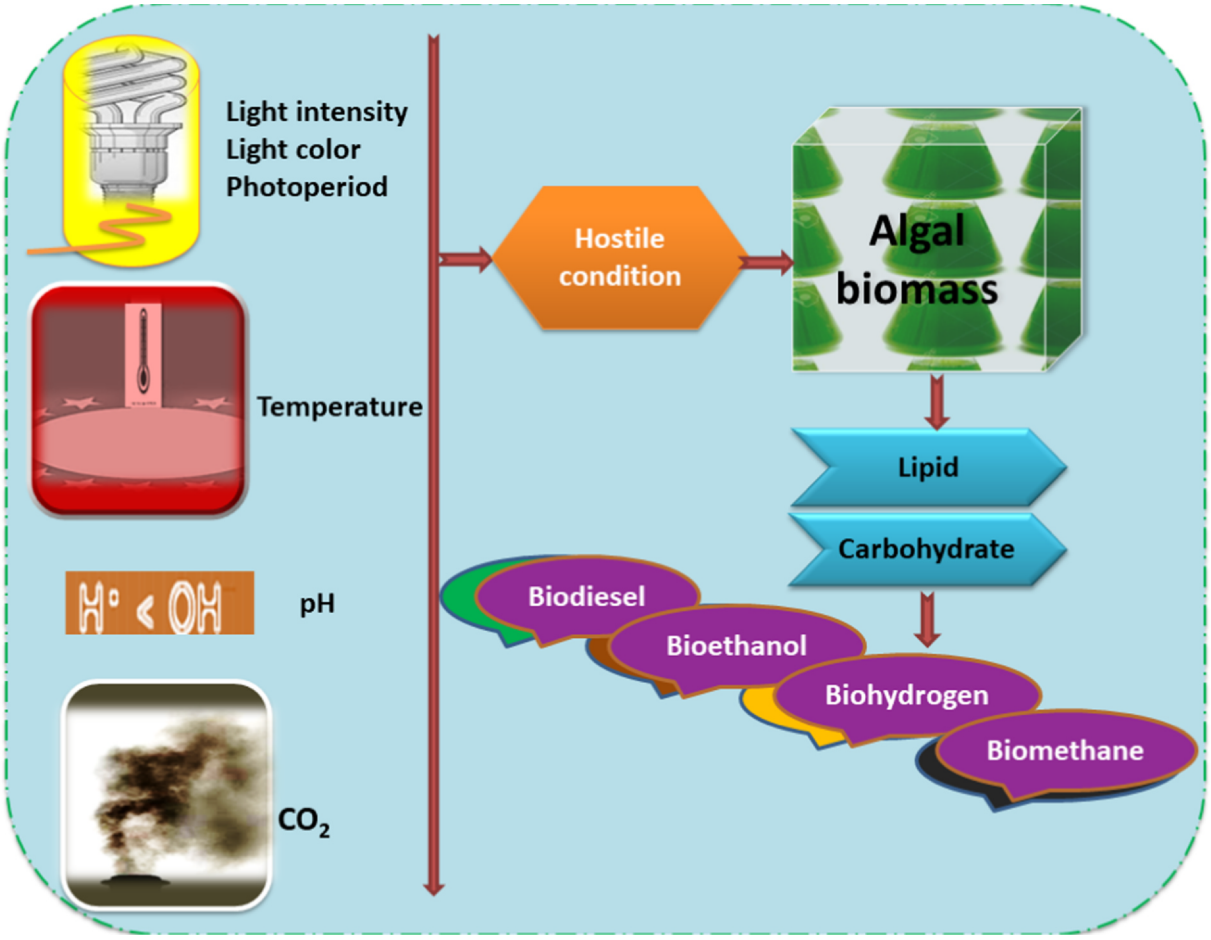

Fig. 1. Effect of various cultivation conditions on microalgae.

Table 1
Impact of different temperatures on the lipid content and fatty acid profile of microalgae.

Algae	Temperature	Response	Reference
Nannochloropsis oculata	Shift – 20 °C to 25 °C	14.92% increase in total lipid	[10]
Chaetoceros sp.	Grew at 25 °C	16.8% increase in total lipid	[63]
Botryococcus braunii	Supra-optimal (32 °C)	Intracellular lipid synthesis decreased 5%	[29]
Chlorella vulgaris	25 to 30 °C	Lipid content decreased to about 6 from15%	[10]
Ochromonas danica	15 °C to 30 °C	Increase in total lipids	[63]
Nannochloropsis oculata	> 30 °C	Neutral lipid decreased and polar lipid increased	[77]
Nitzschia laevis	15 to 23 °C	TAG increased and phospholipid decreased	[8]
Spirulina platensis	High temperature	Reduction of UFA's	[70]
Chlorella vulgaris, Botryococcus braunii			
Dunaliella salina	30 °C to 12 °C	UFA's increased	[63]
Thermophilic *Synechococcus Lividus*	55 °C to 38 °C	16:1 & 18:1 increased	[18]
Selenastrum capricornutum	25 °C to 10 °C	C 18:1 increased	[40]
Tetraselmis subcordiformis	> 20 °C	Neutral lipid and PUFA were decreased	[77]

2.2. Effect of light intensity

Light (photoperiod and spectral range) is considered to be a crucial environmental factor for microalgal growth [31]. Microalgae grow under various light intensities and wavelengths exhibiting notable changes in their lipid yield, profile and photosynthetic activity (Table 2). According to Liu et al. [37], changes in the intensity of light might alter the quantity and composition of lipids [37]. In filamentous green alga *Cladophora* sp., high light intensity revealed an increase in the TAG content with a concurrent reduction in the polar phospholipid composition [45]. In this connection, changing the light intensity to 1500 from 700 μmol m^{-2} s^{-1} was found to reduce the biomass production of *D. viridis* to 63% [22], while high photon flux density exposure increased its extracellular polysaccharide content [26]. Dark incubation (absence of light) of *D. virdis* was found to increase its total lipid content with a simultaneous reduction in triglycerides and free fatty acids composition [67]. Similarly, low light intensity (35 μE $m^{-2}s^{-1}$) grown *Nannochloropsis* sp., showed 40% galactolipid and 26% TAG of total lipid. In the same system, as expected, high TAG content with a parallel reduction in galactolipid was observed under 550 μE $m^{-2}s^{-1}$, i.e. high light conditions [69]. As shown in Table 2, various strains showed high lipid content under high light illumination. For example, the unicellular microalga *Scenedesmus* sp. grown under 250–400 μmol m^{-2} s^{-1} light conditions unveiled high storage lipid content [36]. Increasing the photon flux up to 150 μmol $m^{-2}s^{-1}$ increases the total lipid of *Isochrysis galbana* LB987, *N. oculata* CCAP849/

Table 2
Influence of different light intensities, light color, light period on the lipid content and fatty acid profile of microalgae.

Algae	Light intensity/color	Response	Reference
Chlorella vulgaris	2700 lx light	Total lipid 19%	[15]
	3300 lx light	Total lipid 13% unaltered	
Chlorella vulgaris	Red color light	Lipid content increased	[15]
	Green light	Lipid content decreased 5%over control	
Chlorella vulgaris	24 h photoperiod	Lipid production increased/ decreased	[15]
	16:8 L/D		
Pavlova lutheri	High light intensities	Increased total lipid content	[63]
Isochrysis galbana, Nannochloropsis oculata	High light intensity > 100 $\mu molm^2s^{-1}$	Total lipid content increased over control	[20]
Scenedesmus sp.	250–400 $\mu molm^2s^{-1}$	Neutral lipid content increased	[36]
Marine chlorella sp. *Nannochloropsis* sp.	10,000 lx	Lipid content decreased	[6]
Isochrysis galbana	Shorter light/dark regime	PUFA's increased	[63]
Selenastrum capricornutum	Dark treatment	Increase in 18:3 and decrease in 18:1	[40]

1, and *D. salina*, respectively [20]. In contrast, two marine microalgae, *Chlorella* sp. and *Nannochloropsis* sp., grew under high 10,000 lx photon density exhibited low total lipid productivity [6]. Effect of various light colours, light intensities on lipid yield of axenic microalga *Chlorella* sp. was investigated by Durairaj et al. [15]. The highest lipid content of 19.4% was obtained under 2700 lx, which is higher than other light intensities exposed cultures. Regarding the impact of light colours on the lipid content, culture grown under the red light showed high lipid content compared to yellow and white light (control) grown culture; at the same time, the culture grown under green light showed low lipid content of 7.25% in the same system.

Different strains/species respond contrarily to photo stress even though both photosynthesis and photo acclimatization processes are driven by light. For example, *Chlorella* sp. grown under continuous illumination of light (24 h) indicated high biomass and lipid yield of 0.54 g L^{-1} and 0.0791 g L^{-1}, respectively [15]). Similar results were noticed with *P. lutheri* [61], *S. obliquus* and *B. braunii* [32]. Furthermore, *Chlorella Santissima* that was grown under a 24 h photoperiod (200–400 $\mu E/m^2s$ of light intensity) did not show considerable differences in its fatty acid composition [71]. With reference to the impact of light intensity on the fatty acid profile of microalgae, various studies have suggested that PUFA level decreases with increase in the light intensity [28]. As reported by Al-Qasmi et al. [1], algae cultivated under high photon intensity and prolonged light duration was found to possess increased levels of saturated fatty acids (SFA) coupled with a decline in the content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). In addition, photoperiod also has a considerable influence on the lipid content of microalgae. Light/dark cycles on fatty acid profile of *Thalassiosira pseudonana* disclosed high SFA, MUFA and lesser PUFA content under a 12:12 h photoperiod [5]. Contradictorily, when the photon flux of light intensity is increased from 50 to 150 $\mu mol\ m^{-2}\ s^{-1}$, the fatty acid content of algal biofilms was also shown to increase, but increasing the photon flux beyond the threshold i.e., > 300 and 600 $\mu mol\ m^{-2}\ s^{-1}$, the fatty acid profile was found to be statistically similar [60]. The interactive impact of light intensity (0 to 1920 $\mu mol\ m^{-2}\ s^{-1}$) and CO_2 concentration (1–5%) on the lipid content and fatty acid profile of *Synechocystis* sp. PCC6803 in continuous-flow photobioreactor was reported [11]. The total lipid content increased linearly with increasing light intensity to 1920 $\mu mol\ m^{-2}\ s^{-1}$ and 3% CO_2 whereas increased light illumination did not have any influence on the fatty acid composition.

The growth and lipid content of algae is strain specific. High light intensity above the threshold limit causes photoinhibition due to the chloroplast lamellae disruption and inactivation of CO_2 fixing enzymes [28]. The phenomenon of photoinhibition is explained as a damage of the photosynthetic receptor system due to the high illumination intensities above the light compensation point [75]. Physiological or internal acclimatization of microalgae to light intensity is accomplished by variety of mechanisms such as pigment types, dark respiration rate and the presence of essential fatty acids [16], whereas morphological photoacclimation occurs by an alteration in the cell density or volume, density and number of photosynthetic apparatus, i.e., thylakoid membranes. Further, it is stated that algae surpass the low light intensity (limitation) through desaturation of chloroplast membranes [41]. Dissipation of surplus photon energy and deterrence of photochemical destruction to algal cells could have been aided by ATP and NAD(P)H (produced by photosynthesis), which are required in large amount for the synthesis of TAG. Therefore, the carbon flux generated through photosynthesis is directed to lipid synthesis when the algae are kept under high light illumination conditions [36,68]. On the other hand, in the case of growth, cells absorbs the excess excitation energy, which causes photodamage to cells (reduction of acceptors of PS II) and particularly, reaction centre polypeptide is more liable to damage during increased light exposure [24]. During acclimatization to high light intensities, the ratio of PS I/PSII is about 5 in microalgae like *Synechocystis* sp. PCC6883. This abundance was related to an oxidized plastoquinone and thus, photo damage could be minimized [11]. It is inferred that light requirement is strain/species dependent and it is essential for storage lipid synthesis, and in general, the light will elicit fatty acid biosynthesis, culture growth and chloroplast membrane formation [30].

2.3. Effect of CO_2

The CO_2 concentration in the environment is reduced by carbon capture and sequestration; in this regard, the sequestration of CO_2 through microalgae is of interest to researchers. Microalgae can mitigate carbon dioxide emissions either from the atmosphere or more concentrated sources, such as power plants and industrial flue gases through CO_2 bio-fixation. While the former is easy to accomplish but ineffective due to a low carbon concentration (few hundred ppm), the latter is more promising, since higher CO_2 level (up to 20%) enhances the carbon sequestration efficiency, with added environmental benefits. Green algae can be grown easily in higher CO_2 levels and *Chlorella* offers high photosynthetic efficiency during CO_2 sequestration [66].

Therefore, the cultivation of microalgae using point-source real flue gases seems to be very attention-grabbing. Notwithstanding that, flue gases might contain harmful substances such as NO_x and SO_x, which deserves further attention [5]. In detail, the carbon dioxide level might affect two characteristics: first, the growth rate, and second, the lipid (fatty acids) content. In particular, the latter characteristic is important to optimize the further conversion to a specific kind of biofuel. In this regard, several authors had investigated the influence of carbon dioxide on the growth, lipid, and fatty acid of many microalgal strains. Yoo et al. [79] investigated the response of three different strains to various carbon dioxide concentration, i.e. up to 10% [79]. In detail, the species taken for the experiments were: *Botryococcus braunii*, *Chlorella vulgaris* and *Scenedesmus* sp. As a first outcome, the authors confirmed that all three species are able to grow up to the maximum carbon dioxide levels tested in the study (10%) and after a 14-day cultivation period, different biomass and total lipid productivity was observed for the three

Table 3
Effect of carbon dioxide concentration in aeration medium on the biomass and lipid productivity of several microalgae strains.

Microalgae strain	CO_2 concentration	Biomass productivity	Hydrocarbon yield	Total lipid productivity	Reference
Botryococcus braunii	10%	26.55 mg_{dw}/L/d	–	5.51 mg /L/d – about 21%	[79]
	6%	87 mg_{dw}/L/d	–		[13]
	18%	The growth has been demonstrated.			
	5.5% (flue gases)	77 mg_{dw}/L/d	–	21 mg /L/d - about 24%	[79]
Botryococcus braunii 765	2%	–	16.43%	10.41%	[19]
	5%	–	18.25%	11.21%	[19]
	10%	–	21.03%	12.41%	[19]
	20%	–	24.45%	12.71%	[19]
Chlorella vulgaris	10%	104.76 mg_{dw}/L/d	–	6.91 mg /L/d	[79]
Chlorella vulgaris	5% with 0.5 vvm aeration	–	–	157 mg L^{-1} d^{-1}	[81]
	6	85 mg_{dw}/L/d	–	–	[12]
Scenedesmus obliquus	6%	100 mg_{dw}/L/d			[12]
	12%	140 mg_{dw}/L/d	–		[12]
Scenedesmus sp.	10%	217.50 mg_{dw}/L/d	–	20.65 mg /L/d – 9%w	[79]
	5.5% (flue gases)	203 mg_{dw}/L/d	–	39 mg /L/d − 18%w)	[53,79]

strains. Specifically, *Botryococcus braunii* exhibited a biomass productivity of about 26.55 mg L^{-1} d^{-1} and a total lipid productivity of about 5.51 mg L^{-1} d^{-1}. Similarly, the same parameters were estimated for *Chlorella vulgaris*, resulting in 104.76 mg L^{-1} d^{-1} and 6.91 mg L^{-1} d^{-1} and for *Scenedesmus* sp., 217.50 mg L^{-1} d^{-1} and 20.65 mg L^{-1} d^{-1}, respectively. In the same study, two of the mentioned strains were tested under a real flue gases atmosphere, featuring a carbon dioxide concentration of 5.5%. Under these conditions, the biomass productivity of both species was found to be enhanced and notably, the lipid productivity of *Scenedesmus* sp. exhibited a 3.7-fold increase, i.e. 21 mg L^{-1} d^{-1}, which is equivalent to 24%$_w$. In another study, Ge et al. [19] performed experiments using *Botryococcus braunii* 765 and tested up to 20% CO_2 [19]. According to their findings, the biomass productivity increases with an increase in the CO_2 concentration. Specifically, varying the CO_2 concentration from 2% up to 20% showed hydrocarbon yield variation in the range of 16.43–24.45% (on a weight basis), while the total lipids varied from 10.41–12.71% (on a weight basis). More details are shown in Table 3. *Chlorella vulgaris* grown under different CO_2 concentration showed that, 5% CO_2 with 0.5 vvm aeration rate revealed biomass concentration and lipid productivity of 3.83 g L^{-1} and 157 mg L^{-1} d^{-1}, respectively [81].

As reported by Ranga Rao et al. [51], among 0.5, 1.0, and 2.0% CO_2 concentrations, 2% CO_2 has shown a two-fold increase in the growth and carotenoid content of *Botryococcus braunii* compared to control culture with no CO_2 supply. Further, 1% and 2% CO_2 has increased the palmitic acid and oleic acid levels by 2.5 to 3 folds, respectively, in *B. braunii* LB-572. Further, the duration of CO_2 supply to the culture is also considered as a crucial factor in determining the amount of fatty acid [44] and particularly, a one-day long increase in CO_2 from 2% to 10% resulted in a slight increase of the total fatty acids (+30%) in *Dunaliella salina*, while after a 7-day long increase, the fatty acid quantity was magnified by a factor 2.7. Generally, increasing the carbon dioxide concentration in the culture media has a beneficial effect on the growth of microalgae. However, this is strongly related to the specific species. In fact, as witnessed by Chiu et al. [9] there are species (*Chlorella* sp.) which exhibit a decreasing productivity as long as the carbon dioxide concentration goes up. For instance, investigating the CO_2 concentration in the range of 2%-15%, *Chlorella* sp. showed a maximum biomass productivity and lipid productivity of 422 mg L^{-1} d^{-1}, and 143 mg L^{-1} d^{-1}, respectively, at a CO_2 concentration of 2%. Nonetheless, it was observed that, the lipid fraction did not vary with the carbon dioxide concentration. A similar trend was observed in *Dunaliella salina*, as reported by Ying et al. [78].

The summary of literature reports is given in Table 3, where for each strain considered; main experimental outcomes (total biomass productivity, hydrocarbon yields and total lipid productivity) are reported. To conclude, once the tolerance threshold to carbon dioxide has been assessed, it is a good criterion to push carbon dioxide concentration in the culture media to the level required for the maximum growth rate of the specific strain. From an environmental benefit view point, in many studies, inorganic CO_2 source by means of untreated and desulphated flue gas were used and interestingly, no remarkable difference in the biomass content of *Nannochloropsis salina and Phaeodactylum tricornutum* was noticed. Further, the presence of trace amount of nickel, vanadium and mercury in the flue gas might affect the productivity while NOx was taken up as a nitrogen source by cells [42]. The comparative tolerance level of *T. suecica* and *Chlorella* sp. (regulated by pH) to CO_2 and flue gas was ascertained, in which higher lipid productivity of 14.8 mg lipid L^{-1} day^{-1} and 13.70 mg lipid L^{-1} day^{-1} was recorded for *T. suecica* and *Chlorella* sp., respectively, when the CO_2 was purged to the algal suspension and the same results were also observed for flue gas purged cells [42]. In another study, the total lipid content increased to 21% when *D. viridis* was grown under higher CO_2 concentration and nitrogen limitation condition. At higher CO_2 concentrations, carbon incorporation is enforced under nitrogen deficiency and at the same time, carbon skeletons were not integrated into the proteins to let the cells grow, and thus lipid synthesis pathway can be engaged as the major carbon sink [23].

2.4. Effect of pH

The pH is one of the key physical factors as it governs the availability and distribution of inorganic carbon source and the essential nutrients [7]. pH is a basic operational factor driving microalgal growth since pH affects the photosynthetic process, the availability presence of inorganic nutrients through carbon and metal speciation, the lipid production rate, and the enzyme activities within cell [27]. Microalgal metabolism is also influenced in response to pH and optimum pH range of algae is strain or species specific [42]. The biomass and lipid yield of algae is also altered in response to change in the pH of the cultivation medium. Algal growth medium is initially kept at neutral pH at the time of inoculation. As the carbonaceous species in medium is regulated by the pH, the pH of the algal suspension increases steadily during the day time owing to the utilization of inorganic carbon, however, extreme pH of the cultivation system hampers the availability of CO_2, and therefore hinders the microalgal growth [49]. Both increase or decrease or no change in the lipid content or productivity of algae has been reported in the literature. Various researchers have ascertained the influence of pH on biomass and lipid content of algae (Table 4). For *Chlorella protothecoides*, the optimal pH was found to be 6.5 [33]

The impact of different pre-set pH ranges on the growth of *Skeletonema costatum* was tested in a semicontinuous mode, and the results showed that at pH 6.0 − 9.0, growth was constant, whereas inferior growth was observed at pH 9.0; this is due to the lower rate of

Table 4
Effect of different pH levels on the lipid content of various algae.

Algae	Optimal pH identified	Response	Impact	References
Chlorella sp.	8.0	Lipid content 23%	Positive	[50]
C. vulgaris	7.5	53.43	Positive	[55]
Chlorella protothecoides	6.5 (simulating gauss function)	3.75 g/l lipid yield	Positive	[33]
Chlorella vulgaris	6.0, 7.0, 9.0	No change. 22% (lipid content unaltered)	Nil	[39]
C. vulgaris	7.0, 8.0, 9.0, and 10.0.	No change in the cell composition	Nil	[21]
Pavlova lutheri	8.0	35% lipid content	Positive	[61]
T. suecica	9.0	Neutral lipid content increased	Positive	[3]
Nannochloropsis salina	8.0–9.0	21.8	Positive	[4]
Chroococcus minor	9.0	22	Positive	[2]
B. braunii	6.5	2.2 g m^{-2} d^{-1} areal lipid productivity	positive	[27]

certain key biochemical reactions, as well change in the membrane properties [72]. Biomass and lipid productivities of *Chlorella* sp. and *Tetraselmis suecica* CS-187 was studied in response to different pH and inorganic carbon sources. High biomass and lipid productivities of 320 mg $L^{-1}day^{-1}$ and 92 mg $L^{-1}day^{-1}$ was observed for *T. suecica* at pH 7.5, respectively, while 407 mg $L^{-1}day^{-1}$ and 99 mg $L^{-1}day^{-1}$ was obtained for *Chlorella* sp., respectively, at pH 7.0 [42]. Further, the impact of different pH levels (6.0, 7.0, 8.0, 9.0, and 10.0) on the biomass and lipid accumulation of *Nannochloropsis salina* was evaluated and the data revealed that *N. salina* showed its highest growth rates at pH 8.0 and 9.0 and lipid content of unbuffered system was 21.8% FAME (on mass basis). Further, that study concluded that the maximal lipid production and minimal invading organisms was observed at that pH range [4]. In heterotrophically cultivated *C. vulgaris*, the optimal pH that aids maximal specific growth rate (0.541 $days^{-1}$) and lipid content (53.43%) under sulfur limitation was 7.5. Cells did not survive at pH values of 3.0, 4.0 and 11.0 and aggregation of algal cells was noticed at pH 9.5. Further, fatty acid composition of *C. vulgaris* was found to be unaffected by the pH values [55].

Chlorella sp. HQ was grown under initial pH ranges from 5.0 to 11.0 to assess its biomass and lipid production capability [80]. Alkaline pH was noticed after 8 days of incubation due to the CO_2 absorption and nitrate consumption, and release of algal cells was observed at its stationary phase. After 30 days, higher lipid content (32.8%), and lipid yield (168 mg L^{-1}) was observed at an initial pH of 7.0, whereas the maximum TAG content of 63% was obtained at a lower initial pH of 5.0. A similar study reported that among the wide ranges of pH tested (6.0, 6.5, 7.0, 7.5, 8.0, and 8.5), higher biomass production was obtained at pH 6.5 and 7.0 and maximal lipid production was observed in the range of 7.0–8.5 [76]. The optimal pH for marine microalga *Dunaliella salina* is around 11.5, whereas the pH is 3.0 for *Dunaliella acidophila* [74]. The high levels of PUFA was seen in *Pinguiococcus pyrenoidosus* at a pH of 6.0 [56]. In another study, higher lipid yield of 0.1995 g L^{-1} and lipid content of 23% was reported under pH 8.0 for *Chlorella* sp. [50]. The effect of pH 7.0, 8.0, and 9.0 was tested each week from 1 to 4 weeks after inoculation of *T. suecica,* and the maximum neutral lipid levels measured in terms Nile red fluoresce was noticed at pH 9.0 for 2 weeks [3]. In a review by Moheimani [42], adjusting the pH 7.0–7.5 in the algal suspension using CO_2 augmented the lipid productivity of *Nannochloropsis salina* and *Phaeodactylum tricornutum* when cultivated in paddle wheel driven raceway pond.

3. Conclusions

Necessity for replacing fossil based fuels is the most urgent requirement in recent times. Biofuels from microalgae are considered as a potential substitute to fossil-based fuel and biofuels are considered as non-toxic and renewable fuels. Algae are easily available source for generating biofuels like bioethanol, biobutanol, biodiesel, biogas, and biohydrogen. However, to meet the present day fuel requirement, lipid yield from microalgae is not adequate; therefore, researchers have focused their attention on enhancing the lipid yield through altering the cultivation conditions. Most important cultivation conditions such as temperature, light intensities, light color, light duration, CO_2, and pH are the major requirement for efficient microalgal growth and their production. Therefore, this review has given a comprehensive discussion on increasing the biomass, and lipid content in microalgae. Besides, this article has provided a detailed review on the effect of cultivation parameters influencing the lipid productivity and fatty acid composition of microalgae.

CRediT authorship contribution statement

Kathirvel Brindhadevi: Conceptualization, Methodology, Writing - review & editing. **Thangavel Mathimani:** Investigation, Writing - original draft. **Eldon R. Rene:** Writing - review & editing. **Sabarathinam Shanmugam:** Writing - review & editing. **Nguyen Thuy Lan Chi:** Supervision, Project administration. **Arivalagan Pugazhendhi:** Supervision, Project administration.

Declaration of Competing 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] Al-Qasmi M, Raut N, Talebi S, Al-Rajhi S, Al-Barwani T. A review of effect of light on microalgae growth, Proceedings of the world congress on engineering, pp. 4–6, 2012.
[2] Al-Safaar AT, Al-Rubiaee GH, Salman SK. Effect of pH condition on the growth and lipid content of microalgae Chlorella vulgaris & Chroococcus minor. Int J Sci Eng Res 2016;7.
[3] Almutairi A, Toulibah H. Effect of salinity and ph on fatty acid profile of the Green Algae Tetraselmis suecica. J Pet Environ Biotechnol 2017;8:3–8.
[4] Bartley ML, Boeing WJ, Dungan BN, Holguin FO, Schaub T. pH effects on growth and lipid accumulation of the biofuel microalgae Nannochloropsis salina and invading organisms. J Appl Phycol 2014;26:1431–7.
[5] Brown MR, Dunstan GA, Norwood SJ, Miller KA. Effects of harvest stage and light on the biochemical composition of the diatom Thalassiosira pseudonana 1. J Phycol 1996;32:64–73.
[6] 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;110:510–6.
[7] Chen CY, Durbin EG. Effects of pH on the growth and carbon uptake of marine phytoplankton. Marine Ecology-Progress Series 109, 83-83, 1994.
[8] Chen G-Q, Jiang Y, Chen F. Variation of lipid class composition in Nitzschia laevis as a response to growth temperature change. Food Chem 2008;109:88–94.
[9] Chiu S-Y, Kao C-Y, Chen C-H, Kuan T-C, Ong S-C, Lin C-S. Reduction of CO2 by a high-density culture of Chlorella sp. in a semicontinuous photobioreactor. Bioresour Technol 2008;99:3389–96.
[10] Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M. Effect of temperature and nitrogen concentration on the growth and lipid content of Nannochloropsis oculata and Chlorella vulgaris for biodiesel production. Chem Eng Process Process Intensif 2009;48:1146–51.
[11] Cuellar-Bermudez SP, Romero-Ogawa MA, Vannela R, Lai YS, Rittmann BE, Parra-Saldivar R. Effects of light intensity and carbon dioxide on lipids and fatty acids produced by Synechocystis sp. PCC6803 during continuous flow. Algal Res 2015;12:10–6.

[12] De Morais MG, Costa JAV. Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 2007;129:439–45.
[13] de Morais MG, Costa JAV. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. Energy Convers Manage 2007;48:2169–73.
[14] Dellomonaco C, Fava F, Gonzalez R. The path to next generation biofuels: successes and challenges in the era of synthetic biology. Microb Cell Fact 2010;9:1–15.
[15] Durairaj B, Muthu S, Xavier T. Antimicrobial activity of Aspergillus niger synthesized titanium dioxide nanoparticles. Adv Appl Sci Res 2015;6:45–8.
[16] Fábregas J, Maseda A, Domínguez A, Otero A. The cell composition of Nannochloropsis sp. changes under different irradiances in semicontinuous culture. World J Microbiol Biotechnol 2004;20:31–5.
[17] Fakhry EM, El Maghraby DM. Lipid accumulation in response to nitrogen limitation and variation of temperature in Nannochloropsis salina. Botanical Studies 2015;56:6.
[18] Fork DC, Murata N, Sato N. Effect of growth temperature on the lipid and fatty acid composition, and the dependence on temperature of light-induced redox reactions of cytochrome f and of light energy redistribution in the thermophilic blue-green alga Synechococcus lividus. Plant Physiol 1979;63:524–30.
[19] Ge Y, Liu J, Tian G. Growth characteristics of Botryococcus braunii 765 under high CO2 concentration in photobioreactor. Bioresour Technol 2011;102:130–4.
[20] Gim GH, Ryu J, Kim MJ, Kim PI, Kim SW. 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;43:605–16.
[21] Gong Q, Feng Y, Kang L, Luo M, Yang J. Effects of light and pH on cell density of Chlorella vulgaris. Energy Procedia 2014;61:2012–5.
[22] Gordillo FJ, Goutx M, Figueroa FL, Niell FX. Effects of light intensity, CO2 and nitrogen supply on lipid class composition of Dunaliella viridis. J Appl Phycol 1998;10:135–44.
[23] Gordillo FJ, Goutx M, Figueroa FL, Niell FX. Effects of light intensity, CO 2 and nitrogen supply on lipid class composition of Dunaliella viridis. J Appl Phycol 1998;10:135–44.
[24] He Q, Dolganov N, Björkman O, Grossman AR. The high light-inducible polypeptides in synechocystis PCC6803 expression and function in high light. J Biol Chem 2001;276:306–14.
[25] Huang G, Chen F, Wei D, Zhang X, Chen G. Biodiesel production by microalgal biotechnology. Appl Energy 2010;87:38–46.
[26] Iqbal M, Zafar S. Effects of photon flux density, CO 2, aeration rate, and inoculum density on growth and extracellular polysaccharide production by Porphyridium cruentum. Folia Microbiol 1993;38:509–14.
[27] Jin J, Dupré C, Legrand J, Grizeau D. Extracellular hydrocarbon and intracellular lipid accumulation are related to nutrient-sufficient conditions in pH-controlled chemostat cultures of the microalga Botryococcus braunii SAG 30.81. Algal Res 2016;17:244–52.
[28] Juneja A, Ceballos RM, Murthy GS. Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: a review. Energies 2013;6:4607–38.
[29] Kalacheva G, Zhila N, Volova T, Gladyshev M. The effect of temperature on the lipid composition of the green alga Botryococcus. Microbiology 2002;71:286–93.
[30] Khotimchenko SV, Yakovleva IM. Lipid composition of the red alga Tichocarpus crinitus exposed to different levels of photon irradiance. Phytochemistry 2005;66:73–9.
[31] Koller M, Salerno A, Tuffner P, Koinigg M, Böchzelt H, Schober S, et al. Characteristics and potential of micro algal cultivation strategies: a review. J Cleaner Prod 2012;37:377–88.
[32] Krzemińska I, Pawlik-Skowrońska B, Trzcińska M, Tys J. Influence of photoperiods on the growth rate and biomass productivity of green microalgae. Bioprocess Biosyst Eng 2014;37:735–41.
[33] Liang G, Mo Y, Tang J, Zhou Q. Improve lipid production by pH shifted-strategy in batch culture of Chlorella protothecoides. African J Microbiol Res 2011;5:5030–8.
[34] Liu J, Huang J, Sun Z, Zhong Y, Jiang Y, Chen F. Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic Chlorella zofingiensis: assessment of algal oils for biodiesel production. Bioresour Technol 2011;102:106–10.
[35] Liu J, Song Y, Qiu W. Oleaginous microalgae Nannochloropsis as a new model for biofuel production: review & analysis. Renew Sustain Energy Rev 2017;72:154–62.
[36] Liu J, Yuan C, Hu G, Li F. Effects of light intensity on the growth and lipid accumulation of microalga Scenedesmus sp. 11–1 under nitrogen limitation. Appl Biochem Biotechnol 2012;166:2127–37.
[37] Liu Z-Y, Wang G-C, Zhou B-C. Effect of iron on growth and lipid accumulation in Chlorella vulgaris. Bioresour Technol 2008;99:4717–22.
[38] Mathimani T, Nair BB. Evaluation of microalga for biodiesel using lipid and fatty acid as a marker–a central composite design approach. J Energy Inst 2016;89:436–46.
[39] Mathimani T, Uma L, Prabaharan D. Formulation of low-cost seawater medium for high cell density and high lipid content of Chlorella vulgaris BDUG 91771 using central composite design in biodiesel perspective. J Cleaner Prod 2018;198:575–86.
[40] McLarnon-Riches CJ, Rolph CE, Greenway DL, Robinson PK. Effects of environmental factors and metals on Selenastrum capricornutum lipids. Phytochemistry 1998;49:1241–7.
[41] Mock T, Kroon BM. Photosynthetic energy conversion under extreme conditions—II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. Phytochemistry 2002;61:53–60.
[42] Moheimani NR. Inorganic carbon and pH effect on growth and lipid productivity of Tetraselmis suecica and Chlorella sp (Chlorophyta) grown outdoors in bag photobioreactors. J Appl Phycol 2013;25:387–98.
[43] Mujtaba M, Kalam M, Masjuki H, Gul M, Soudagar MEM, Ong HC, et al. Comparative study of nanoparticles and alcoholic fuel additives-biodiesel-diesel blend for performance and emission improvements. Fuel 2020;279:118434.
[44] Muradyan E, Klyachko-Gurvich G, Tsoglin L, Sergeyenko T, Pronina N. Changes in lipid metabolism during adaptation of the Dunaliella salina photosynthetic apparatus to high CO 2 concentration. Russ J Plant Physiol 2004;51:53–62.
[45] Napolitano GE. The relationship of lipids with light and chlorophyll measurements in freshwater algae and periphyton 1. J Phycol 1994;30:943–50.
[46] Ota M, Kato Y, Watanabe H, Watanabe M, Sato Y, Smith Jr RL, et al. Fatty acid production from a highly CO2 tolerant alga, Chlorococcum littorale, in the presence of inorganic carbon and nitrate. Bioresour Technol 2009;100:5237–42.
[47] Prabakar D, Manimudi VT, Sampath S, Mahapatra DM, Rajendran K, Pugazhendhi A. Advanced biohydrogen production using pretreated industrial waste: Outlook and prospects. Renew Sustain Energy Rev 2018;96:306–24.
[48] Pugazhendhi A, Mathimani T, Varjani S, Rene ER, Kumar G, Kim S-H, et al. Biobutanol as a promising liquid fuel for the future-recent updates and perspectives. Fuel 2019;253:637–46.
[49] Qiu R, Gao S, Lopez PA, Ogden KL. Effects of pH on cell growth, lipid production and CO2 addition of microalgae Chlorella sorokiniana. Algal Res 2017;28:192–9.
[50] Rai MP, Gautom T, Sharma N. Effect of salinity, pH, light intensity on growth and lipid production of microalgae for bioenergy application. OnLine J Biol Sci 2015;15:260.
[51] Ranga Rao A, Sarada R, Ravishankar G. Influence of CO~ 2 on Growth and Hydrocarbon Production in Botryococcus braunii. J Microbiol Biotechnol 2007;17:414.
[52] Renaud SM, Thinh L-V, Lambrinidis G, Parry DL. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 2002;211:195–214.
[53] Rodolfi L, Chini Zittelli G, Bassi N, Padovani G, Biondi N, Bonini G, et al. Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnol Bioeng 2009;102:100–12.
[54] Roleda MY, Slocombe SP, Leakey RJ, Day JG, Bell EM, Stanley MS. Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresour Technol 2013;129:439–49.
[55] Sakarika M, Kornaros M. Effect of pH on growth and lipid accumulation kinetics of the microalga Chlorella vulgaris grown heterotrophically under sulfur limitation. Bioresour Technol 2016;219:694–701.
[56] Sang M, Wang M, Liu J, Zhang C, Li A. Effects of temperature, salinity, light intensity, and pH on the eicosapentaenoic acid production of Pinguiococcus pyrenoidosus. J Ocean Univ China 2012;11:181–6.
[57] Saravanan AP, Mathimani T, Deviram G, Rajendran K, Pugazhendhi A. Biofuel policy in India: a review of policy barriers in sustainable marketing of biofuel. J Cleaner Prod 2018;193:734–47.
[58] Saravanan AP, Pugazhendhi A, Mathimani T. A comprehensive assessment of biofuel policies in the BRICS nations: Implementation, blending target and gaps. Fuel 2020;272:117635.
[59] Sayegh FA, Montagnes DJ. Temperature shifts induce intraspecific variation in microalgal production and biochemical composition. Bioresour Technol 2011;102:3007–13.
[60] Schnurr PJ, Espie GS, Allen GD. The effect of photon flux density on algal biofilm growth and internal fatty acid concentrations. Algal Res 2016;16:349–56.
[61] Shah SMU, Radziah CC, Ibrahim S, Latiff F, Othman MF, Abdullah MA. Effects of photoperiod, salinity and pH on cell growth and lipid content of Pavlova lutheri. Ann Microbiol 2014;64:157–64.
[62] Sharma J, Kumar SS, Bishnoi NR, Pugazhendhi A. Enhancement of lipid production from algal biomass through various growth parameters. J Mol Liq 2018;269:712–20.
[63] Sharma KK, Schuhmann H, Schenk PM. High lipid induction in microalgae for biodiesel production. Energies 2012;5:1532–53.
[64] Sheng J, Kim HW, Badalamenti JP, Zhou C, Sridharakrishnan S, Krajmalnik-Brown R, et al. Effects of temperature shifts on growth rate and lipid characteristics of Synechocystis sp. PCC6803 in a bench-top photobioreactor. Bioresour Technol 2011;102:11218–25.
[65] Shuping Z, Yulong W, Mingde Y, Kaleem I, Chun L, Tong J. Production and characterization of bio-oil from hydrothermal liquefaction of microalgae Dunaliella tertiolecta cake. Energy 2010;35:5406–11.
[66] Singh S, Singh P. Effect of CO2 concentration on algal growth: a review. Renew Sustain Energy Rev 2014;38:172–9.
[67] Smith RE, Cavaletto JF, Eadie B, Gardner WS. Growth and lipid composition of high Arctic ice algae during the spring bloom at Resolute, Northwest Territories, Canada. Mar Ecol Progress Series 1993:19–29.
[68] Solovchenko A, Khozin-Goldberg I, Didi-Cohen S, Cohen Z, Merzlyak M. Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga Parietochloris incisa. J Appl Phycol 2008;20:245–51.
[69] Sukenik A, Carmeli Y, Berner T. Regulation of fatty acid composition by irradiance level in the eustigmatophyte Nannochloropsis sp. 1. J Phycol 1989;25:686–92.
[70] Sushchik N, Kalacheva G, Zhila N, Gladyshev M, Volova T. A temperature dependence of the intra-and extracellular fatty-acid composition of green algae and cyanobacterium. Russ J Plant Physiol 2003;50:374–80.
[71] Tang H, Chen M, Garcia M, Abunasser N, Ng KS, Salley SO. Culture of microalgae Chlorella minutissima for biodiesel feedstock production. Biotechnol Bioeng 2011;108:2280–7.
[72] Taraldsvik M, Myklestad S. The effect of pH on growth rate, biochemical composition and extracellular carbohydrate production of the marine diatom Skeletonema costatum. Eur J Phycol 2000;35:189–94.

[73] Van Wagenen J, Miller TW, Hobbs S, Hook P, Crowe B, Huesemann M. Effects of light and temperature on fatty acid production in Nannochloropsis salina. Energies 2012;5:731–40.
[74] Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP. Extremophilic microalgae and their potential contribution in biotechnology. Bioresour Technol 2015;184:363–72.
[75] Wahidin S, Idris A, Shaleh SRM. The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. Bioresour Technol 2013;129:7–11.
[76] Wang C, Li H, Wang Q, Wei P. Effect of pH on growth and lipid content of Chlorella vulgaris cultured in biogas slurry. Sheng wu gong cheng xue bao = Chin J Biotechnol 2010;26:1074–9.
[77] Wei L, Huang X, Huang Z. Temperature effects on lipid properties of microalgae Tetraselmis subcordiformis and Nannochloropsis oculata as biofuel resources. Chin J Oceanol Limnol 2015;33:99–106.
[78] Ying K, Zimmerman W, Gilmour D. Effects of CO and pH on growth of the microalga Dunaliella salina. J Microbial Biochem Technol 2014;6:167–73.
[79] Yoo C, Jun S-Y, Lee J-Y, Ahn C-Y, Oh H-M. Selection of microalgae for lipid production under high levels carbon dioxide. Bioresour Technol 2010;101:S71–4.
[80] Zhang Q, Wang T, Hong Y. Investigation of initial pH effects on growth of an oleaginous microalgae Chlorella sp. HQ for lipid production and nutrient uptake. Water Sci Technol 2014;70:712–9.
[81] Zheng H, Gao Z, Yin F, Ji X, Huang H. Effect of CO2 supply conditions on lipid production of Chlorella vulgaris from enzymatic hydrolysates of lipid-extracted microalgal biomass residues. Bioresour Technol 2012;126:24–30.