Chapter 6
Influence of Culture Conditions on the Microalgal Biomass and Lipid Accumulation

Manisha Verma and Vishal Mishra

Abstract In the current situation, almost every industrial and transportation activities are dependent on fossil fuels for their energy requirements. Fossil fuels are an overpriced and non-renewable source of energy. Besides these fossil fuels, combustion emits greenhouse gases that cause air pollution and global warming. Nowadays, microalgae-originated biofuels gained huge attention from researchers as they are expected to be a potential alternative to conventional fuels. Microalgal biofuel sector considers various strategies to enhance lipid content and biomass productivity in different habitats. To attain high lipid content from microalgae, lipid triggering circumstances are required to be optimized. This chapter summarizes various cultivation conditions, including pH, light intensity, and temperature for raised lipid accumulation inside microalgal cells. Different levels of physical factors affecting microalgal growth and lipid yield have been discussed in this chapter. The influence of the cultivation conditions such as CO_2 concentration, temperature, light colour, and light intensity on lipid accumulation is evaluated comprehensively. Also, very recent progress and research studies on microalgal biomass and biodiesel production are discussed and summarized.

Keywords Microalgae · Lipid accumulation · Algal cultivation · Algal biomass · Biodiesel

Abbreviations

ATP	Adenosine triphosphate
CO_2	Carbon dioxide
MUFA	Monounsaturated fatty acid
NADH	Nicotinamide adenine dinucleotide

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N. Srivastava, P. K. Mishra (eds.), *Technological Advancement in Algal Biofuels Production*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-6806-8_6

PBR	Photobioreactor
PS I	Photosystem I
PS II	Photosystem II
PUFA	Polyunsaturated fatty acid
Q_{10}	Temperature coefficient
SFA	Saturated fatty acid
TAG	Triacylglycerol

6.1 Introduction

In the present, continuous depletion in fossil fuel reservoirs causes scarcity of energy resources. Conventional fuels will be going to become exhausted and unable to satisfy the growing demand for energy. Continuously inflated fuel prices, global warming, and greenhouse gas emission bring the requirement to upgrade sustainable energy resources. Algae-derived biofuels are alternative to replace conventional fuels. Third-generation biofuels include microalga biomass for the production of ethanol, butanol, and biodiesel. Algae have huge potential to replace first- and second-generation agricultural feedstocks as microalgae are rich in carbohydrate-lipid content and have a rapid growth rate and high biomass yield (Verma and Mishra 2020). In fourth-generation biofuels, upcoming advances bring the metabolic and gene engineering approaches to design custom microalgae to increase biofuel production (Abdullah et al. 2019; Moravvej et al. 2019). Brazil, Germany, France, Sweden, and the United States are leading countries in the consumption and development of biofuels (Adenle et al. 2013). It is observed that genetically modified algae shown significant enhancement in biomass, lipid accumulation, and carbon capturing capacity (Beacham et al. 2017; Shuba and Kifle 2018; Dutta et al. 2014; Levitan et al. 2014). Algal species are found in many shapes and sizes, extending from single-cell microalgae to multicellular filaments macroalgae in different aquatic habitats (Shafik et al. 2015). Most of the algal species are found in extreme environmental conditions in various aquatic habitats (Adeniyi et al. 2018). Based on their cellular organization and development, algae are classified into cyanobacteria and eukaryotes. Cyanobacteria are differentiated by the lack of chloroplasts and an adequate nucleus. Based on cell wall composition, storage products, and pigments, eukaryotic algae can be classified into Chrysophyceae, Chlorophyceae, Euglenophyceae, Phaeophyceae, Pyrrophyceae, Rhodophyceae, and Xanthophyceae. Algae achieve its economic significance in biofuel generation, bioremediation, wastewater treatment, nutraceuticals, animal feed, and biofertilizer (Sirakov et al. 2015; Suparmaniam et al. 2019; Pulz and Gross 2004). Additionally, algae are rich in several metabolites, including fatty acids, nutraceuticals, proteins, pigments, and vitamins (Sirakov et al. 2015; Pulz and Gross 2004). Under optimized conditions, algae can grow rapidly and produce two to ten-fold higher lipid content as compared to soybean, jatropha, and rapeseed (Rawat et al. 2013; Chisti 2007;

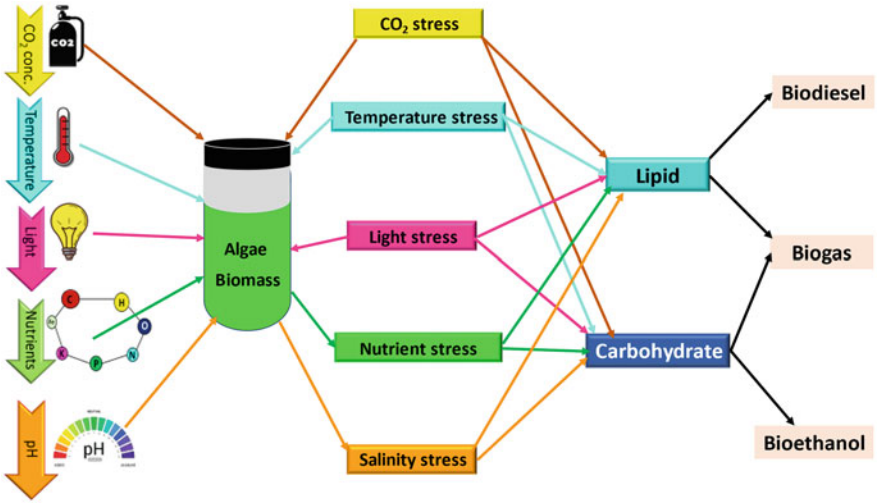

Fig. 6.1 Influence of culture condition on biofuel production

Lam and Lee 2011; Tsukahara and Sawayama 2005). This chapter summarizes various cultivation conditions, including pH, light intensity, and temperature for raised lipid accumulation inside microalgal cells. Different levels of physical factors affecting microalgal growth and lipid yield have been discussed in this chapter; the impact of the culture conditions like CO_2 concentration, temperature, light colour, and light intensity on lipid accumulation is evaluated comprehensively. Also, very recent progress and research studies on microalgal biomass and biodiesel production are discussed and summarized. Figure 6.1 represents crucial factors affecting microalgae culture and ultimately affect biofuel production.

6.2 Microalgal Cultivation

Algae have higher growth rates, photosynthetic levels, and CO_2 sequestering efficiency. Microalgae are significantly considered organisms for reducing the nutrient load (nitrogen and phosphorous) from agricultural, industrial, municipal, and domestic wastewater. They consist of considerable quantities of fatty acids that yield sustainable biodiesel after transesterification. Microalgal cultivation for lipid recovery and biofuel generation is a moderate process. Meanwhile, they need quite simple light and aeration arrangements to grow. However, some factors alter the optimum growth and total lipid yield in microalgae. These factors include macro- and micronutrients (concentration and availability), CO_2, pH, light (photoperiod/intensity), and temperature. Algae have higher growth rates, photosynthetic levels, and CO_2 sequestering efficiency. Microalgae are significantly considered organisms for reducing the nutrient load (N and P) from industrial, agricultural, municipal, and

domestic wastewater. They consist of considerable quantities of fatty acids that yield sustainable biodiesel after transesterification. Microalgal biomass cultivation for biodiesel production is a moderate process; meanwhile, they need quite simple light and aeration arrangements to grow. Some factors like nutrients availability, pH, temperature, CO_2, photoperiod, and light intensity might affect biomass yield and lipid accumulation inside microalgal cells. Among all these conditions, light and temperature are primarily significant for algal growth. A temperature that ranges from 20 to 30 °C is preferable for most microalgal species. Highly raised temperature results in physiological adaptations in microalgal cells that cause lesser unsaturated fatty acid production (Van Wagenen et al. 2012). Biodiesel properties like cetane number, heating point, oxidative stability, lubricity, and melting point are primarily based on the type of precursor fatty acid. Palmitoleic acid, stearic acid, linolenic acid, oleic acid, linoleic acid, and palmitic acid are the most suitable fatty acids for biodiesel generation (Zheng et al. 2013). The lipid synthesis pathway in microalgae might be altered in several limiting conditions and synthesize a higher amount of triacylglycerol (TAG) in lipid bodies (Hu et al. 2008). It is necessary to observe loads of microalgal species for their high lipid productivity; then selected algal species are optimized for different factors to attain maximum biodiesel production. The cultivation system is an essential factor as it affects the biofuel yield and photosynthesis efficiency of microalgae. Generally, algal cultivation is preferred in two ways: (1) open cultivation system and (2) closed cultivation system. Photobioreactors (PBR) are a closed system for microalgal cultivation. Inside PBR, microalgal cultural conditions as light intensity, aeration, and stirring are customized according to the microalgal species (Liao et al. 2018; Lee and Lee 2016). Glass or plastic material is used to construct PBR with different designs such as flat plate, airlift, and tubular bioreactor and bubble column (Brennan and Owende 2010). Airlift bioreactor is widely appreciated to eliminate the chance of contamination and to obtain high algal biomass (Huang et al. 2010). High cost, oxygen build-up, biofouling, overheating, cell damage, and difficulties in scale-up are major limitations of PBR (Adeniyi et al. 2018; Brennan and Owende 2010). In comparison, open cultivation systems like the shallow pond, unstirred pond, raceway pond, closed pond, and circular pond are less optimized but provide affordable, lower maintenance cost, less operational cost, and easy scale-up. Open systems are highly affected by water evaporation, temperature, and light variability. PBR exhibited highly customized growth conditions and ensured the availability of adequate light intensity; hence, PBR provides a higher biomass productivity rate than the open system (Leite et al. 2013). CO_2 consumption as a carbon source is the major pros of utilizing photoautotrophic systems. Furthermore, chances of contamination are significantly less while using photoautotrophs (Mata et al. 2010). A combined design of an open pond and closed PBR system is applied to achieve elevated biomass productivity and excellent nutrient removal. A combined setup of closed and open systems is termed as hybrid algal cultivation method, which is adequate for massive microalga cultivation (Razzak et al. 2017; Rawat et al. 2013). Hybrid systems are customized to eliminate drawbacks of open systems and to minimize the operational cost of photobioreactors. Such a hybrid cultivation system

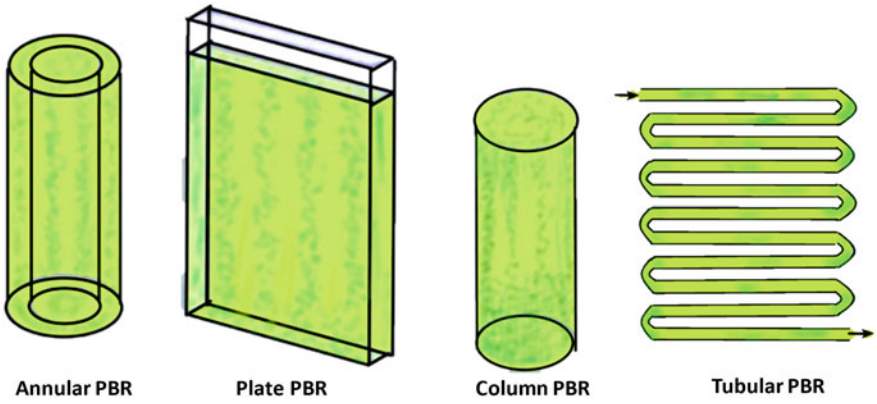

Fig. 6.2 Closed photobioreactor system for algal cultivation

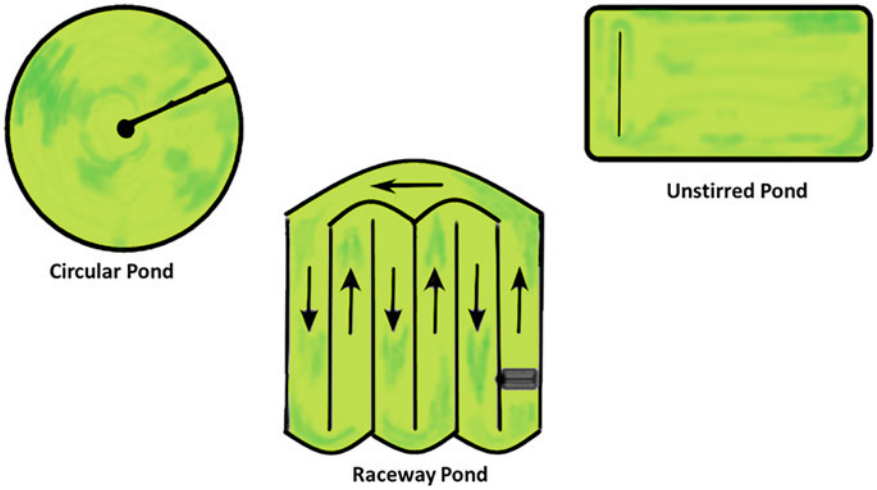

Fig. 6.3 Open pond system for algal cultivation

preferred the initial stage of microalgal culture inside photobioreactors, and then it will be transferred to ponds (Schenk et al. 2008) (Figs. 6.2 and 6.3).

Concerning metabolic activity, algal cells can be classified into main categories that are heterotrophic, photoautotrophic, autotrophic, and mixotrophic (Daliry et al. 2017). The heterotrophic mode of nutrition needs the presence of organic carbon as a substrate, whereas photoheterotrophs need both light and organic carbon to survive (Chen and Durbin 1994). Autotrophic microalgae convert inorganic source of carbon into chemical energy in the availability of sunlight, while mixotrophic algae can survive in both heterotrophic and autotrophic modes dependent on the presence/absence of carbon source and light (Burkholder et al. 2008). Scarsella et al. (2010) found that mixotrophy nutrition mode in microalgae is suitable for yielding

maximum lipid and biomass. Heterotrophic metabolism has a better growth rate than autotrophic metabolism (Martinez et al. 1991).

6.3 Impact of Cultural Conditions on the Algal Lipid Content

Photoautotrophs like green algae can synthesize proteins, lipids, carbohydrates, and proteins. Lipid composition in algae is highly affected by its life cycle, whereas variation in lipid content depends on the cultivation medium and some other physical factors (Scarsella et al. 2010). Temperature, pH level, photoperiod, light intensity, and CO_2 highly affect photosynthesis in green algae (Deng et al. 2014). Figure 6.4 depicts impact of stress condition on algal cells.

6.3.1 Effect of Temperature

It is evident to evaluate the influence of temperature to achieve high microalgal growth rate at broad-scale outdoor cultivation systems. Microalgal culture in outdoor open ponds is expected to undergo extreme surrounding temperatures and produce high algal biomass for economic lipid production. Surrounding temperature is a crucial parameter that affects lipid composition and content in algal cells (Ras et al. 2013). Temperature modifies metabolic pathways and causes acceleration or retardation of biochemical reaction rate in algal cells (Afify et al. 2010). The dependency

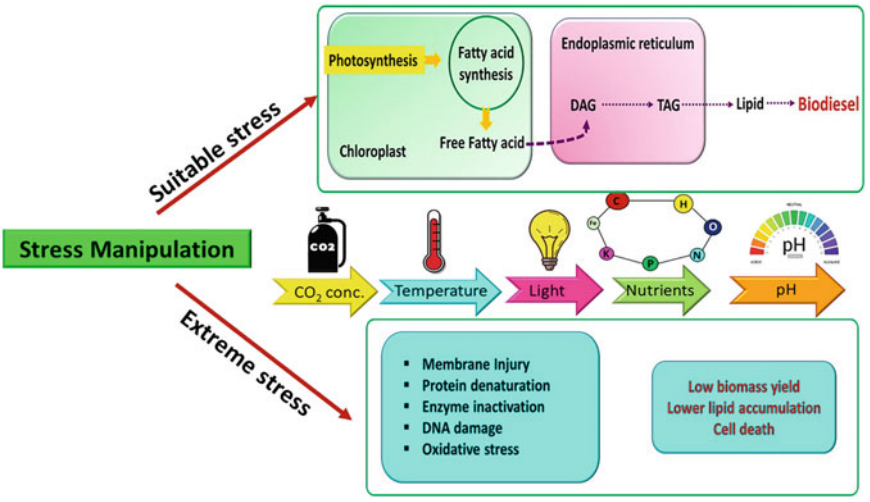

Fig. 6.4 Impact of stress manipulation on algal cells

of biochemical reactions rate on temperature can be described by Arrhenius function and Q_{10} (temperature coefficient). Q_{10} is a factor by which any biological processes or biochemical reaction rate increases if the temperature is raised by 10 units (Teoh et al. 2010). The relationship among temperature and algal growth rate follows the Arrhenius relationship. Q_{10} and Arrhenius function are equally expected to achieve accelerated growth rates with rising temperatures. However, microalgal growth rate accelerates up to an optimum temperature, but crossing that optimum value can cause retardate growth (Suzuki and Takahashi 1995; Montagnes and Franklin 2001). Table 6.1 present different studies observing the effect of temperature on different algal species. It has been seen that raised temperatures in *Nannochloropsis salina* and *Ochromonas Danica* cultivation cause correspondence increase in their lipid content, whereas *Chlorella sorokiniana* behave contradictory and show no variation in lipid content corresponding to raised temperature (Fakhry and El Maghraby 2015). Kalacheva et al. (2002) use *Botryococcus braunii* cultivation to observe the effect of different temperatures (18 °C, 25 °C, and 32 °C) on cellular lipid content. At 25 °C (optimum temperature), lipid content was estimated up to 22%, whereas crossing 32 °C cause 5% less cellular lipid content than optimum temperature (Kalacheva et al. 2002). Similarly, 25 °C is the optimum growth temperature for *Chlorella vulgaris* and *Nannochloropsis oculata*. A study found that increasing temperature from 20 to 25 °C *Nannochloropsis oculata* cultivation results in the twofold increment of lipid accumulation (7.90–14.92%) (Converti et al. 2009). However, in the case of *C. vulgaris*, raising temperature more than optimum (from 25 to 30 °C) causes approximately 7% reduction (14.71–5.90%) in lipid content (Converti et al. 2009). Researchers found that temperatures ranging from 15 to 35 °C might be effective in raising lipid accumulation inside microalgal cells (Converti et al. 2009; Kalacheva et al. 2002; Fakhry and El Maghraby 2015). Some studies were performed to observe the additive impact of nutrient availability and temperature on the cellular lipid contents of algae. To examine the synergistic effect of temperature and nutrient availability, microalgal species such as *Chromulina ochromonoides*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Nannochloropsis oculata*, *Odontella aurita*, and *Thalassiosira pseudonana* were cultivated at 10 and 20 °C with two different conditions (nutrient starved and sufficient) (Roleda et al. 2013; Shuping et al. 2010). Results revealed that adding multiple stress conditions would not be able to give additional impact to enhance lipid content, and only the nutrient-deficient condition is favourable to improve lipid accumulation (Shuping et al. 2010; Roleda et al. 2013). A study on microalgae was performed to analyse the impact of temperature on fatty acid composition. *Nannochloropsis oculata* and *Tetraselmis subcordiformis* were cultivated under several temperatures ranging from 15 to 35 °C (Wei et al. 2015). Results showed that high temperatures cause more MUFA accumulation than PUFA and neutral lipids (Wei et al. 2015). *Dunaliella salina*, a marine microalga, was studied under a temperature range shifting from 30 to 12 °C to analyse temperature shifting effect on lipid composition. Under lower temperatures, unsaturated fatty acid accumulation is raised by 20% (Sharma et al. 2012). Generally, low temperatures lead to high membrane fluidity, more unsaturated fatty acids, and less saturated fatty acids associated with algal cell

Table 6.1 Influence of temperature on different algal species

Algae	Temperature (°C)	Other culture conditions	Effects observed	References
N. salina	15	Photoperiod: 12 h:12 h (light: dark) Light intensity: 150 µmol m^{-2} s^{-1}, pH 7.5	Dry cell weight: 0.42 ± 0.0 gL^{-1} Lipid productivity: 0.31 ± 0 gL^{-1} day^{-1}	Fakhry and El Maghraby (2015)
N. salina	20	Photoperiod: 12 h:12 h (light: dark) Light intensity: 150 µmol m^{-2} s^{-1}, pH 7.5	Dry cell weight: 0.45 ± 0.0 gL^{-1} Lipid productivity: 0.35 ± 0 gL^{-1} day^{-1}	Fakhry and El Maghraby (2015)
N. salina	25	Photoperiod: 12 h:12 h (light: dark) Light intensity: 150 µmol m^{-2} s^{-1}, pH 7.5	Dry cell weight: 0.50 ± 0.04 gL^{-1} Lipid productivity: 0.37 ± 0.01 gL^{-1} day^{-1}	Fakhry and El Maghraby (2015)
N. salina	30	Photoperiod: 12 h:12 h (light: dark) Light intensity: 150 µmol m^{-2} s^{-1}, pH 7.5	Dry cell weight: 0.53 ± 0.02 gL^{-1} Lipid productivity: 0.43 ± 0.0 gL^{-1} day^{-1}	Fakhry and El Maghraby (2015)
N. salina	35	Photoperiod: 12 h:12 h (light: dark) Light intensity: 150 µmol m^{-2} s^{-1}, pH 7.5	Dry cell weight: 0.51 ± 0.02 gL^{-1} Lipid productivity: 0.41 ± 0.01 gL^{-1} day^{-1}	Fakhry and El Maghraby (2015)
B. braunii	18	Illumination: 20 W/m^2 illumination period: 24 h (14 h day and 10 h night) 1 vol % CO_2	Dry biomass weight: 0.43 g/L Total intracellular lipids: 50% TGA content: 9.3%	Kalacheva et al. (2002)
B. braunii	25	Illumination: 20 W/m^2 illumination period: 24 h (14 h day and 10 h night) 1 vol % CO_2	Dry biomass weight: 0.44 g/L Total intracellular lipids: 50% TGA content: 2%	Kalacheva et al. (2002)
B. braunii	32	Illumination: 20 W/m^2 illumination period: 24 h (14 h day and 10 h night) 1 vol % CO_2	Dry biomass weight: 0.7 g/L Total intracellular lipids: 50% TGA content: 13.8%	Kalacheva et al. (2002)

(continued)

Table 6.1 (continued)

Algae	Temperature (°C)	Other culture conditions	Effects observed	References
C. vulgaris	25	14 days Photon flux density: 70.0 μE m^{-2} s^{-1}	Lipid productivity: 20.22 ± 0.60 mg lipids L^{-1} $days^{-1}$	Converti et al. (2009)
C. vulgaris	30	14 days Photon flux density: 70.0 μE m^{-2} s^{-1}	Lipid productivity: 8.16 ± 0.65 mg lipids L^{-1} $days^{-1}$	Converti et al. (2009)
C. vulgaris	35	14 days Photon flux density: 70.0 μE m^{-2} s^{-1}	Lipid productivity: 8.21 ± 0.17 mg lipids L^{-1} $days^{-1}$	Converti et al. (2009)
C. vulgaris	38	14 days Photon flux density: 70.0 μE m^{-2} s^{-1}	Lipid productivity: −2.72 ± 1.62 mg lipids L^{-1} $days^{-1}$	Converti et al. (2009)

adaptation in stress conditions (Mathimani and Nair 2016). Regardless of cellular organization, eukaryotic green microalgae *B. braunii*, *C. vulgaris*, and prokaryotic algae *S. platensis* showed variation in lipid yield over raised temperatures. *B. braunii*, *C. vulgaris*, and *S. platensis* accumulated a lower amount of unsaturated fatty acids as compared to saturated fatty acids at high temperatures (Sushchik et al. 2003). Researchers are not able to conclude a similar trend of temperature variation for its impact on lipid accumulation in algal cells. High temperature enhances lipid accumulation as a storage product of algal cells due to its maximum use (Sayegh and Montagnes 2011). Stress conditions are applied to the activity of photosystem II when microalgal cultivation is done at more than optimum temperature and results in a lower yield of biomass and lipid (Sheng et al. 2011), whereas at extreme temperatures, microalgal growth is obstructed due to enzyme denaturation and termination of metabolic reactions (Renaud et al. 2002).

6.3.2 *Effect of Light Intensity*

Alga is a widespread photosynthetic living being. Light is a vital aspect of the autotrophic activity, photosynthesis, and growth of microalgal species. Algal cells consist of numerous photon harvesting pigments, where chlorophyll 'a' and chlorophyll 'b' are most abundant pigments that have sensitivity towards red and blue wavelengths. Light is a prominent aspect for algal growth. Algae can grow at different photoperiods, wavelengths, and light intensities; however, different light conditions may cause the difference in their photosynthetic activity and lipid accumulation. In addition, light intensity might influence the lipid yield and alteration in the fatty acid composition (Liu et al. 2008). In a study, a *Cladophora* species (filamentous algae) was cultivated using extreme light intensities. Results revealed that the composition of polar phospholipids was significantly reduced, whereas

triacylglyceride accumulation was enhanced (Napolitano 1994). Table 6.2 present different studies observing the influence of light intensity on several algal species. Similarly, *Desmarestia viridis* was cultivated under varying light intensities. When light intensity shift from 700 to 1500 μmol m^{-2} s^{-1}, 63% biomass reduction was observed (Gordillo et al. 1998a, b). Total lipid content is amplified when *Desmarestia viridis* was cultivated under dark conditions; however, triglyceride contents are reduced simultaneously (Smith et al. 1993). An increment in extracellular polysaccharide content was observed while raising proton flux density (Iqbal and Zafar 1993). Algae that are grown under enhanced intensity result in more lipid accumulation. *Scenedesmus* sp. was cultivated in 250–400 μmol m^{-2} s^{-1} range of light intensity and showed enhanced lipid accumulation (Liu et al. 2012). Despite that, two marine algal species *Nannochloropsis* and *Chlorella* sp. were grown under an extreme photon density of 10,000 lx, demonstrating lower lipid production (Cheirsilp and Torpee 2012). de Mooij et al. (2016) cultivate *C. reinhardtii* to study its productivity in different light colour. *C. reinhardtii* cultivation done inside a photobioreactor has light intensity of 1500 μmol m^{-2} s^{-1}, where yellow light results in highest productivity (54 g m^{-2} day^{-1}) as compared to red and blue light (de Mooij et al. 2016). Krzemińska et al. (2014) use five different algae (*B. braunii*, *N. conjuncta*, *N. texensis*, *N. terrestris*, and *S. obliquus*) to observe consequences of two different photoperiods (12:12 light/dark hour cycle and 24-h uninterrupted illumination) on biomass productivity and growth rate. 12:12 light/dark hour cycle was favourable to stimulate growth rate in *Neochloris* sp., while continuous stimulation is found effective for higher growth rates in case of *S. obliquus* and *B. braunii* (Krzemińska et al. 2014). Also, a study observes the effect of continuous illumination on the fatty acid composition of a green microalga *Chlorella minutissima*; however, there is no significant difference occurred in fatty acid composition at a light intensity of 200–400 μE m^{-2} s^{-1} (Tang et al. 2011). Although several findings relate the fatty acid composition to the light intensity, it was observed that PUFA content was dropped down with raised light intensity (Juneja et al. 2013). Continuous illumination, longer photoperiods, and high photon intensity reduce MUFA and PUFA content and elevate saturated fatty acid levels (Al-Qasmi et al. 2012). *Thalassiosira pseudonana* cultivated in 12:12 h light/dark photoperiod revealed lower PUFA and elevated SFA and MUFA levels (Brown et al. 1996). Inconsistently, intensified photon flux of 50–150 μmol m^{-2} s^{-1} light intensity increases fatty acid accumulation in algal cells (Schnurr et al. 2016). No considerable alteration occurs in the fatty acid profile when raising the photon flux beyond 300–-600 μmol m^{-2} s^{-1} (Schnurr et al. 2016). Cuellar-Bermudez et al. (2015) studied the combined effect of CO_2 and light intensity on the fatty acid composition of *Synechocystis* sp. *PCC6803*. Total lipid content enhanced when *Synechocystis* sp. cultivated under 1920 μmol m^{-2} s^{-1} light intensity with 3% CO_2 concentration; however increasing light illumination does not affect fatty acid composition (Cuellar-Bermudez et al. 2015). It is considered that growth and lipid yield is varying with different algal strains. Beyond the threshold limit of light, intensity triggers lamellae disruption within the chloroplast and destined photoinhibition or inactivation of CO_2 fixing enzymes (Juneja et al. 2013). Extreme illumination intensities

Table 6.2 Impact of light on various microalgal species

Algae	Light conditions	Other growth condition	Effects observed	References
C. vulgaris	Light intensity: 300, 150, and 50 μmol m^{-2} s^{-1}	16:8 h (light/dark) Temperature: 25 °C Shaking: 150 rpm	Higher light intensity results greater biomass	Nzayisenga et al. (2020)
Desmodesmus sp.	Light intensity: 300, 150, and 50 μmol m^{-2} s^{-1}	16:8 h (light/dark) Temperature: 25 °C Shaking: 150 rpm	Higher light intensity results in greater biomass and raised fatty acid content	Nzayisenga et al. (2020)
E. pseudoalveolaris	Light intensity: 300, 150, and 50 μmol m^{-2} s^{-1}	16:8 h (light/dark) Temperature: 25 °C Shaking: 150 rpm	Higher light intensity results in more biomass	Nzayisenga et al. (2020)
S. obliquus	Light intensity: 300, 150, and 50 μmol m^{-2} s^{-1}	16:8 h (light/dark) Temperature: 25 °C Shaking: 150 rpm	Higher light intensity results in high biomass and raised fatty acid content	Nzayisenga et al. (2020)
I. galbana LB987	Photoperiod: 12 h/12 h (day/night) Light intensity: 0–200 μmol m^{-2} s^{-1}, 10 days	pH 8.0, Temperature: 25 °C mixotrophic condition	Optimal light intensity: 80–150 μmol m^{-2} s^{-1} Stimulated lipid production and chlorophyll synthesis Total lipid content: 30%	Gim et al. (2016)
N. oculata CCAP849/1	Photoperiod: 12 h/12 h (day/night) Light intensity: 0–200 μmol m^{-2} s^{-1}, 10 days	pH 8.0, Temperature: 25 °C mixotrophic condition	At 150 μmol/m^2/s, fatty acid concentrations increased slightly Total lipid content: 37.3%	Gim et al. (2016)
D. salina	Photoperiod: 12 h/12 h (day/night) Light intensity: 0–200 μmol m^{-2} s^{-1}, 10 days	pH 8.0, Temperature: 25 °C, mixotrophic condition	At 150 μmol/m^2/s, fatty acid concentrations increased slightly Total lipid content: 31.3 %	Gim et al. (2016)
Scenedesmus sp.	Light intensity: 50 μmol/m^2/s	Temperature: 25 °C, CO_2: 2% v/v	Biomass yield: 2.55 g/L Lipid content: 26.2%	Liu et al. (2012)

(continued)

Table 6.2 (continued)

Algae	Light conditions	Other growth condition	Effects observed	References
Scenedesmus sp.	Light intensity: 250 μmol/m^2/s	Temperature: 25 °C, CO_2: 2% v/v	Biomass yield: 3.62 g/L Lipid content: 39.2%	Liu et al. (2012)
Scenedesmus sp.	Light intensity: 400 μmol/m^2/s	Temperature: 25 °C, CO_2: 2% v/v	Biomass yield: 3.88 g L^{-1} Lipid content: 41.1 %	Liu et al. (2012)

(beyond threshold level) disrupt the photosynthetic receptor system and trigger photoinhibition (Wahidin et al. 2013). Availability of essential fatty acids, different pigments, and dark respiration rate are the severe factors behind the physiological photo-acclimatization, whereas changes in volume, number, and density of photosynthetic apparatus can cause morphological photo-acclimation (Fábregas et al. 2004). It is found that desaturated chloroplast membranes of algae can exceed the limitation of low light intensity (Mock and Kroon 2002). Prevention of photoinhibition and dissipation of excess photon energy could assist by NADH and ATP generated during photosynthesis. Also, triacylglyceride synthesis needs high NADH and ATP levels; hence the carbon flux is generated during photosynthesis when microalgal cells are cultivated under extreme light conditions (Solovchenko et al. 2008; Liu et al. 2012). Despite this, when algal cells absorb the surplus level of excitation energy, it results in photodamage (destruction of photoreceptors of PS II); however, polypeptides of the reaction centre are more stable during extreme light exposure (He et al. 2001). When *Synechocystis* sp. *PCC6883* was acclimatized under extreme light intensities, plastoquinone oxidation takes place, and the PS I/PS II ratio reaches up to 5, which was advantageous to minimize photodamage (Cuellar-Bermudez et al. 2015). The light could provoke algal culture growth, chloroplast membrane development, and lipid synthesis; hence light is a crucial factor for lipid accumulation in microalgal cells, but light intensity/photoperiod requirements are dependent and varies with algal strains.

6.3.3 Effect of Salinity

Salinity (NaCl concentration) is the vital factor that modifies the composition of biochemical compounds inside microalgal cells. Too low or high salinity exposure of algal cells cause alteration in their natural growth rate and biochemical composition. Studies revealed that high salinity can cause increased lipid content in algae (Zhila et al. 2011; Renaud and Parry 1994). In a study, marine algae *Dunaliella* was cultivated under different saline concentrations ranging from 0.4 to 4 M; results reported an increase in SFA and MUFA with increased salinity up to 4 M (Xu and

Beardall 1997). Similarly, an increase in triglyceride (40–56%) and lipid content (60–67%) was reported when *Dunaliella tertiolecta* was cultivated under saline concentration raised from 0.5 to 1.0 M NaCl (Takagi and Yoshida 2006). *Botryococcus braunii* cultivation under high salinity level results in higher growth rate, with increase in lipid and carbohydrate content (Rao et al. 2007). Correspondingly, *Botryococcus braunii* was cultivated in two salinity conditions (without NaCl and with 0.50 NaCl). This study reported higher lipid content in a media with 0.50 NaCl; however, carbohydrates, proteins, and pigments were less (Ben-Amotz et al. 1985). A study reported reduced protein content whereas no variation in lipid and carbohydrate content in *Botryococcus braunii* under high saline levels. However, growth rate was reduced as algal cells was unable of adapt in high saline concentration (Vazquez-Duhalt and Arredondo-Vega 1991). Accordingly, another study supported reduced protein content per algal cells in *Tetraselmis suecica* with increased NaCl concentration (Fabregas et al. 1984).

6.3.4 Effect of Nutrients

Varying nutrient composition using different nutrient limitations could be helpful to identify which nutrient or substrate affect algal growth up to what extent. In general, Michaelis-Menten equation described nutrient uptake rate. At optimal culture conditions, algal growth is proportional to the utilization rate of highly limiting substrate. N and P are crucial macronutrients for metabolism and growth of microalgal cells. Nitrogen is basic element to form nucleic acid and proteins. Similarly, phosphate plays a major role as energy currency in the form of ATP in all living beings. Despite that, phosphorous is key element found in RNA, DNA, and phospholipids. Limitation of nitrogen and phosphorus will shift metabolic system of microalgal cells. Starvation of N and P shifts the lipid metabolism and tends towards lipid storage instead of membrane lipid synthesis. Hence the total lipid content increases in microalgae under N- and P-starved conditions (Hirano et al. 1997). Specific discussion on all limiting substrate is reviewed below.

6.3.4.1 Carbon

C, H, and O are crucial non-mineral nutrients. Carbon is most essential nutrient for algal growth, photosynthesis, and reproduction. Carbon uptake is generally fixed by three ways: (1) as energy resource; (2) as respiration; and (3) as a raw material for cell formation (Berman-Frank and Dubinsky 1999). Lower carbon fixing rate results in lesser growth rate in microalgal cells. Algal cells need inorganic carbon substrate for photosynthesis which is available in the form of carbon dioxide (CO_2) or carbonate in autotrophic mode of nutrition. CO_2 dissolved in water are convertible into carbonate or bicarbonate depending on nutrient composition, pH, and temperature. With rise in pH level, carbonate content rises as compared to carbon dioxide

and bicarbonate (Chen and Durbin 1994). Riebesell et al. (2000) observe *Emiliania huxleyi* to analyse the influence of CO_2 concentration on lipid composition. CO_2 concentration significantly influences the composition of PUFA and alkenones. Study revealed low concentration of CO_2 tends to increase PUFA 22:6 (n-3), while high CO_2 concentration raises 14:0 fatty acids composition (Riebesell et al. 2000). A study reported that increased CO_2 concentration results in enhanced unsaturation and fatty acids content (Tsuzuki et al. 1990). Similarly, in *Dunaliella salina*, increased CO_2 levels leads to elevated level of fatty acid accumulation (Muradyan et al. 2004).

6.3.4.2 Nitrogen

Nitrogen is readily consumed by algal cell; algae consume the inorganic source of nitrogen existing in media and turn it into biochemical compounds to carry out their physiological processes appropriately (Ross et al. 2018; Yang et al. 2017). However, it was studied that nitrogen-starved condition leads to high lipid biosynthesis level and enhanced triglyceride accumulation with lesser protein component (Wei et al. 2020; Heraud et al. 2005; Wang et al. 2009). So, the higher lipid and lower protien content could be obtained at the disbursement of algal growth rate (Converti et al. 2009; Li et al. 2008), under nitrogen-limited conditions to redirect their photosynthetic carbon into carbohydrate synthesis (Hu 2004). Nitrogen-depleted media can also cause lower oxygen generation, lesser CO_2 fixation, and decreased tissue and chlorophyll content (Kolber et al. 1988). In a study, sugar phosphates and ammonium were additionally supplied into the growth media of *Chlorella pyrenoidosa* where an increase in amino acid was observed (Holm-Hansen et al. 1959). In algal photosystem II, phycobilisomes act as antennae for light harvesting. In red algae and cyanobacteria, nitrogen-limited culture media cause degradation of phycobilisomes (Collier and Grossman 1992). Photosynthesis remains persistent (with reduced rate) until nitrogen concentration depleted below a threshold level. *Spirulina platensis* cells were grown under high CO_2 availability with nitrogen-depleted medium and depict very less carbon fixation efficiency (Gordillo et al. 1998a, b). Nitrogen depletion may cause variation in enzyme levels inside algal cells and cause lipid synthesis however simultaneously reducing chlorophyll synthesis that generates higher carotenoid accumulation (Zhang et al. 2017).

6.3.4.3 Phosphorus

Phosphorus is a crucial element for adequate growth and metabolism in microalgal cells. Besides nitrogen, phosphorus is primary limiting macronutrient for microalgal cells (Ren et al. 2017). Phosphorus limitation generally cause lesser than needful rate of light assimilation for carbon fixation; also it will result in reduced level of substrate synthesis during Calvin-Benson cycle (Barsanti and Gualtieri 2005). Phosphorus limitation will be an advantageous condition to gain high lipid accumulation

in algal cells. In a study, *Scenedesmus* sp. is grown at 2.0 mg L^{-1} of initial phosphorus concentration and then reduced it to 0.1 mg L^{-1} early phosphorus concentration. It was observed that reduction of phosphorus concentration attains increment in total lipid accumulation to 53% (at 0.1 mg L^{-1}) from 23% (at 2.0 mg L^{-1}) (Xin et al. 2010). Phosphatidylglycerol is a glycerolipid present in chloroplast membrane. It also has significant roles in cell growth, keeping satisfactory levels of chlorophyll protein complex and PS II function. In a study, *Chlamydomonas reinhardtii* was grown in a phosphorus-limited medium; results depict decreased phosphatidylglycerol content (Sato et al. 2000). In phosphorus-deficient medium, protein and *chlorophyll a* content tends to reduce and thus enhanced relative carbohydrates amount in algal cells (Healey and Hendzel 1979). Like nitrogen deficiency, phosphorus limitation tends to reduced phycobilisome content (Collier and Grossman 1992). In *Selenastrum minutum*, phosphorus-deprived condition causes reduction in respiration rate of algal cells (Theodorou et al. 1991).

6.3.4.4 Micronutrients

Trace metals are micronutrients required by living cells in very less quantity (less than 4 ppm), and these trace metals are very crucial for physiology of algal cells. Trace metals commonly include cobalt, copper, manganese, iron, zinc, and nickel (Facey et al. 2019). For algal cells, trace metal accessibility is dependent on free ion concentration, i.e. it is independent of dissolving trace metal concentration in the culture media (Parent et al. 1996). High level of trace metal in culture media can cause reduction in antioxidant levels, cell membrane damage, and photosynthesis impairment; similarly deficiency of these can also inhibit cell growth. Iron plays significant role as catalyst redox reactions of electron transport system, nitrogen assimilation, and photosynthesis in algal cells (Rueler and Ades 1987). Iron deficiency causes reduction in photosynthetic electron transport, which further reduces NADPH availability. Iron deficiency causes reduction in ferredoxin availability and substitutes ferredoxin with flavodoxin (McKay et al. 1999). Subsequently, ferredoxin substitution could be unfavourable because the catalytic activity of flavodoxin is very slow and not comparable and efficient as ferredoxin (McKay et al. 1999). Iron-limited media can bring chlorophyll-deficit condition inside algal cells (Greene et al. 1992). In *C. vulgaris*, effect of elevated iron concentration has been observed in a study and remarks enhanced lipid content (Liu et al. 2008), whereas iron-deficient condition can cause depletion in carotenoid composition (van Leeuwe and Stefels 1998). Algal cell membrane consists of many functional groups like phosphate, carboxylic, and sulfhydryl groups. Such functional groups act as binding site for metal ions. Most of the metabolic reactions will be inhibited by the presence of some non-essential elements like chromium, lead, and cadmium. A very small concentration of these metals can cause severe toxicity within algal cells. Similarly, presence of excess concentration of essential metals like zinc, nickel, and copper can also

cause cellular toxicity (Campanella et al. 2001; Kennish 1992; Rai and Mallick 1993).

6.3.5 *Effect of CO_2*

Carbon dioxide (CO_2) is accessible in the atmosphere and emitted continuously from different industrial sources, vehicles, and power plants. Increasing levels of CO_2 in atmosphere will be harmful for living beings. Algal cells are naturally contributing to mitigate CO_2 by carbon capturing and carbon sequestration. At high CO_2 concentration, *Chlorella* sp. shows higher rate of photosynthesis (Singh and Singh 2014). Mainly CO_2 concentration affects lipid content and microalgal growth rate. Some researchers inspected the impact of CO_2 concentration on fatty acid, lipid composition, and biomass growth in different algae. In a study, *C. vulgaris*, *Scenedesmus* sp., and *B. braunii* are chosen to observe the effect of different CO_2 concentrations (Yoo et al. 2010). At 10% CO_2 level, after 2 weeks, *B. braunii* gain 26.55 mg L^{-1} biomass productivity and 5.51 mg L^{-1} day^{-1} of total lipid productivity, whereas *Chlorella vulgaris* obtain biomass productivity of 104.76 mg L^{-1} day^{-1} and lipid productivity of 6.91 mg L^{-1} day^{-1} (Yoo et al. 2010). On similar culture conditions and 10% CO_2 levels, *Scenedesmus* sp. obtains biomass productivity of 217.50 mg L^{-1} day^{-1} and lipid productivity of 20.65 mg L^{-1} day^{-1} (Yoo et al. 2010). In the same analysis, Yoo et al. (2010) investigate effect of flue gases comprising 5.5% CO_2 levels on *C. vulgaris*, *Scenedesmus* sp., and *B. braunii*. Results show enhanced biomass productivity in all three species, whereas *Scenedesmus* sp. shows 3.7-fold increment in the total lipid productivity (Yoo et al. 2010). In a study, *B. braunii* 765 was cultured with 20% CO_2 availability; findings reported increasing CO_2 levels from 2% to 20% cause enhanced biomass productivity and total lipid productivity varies from 10.41% to 12.71% by weight (Ge et al. 2011). With an environmental perspective, *Phaeodactylum tricornutum* and *Nannochloropsis salina* were cultured using desulphated and untreated flue gas with inorganic source of CO_2. Result observed no significant changes in biomass obtained from *P. tricornutum* and *N. salina*, although the presence of some toxic non-essential metals like mercury, vanadium, and nickel in flue gas can affect the biomass productivity (Moheimani 2013). A study was performed on *D. viridis* which was cultured in nitrogen deficit and high CO_2 availability simultaneously. Result has shown that lipid composition in *D. viridis* is very sensitive towards nitrogen-deficient condition (in presence of high CO_2 concentration) (Gordillo et al. 1998a, b).

6.3.6 *Effect of pH*

pH of any culture media is the essential factor in any metabolic process, so in the microalgal growth, pH plays major role in metal speciation, nutrient availability, and

enzymatic activities within the microalgal cells. Any changes in pH levels may directly influence enzymatic actions and further affected different metabolic pathways like photosynthesis and lipid synthesis (Jin et al. 2016). However, response of algal cells and its metabolic process towards optimum pH or at other pH range is different and strain specific (Moheimani 2013). Varying pH affects lipid and biomass yield in microalgal cells. In a study, during inoculation initial pH of algal growth media was kept neutral because availability of carbon species is depending on the pH. After some time, algal cells consume inorganic carbon present in media, and hence pH of the growth media gradually increases (Rai et al. 2015). Effect of numerous inorganic carbon sources and pH on *Chlorella* sp. and *T. suecica CS-187* was observed for lipid and biomass productivity. At pH 7.5, *T. suecica* gained a biomass productivity of 320 mg L^{-1} day^{-1} and lipid productivity of 92 mg L^{-1} day^{-1}, whereas at pH 7.0, *Chlorella* sp. obtained biomass productivity of 407 mg L^{-1} day^{-1} and lipid productivity of 99 mg L^{-1} day^{-1} (Moheimani 2013). In a study, growth media was set with different pH ranging from 6.0 to 10.0 to observe effect of pH on the lipid accumulation and biomass productivity in *N. salina* (Bartley et al. 2014). Outcomes revealed that pH 8 to pH 9 depicts maximum growth rates in *N. salina* (Bartley et al. 2014). In a study, *C. vulgaris* cultivated in heterotrophic mode at optimum pH (7.5) in sulphur-deficient condition obtain lipid content of 53.43% and maximum specific growth rate of 0.541 $days^{-1}$. Cell death occurs in *C. vulgaris* at pH 3, 4, and 11, whereas at pH 9.5 algal cells start to aggregate (Sakarika and Kornaros 2016). In a different study *Chlorella* sp. was grown at varying pH levels ranging from 5 to 11 to observe impact of pH on biomass growth and lipid content (Zhang et al. 2014). After a month, highest lipid content was 32.8% (lipid yield of 168 mg L^{-1}) and was noticed at pH 7.0, whereas at pH 5.0, maximum triacylglyceride content was 63% (Zhang et al. 2014). For *Dunaliella salina* pH 11.5 and *Dunaliella acidophila* pH 3 is found as optimum pH (Varshney et al. 2015).

6.4 Future Aspects

In this chapter, we discussed influence of different culture conditions like nutrients, and environmental factors like light, pH, CO_2 availability, temperature, etc., on algal biomass and lipid content. Tremendous efforts have been done by the researchers to understand behaviour of algal biomass growth for lipid production and nutrient recovery from wastewater. These efforts include enhanced optimization of the environmental factors and its effects on algal cell growth and composition. With the progress made by biotechnology research and bioinformatics software, it is feasible to acquire an enormous information from genetic data. It is possible now to trace molecular interaction and its effect on metabolism. Genome sequences of *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, and *Dunaliella salina* are known (Smith et al. 2010; Merchant et al. 2007; Blanc et al. 2010). Predictive models and automated control systems for optimum culture condition would be effective solution for huge scale algal biomass and fatty acid production (Coşgun et al. 2021).

Understanding of critical factors affecting algal production will be beneficial for successful and sustainable biomass production for renewable fuel generation.

6.5 Conclusions

In present scenario, increasing demands of conventional fuels bring necessity of alternative sustainable energy resources. Biodiesel, biobutanol, biohydrogen, and bioethanol production from microalgae is promising alternative for fossil fuels. However, lipid yield is still not sufficient to meet biodiesel production as per demand. Hence, most research work is inclined towards enhanced lipid production by altering different culture conditions such different light colours, light intensities, photoperiods, temperature, pH, and inorganic carbon sources. So, this chapter give a comprehensive glance by discussing influence of critical factors for increasing lipid content, fatty acid composition, and biomass growth of microalgae.

Acknowledgement The authors of the manuscript are thankful to the Indian Institute of Technology (BHU) Varanasi, Varanasi, for extending their technical and financial support.

Conflict of Interest The authors have declared no conflict of interest.

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