Chapter 3
Next Generation Biofuels from Macroalgae: Prospects and Challenges

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Abstract Algae are unicellular as well as multicellular organisms and have been called as micro and macroalgae, respectively. Algae offer multiple potential benefits to address the today's need of the renewable feedstocks and energy source. Based on the source of feedstock, biofuels have been classified into first generation, second generation, and latest third/fourth generation biofuels. The latest technologies and scientific studies on algae have discovered the third and fourth generations of biofuels. Currently, researchers' focus is on biofuels, and they have shown that marine macroalgae have substantial stamina to replace the first- and second-generation biofuels as they are eco-friendly and utilize carbon neutral energy. Algal biomass is a sustainable resource of energy and therefore could offer an economic, environment-friendly, and industrially growing area in biofuel research. Algal biofuel is produced from the lipid stored in the algal cells. Algae have very high carbon dioxide fixation rate, low land requirement, and require significantly less area for their cultivation and mass production. In addition, they certainly have high photosynthetic efficiency per area. The challenges in the algal biofuel production lies in the economic large-scale production of the microalgae lipid, which can be modulated by enhancing the lipid content without trailing the growth rate of the strains.

Keywords Algae · Bioethanol · Biofuel · Renewable · Third-generation bioethanol

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3.1 Introduction

Algae are small, single celled, or multicellular. Algae are plant-like organisms, but lack true leaves and root system. Algae have been divided into microalgae and macroalgae based on their cellular morphology. Microalgae are commonly called as phytoplanktons. Approximately, 50,000 species of microalgae are known till date, which are classified based on their ultra-structure, biochemical composition, and life cycle. Algae have been classified into five groups and are believed to be both eukaryotic and prokaryotic (Table 3.1). Major groups are as follows: green algae (Chlorophyceae), cyanobacteria (Cyanophyceae), diatoms (Bacillariophyceae), golden algae (Chrysophyceae), yellow-green algae (Xanthophyceae), red algae (Rhodophyceae), brownalgae (Phaeophyceae), dinoflagellates (Dinophyceae) and ‘pico-plankton’ (Prasinophyceae and Eustigmatophyceae). Blue-green algae also known as cyanobacteria are prokaryotic photosynthetic unicellular microalgae. These algae conduct photosynthesis directly in the cytoplasm as they lack true cell organization. Photosynthetic cyanobacteria are mostly found in water systems such as streams, rivers, lakes, and oceans and carry out carbon assimilation, energy production and rapid polar and nonpolar lipid accumulation in algal biomass in the presence of sunlight. Macroalgae (generally called seaweeds) are multicellular algae that can grow in both fresh and salt water. These are fast-growing species and can reach sizes up to 60 m in length and are broadly classified into three distinct groups on the basis of their pigmentation: Phaeophyceae (brown seaweed), Rhodophyceae (red seaweed), and Chlorophyceae (green seaweed). While considering the general

Table 3.1 Different classes and common characteristics of the different algal groups

S. no.	Class	Common characteristics	Chlorophyll	Storage compound
1.	Bacillariophyceae (golden brown algae)	Diatoms, unicellular/ colonial, silicate cell wall, fucoxanthin	Chl a, c	Triglycerides (TAGs) and carbohydrates
2.	Chrysophyceae (brown algae)	Unicellular, fucoxanthin, cellulose and pectin in cell wall	Chl a, c	Oil droplets and carbohydrates
3.	Eustigmatophyceae (yellow green algae) promising for biofuel production	Eukaryotic, unicellular, presence of polysaccharides in cell wall	Chl a	Large amount of polyunsaturated fatty acids
4.	Chlorophyceae (green algae)	Unicellular, colonial or filamentous, eukaryotic	Chl a, b	Starch and oil droplets
5.	Prymnesiophyceae (golden brown algae)	Mostly marine, fucoxanthin		Chrysolaminarin: a carbohydrate reservoir
6.	Cyanophyceae	Prokaryotic, unicellular, multicellular or colonial, phycocyanin, can fix nitrogen	Chl a	Low level of lipid reserve material

term algae, the number of species has been estimated to be between one and ten million, most of which are microalgae.

Other algae belong to eukaryotes and contain a well-defined nucleus enclosed within a nuclear membrane. All eukaryotic algae contain intracellular chloroplast that comprises chlorophyll inside photosynthetic lamellae, which is the site for photosynthesis. Algal chloroplasts are of various shapes with diverse types of chlorophyll molecules. Different types of microalgae have characteristic color based on the presence of different types of chlorophyll and pigments. Apart from the chloroplast, well developed endoplasmic reticulum is also present in all eukaryotic algae. Pyrenoides are the site for all the enzymatic reactions leading to the glucose conversion to starch. These pyrenoides are generally present within the chloroplast of golden, red, and green algae. Vacuole also exists as a lipid storage organelle within the algal cells.

Chapman classified the algae by a modern approach on the basis of (1) existence of different nucleus, (2) photosynthetic pigments amount and chemical composition, (3) food reservoir type, (4) composition of cell wall, (5) existence, type, number, orientation of flagella, and (6) reproduction mode. Remarkably, all algal species are not likewise important for the production of biofuels. Microalgae are of prime importance for producing biofuels. Amongst the microalgae, the Chlorophyceae taxonomic group is the most potential species for biofuels. They are capable of performing photosynthesis, important for life on the Earth and produce approximately half of the atmospheric oxygen. They also use the greenhouse gas (GHG) carbon dioxide simultaneously to grow photoautotrophically. They represent an almost unexploited resource on the Earth and hence can be utilized immensely for producing third generation bioethanol.

3.2 Third Generation Biofuels

Biofuels, as the name suggests, are referred to the fuels that are obtained from the biomass. Depending on the means they have been utilized to turn as a fuel source, they are grouped in two broad categories—primary and secondary biofuels; the primary being those that can be directly used as a fuel without processing and the secondary ones have to undergo processing to be utilized as biofuels. In the present scenario, where an emerging requirement of fuel sources has to be met, biofuels are the hope of the hour, and thus, the focus on the secondary biofuels is much needed. Biofuels like biodiesel and bioethanol generated from the biomass are processed and then used in vehicles and industries. The secondary biofuels are further categorized into three generations: first, second, and third generation biofuels on the basis of different parameters such as type of processing technology, type of feedstock, or their level of development (Fig. 3.1).

First generation biofuels have certain limitations because it is derived from crops and thus creates a competition for agricultural land (Schenk et al. 2008); and therefore, second generation of biofuels emerged that are produced from

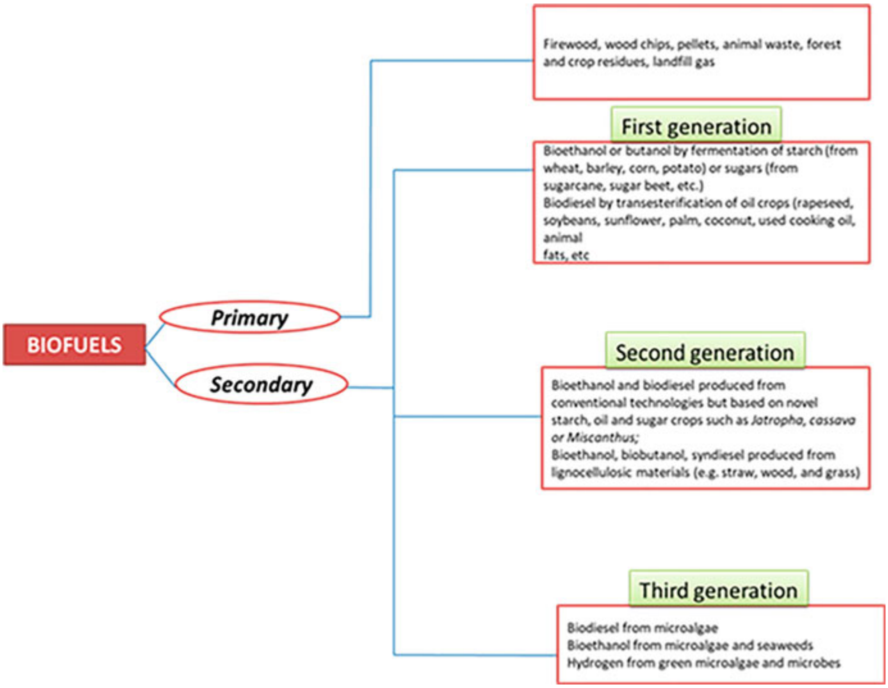

Fig. 3.1 Classification of biofuels

lignocellulosic biomass. Lignocellulosic biomass is the plant residues and hence does not compete with the food production category. Nevertheless, the disadvantage of the second generation is that it is not cost-effective. It requires expensive techniques for processing the lignocellulosic biomass to biofuels, and hence, large-scale production of second-generation biofuels is still noneconomic (Brennan and Owende 2010). Therefore, third generation biofuels that are microalgae derivatives emerged as a feasible alternative energy resource. Third generation biofuels is a recent term that is used for algae derived biofuels. It surpasses the major drawbacks en route with first- and second-generation biofuels (Nigam and Singh 2011; Chisti 2007). Microalgae are capable of producing approximately 15- to 300-fold higher amounts of oil for biodiesel production than the traditional crops on the basis of area. Another major benefit of utilizing microalgae is the shorter harvesting cycle than the conventional crops. The harvesting cycle of microalgae is ≈1–10 days (depending on the process), with significantly higher yields upon harvesting. Other characteristic properties such as algae can be genetically manipulated and oils produced by them can be converted directly in different types of fuels make them a potential source for biofuel production.

3.3 Potential of Macro/Microalgae: Advantages over Traditional Feedstocks

Algae are the potential source of next generation biofuel. The advantages of algal use as biofuels are manifolds. Algae grow very rapidly and produce biomass and oils which can be utilized for the biofuel production. Nearly 70% of the earth surface is covered with water, which can be utilized for the economic cultivation of algae up to five harvests per year. The generation time is less; therefore, algal biomass can be doubled in every few hours and biomass can be harvested. Algae being autotrophs, utilize direct sunlight and CO_2 for their growth. Photosynthetic algae are capable of utilizing almost 2 kg of CO_2/kg of biomass as algae produce about half of the oxygen present in the atmosphere (Williams and Laurens 2010). In contrast to terrestrial crops, microalgae do not require any fertile soil and freshwater supply and can tolerate extreme seasonal conditions (Williams and Laurens 2010). Thus, the cost of biofuel can be highly reduced. These microalgae do not interfere with the growth of terrestrial crops. The use of algae excludes the negative impact of food security by offering high sugar content and low lignin content than the terrestrial plants (Rajkumar et al. 2016). Few classes of algae can utilize heavy metals present in the ocean and accumulate high sugar content as reserve polymers which can easily be converted into biofuels. Finally, the efficacy of algae for photoconversion up to 5% is an added advantage in the direction of third generation biofuel production (Fig. 3.2).

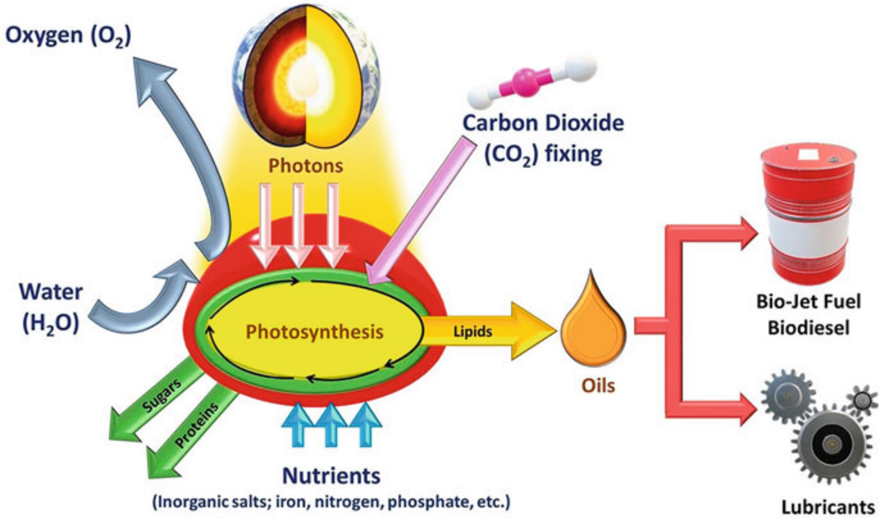

Fig. 3.2 Advantages of macroalgae for biofuel generation

3.4 Bioethanol Production Process from Algae

Microalgal biomass has emerged as the promising source for biofuels and is capable of meeting the demands without compromising either with food products or agricultural land for food production (Chisti 2007). The three main components of the algal biomass are carbohydrates, proteins, and lipids (Um and Kim 2009).

An integrated production of biofuels from microalgae consists of microalgal cultivation, separation of the cells from the growth medium, and subsequent lipid extraction for the biodiesel production through transesterification. Once the oils are obtained, amylolytic enzymes are used that promote fermentable sugar production; then, these are fermented and then distilled using conventional distillation technologies.

3.4.1 Microalgae Cultivation

First, the desired strain has to be selected to obtain the product of interest. The design of bioreactors is a crucial step for commercialization of the product. The cultivation system used for the microalgae should have the following characteristic properties which include high area productivity, high volumetric productivity, cost-effectiveness, and simplified control of the parameters (oxygen, temperature, pH, and turbulence) (Olaizola 2003). Depending on the purpose, the microalgae strain, and the final product, the cultivation system can be selected keeping in mind the various advantages and disadvantages of each kind.

3.4.2 Harvesting Methods

The harvesting of microalgal biomass can be done by physical, chemical, or biological ways. Flocculation is the first dewatering step of harvesting process that considerably lowers the hurdles of the next processing steps. Flocculation is meant to aggregate microalgal cells from the culture (Harun et al. 2010). Flotation can be used in cases where the algae begin to float by the increased lipid content. Centrifugation exploits the centrifugal forces to collect the algal biomass though the high shear forces may damage the cell and process is expensive. Filtration has emerged as the most effective harvesting method with its different forms.

3.4.3 Production of Bioethanol from Algal Biomass

The extraction can be achieved through oil press, liquid/liquid extraction, supercritical fluid extraction (SFE), and ultrasound systems (Harun et al. 2010). Solvent extraction has turned out to be one of the most useful methods, while ultrasound is an another promising way. After extraction, the obtained lipids can be transformed to biofuels via transesterification. The harvested algal biomass almost has 90% of the water content, and further processed using dehydration and extraction procedures, which leads to the formation of bioethanol.

3.4.4 Dehydration of Algal Biomass

Although there are many different methods for reducing the water content, each method has its own set of advantages and disadvantages. The common methods used are sun drying, spring drying, and freeze drying. Sun drying is cost effective, but extended time duration and area requirements makes it less worthy; spring drying is much worthy but not cost-effective. Spring drying is the most effective, but it is quite costly and hence becomes difficult for a large-scale operation (McKendry 2002; Prakash et al. 1997).

3.4.5 Extraction of Bioethanol

After dehydration, the biomass needs to be fermented in order to obtain bioethanol; for this purpose, the microalgal biomass is crushed and the obtained starch is transformed into sugars, which is then mixed with yeast and water in fermenters (Singh et al. 2011). The fermented bioproduct is then processed by distillation to eliminate the other extra matter from the thinned alcohol product. Different methods may be used for the extraction of biofuels as discussed earlier.

3.5 Lipid and Biomass Enhancement Strategies

Today, the population blast is the major concern for the modern generation. The world population is increasing day by day, and as per the current research, it is estimated that, by 2050, the existing population will increase by 1.5% of the current population. At present, the huge demand for energy and fuels is being met mainly through utilization of fossil fuels: oil, natural gas and coal—finite resources (Sajjadia et al. 2018). This could lead to the extinction of all fossil fuel reservoirs and aids in the environmental pollution owing to the heavy release of CO_2. This problem has driven a shift in the research direction toward algae as the novel lipid and biomass resource.

3.5.1 Lipid Content in Algae

Algae are wide lipid producers which cover a variety of lipids, viz. polar and neutral lipids, esters, sterols, and different modified versions of carotenoids. Polar lipids play a vital structural role in cell membrane and are called as high quantity of Poly Unsaturated Fatty Acids (PUFAs). Sterols and phospholipids are key membrane lipids, and some polar lipids act as signal tranducers in algae. Lipids are stored in algae in their late stationary phase in form of triglycerides (TAGs), which include mostly saturated fatty acids (FAs) and a few unsaturated fats. These forms of fats are stored in the cytosolic lipid bodies and can be easily catabolized into energy (Sharma et al. 2012). The fat content in macroalgae can range from 30 to 80% of total biomass; however, as the fat content is increased, the growth rate of algae is dropped rapidly. *Schizochytrium* sp. is known to produce up to 80% of the total mass as lipid which can be extracted and transformed into the next generation biofuels (Deng et al. 2009). In general, Chlorophyceae is the most promising species for algal oil extraction.

3.5.2 Lipid Composition and Suitability of Algae

Suitability of algae in the research field of biofuel is primarily determined by their lipid composition of saturated and unsaturated lipids. Unsaturated fatty acids such as palmitic acid (16:1), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) are the most important components of biofuels. Very little information is available for the use of saturated fatty acids (SFA) in biofuel production. Some microalgae synthesize lipids largely consisting of polyunsaturated fatty acids such as C22:6 (42%) in *Aurantiochytrium* sp., C22:5 + C22:6 (39.4%) in *Schizochytrium limacinum*, and C20:5 (25%) in *Porphyridium cruentum* (Sajjadia et al. 2018). Biofuels obtained from microalgae largely depend on the composition of fatty acids produced by the algal species.

Algal species producing fats predominantly of saturated fatty acids are the most suitable to be used in low temperature. Long chain SFA increases the pouring ability of biodiesel (Sajjadia et al. 2018). In contrast to this, the biofuels produced mainly by algal species producing high unsaturated fatty acids are easily oxidized, which may lead to inadequate engine performance due to the deposition of insoluble particles. For this issue, the proper selection of appropriate algal strain that may produce high yield of lipid along with quality composition of saturated and unsaturated fatty acid is highly desired. Moreover, the study of the impact of extreme environmental conditions on the production and composition of fatty acids by algal strain needs to be considered.

3.5.3 Lipid Content Enhancement Strategies

Metabolic activities influence cellular composition, growth rate, and lipid composition in algae (Fig. 3.3). Nitrogen, phosphorus, and silicate are the most vital nutrient contents in culture medium which are crucial for the growth and metabolic production of lipid in microalgae. Carbon source, salt concentration, intensity of sunlight, and atmospheric temperature are among the other effective factors. The common effective ratio of C:H:O:N:P for optimal algal growth needs to match $C_{106}H_{181}O_{45}N_{16}P$, theoretically so as to sustain the effective algal biomass. N:P ratio is also very crucial, as low N:P ratio (5:1) reflects nitrogen is limiting, whereas a high N:P ratio (30:1) suggests P is a limiting factor.

The research on algae limitation for nutrients is carried out by manipulating the nutrient contents in three different approaches. In starvation, firstly, the algae are allowed to grow in nutrient-rich conditions, and then the culture is transferred to the same nutrient limitation condition. This starvation triggers a sharp biological shock and results in the storage of high energy compounds. During the study of impact of nutrient limitation, metabolic shift in algae can be carried out by growing the algae under nutrient limitation in continuous culture. In nutrient-limited growth, the algae are grown in a medium in which all the macro and micronutrients are abundant except for the one limiting nutrient. This limiting nutrient can limit the yield of algal biomass due to the physiological reaction toward limiting nutrient, a phenomenon known as “law of the minimum” in microbiology. The effects of nutrient depletion

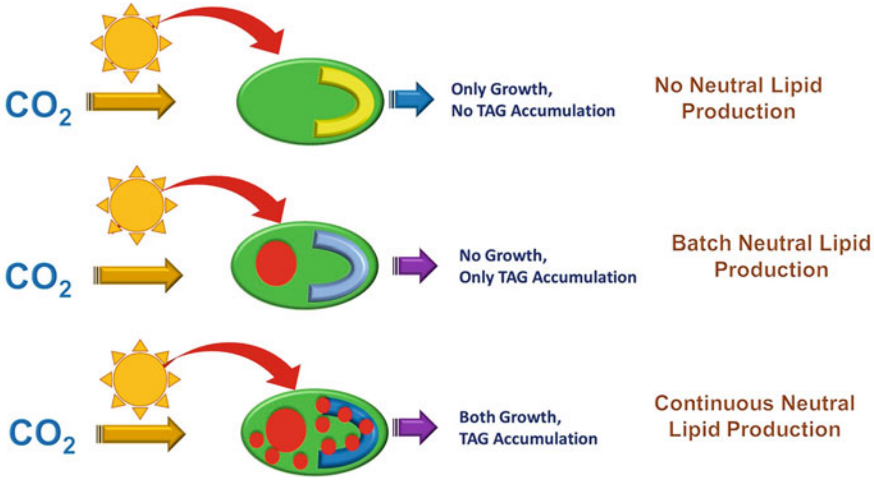

Fig. 3.3 Modulation of lipid content from microalgae for biodiesel production

can be studied by monitoring the growth of algae in small batches under nutrient depletion condition. The depleted nutrient is believed to specify the biomass yield. In this study, the algal cells are first grown in an environment which is enriched with required nutrients. Following the rise in biomass of algae due to growth, the nutrient depletion occurs. Due to this nutrient depletion, the growth rate and photosynthesis rate are reduced and the metabolic processes are shifted so as to adjust the organism to this nutrient depleted state.

3.6 Enhancement Parameters on Lipid Productivity

3.6.1 Nitrogen and Algal Lipid Content

Nitrogen is required for the biosynthesis of the major macromolecule, i.e., protein. It is also a common component for the buildup of structural and functional processes of the cell. Nitrogen is a crucial factor for lipid content and algal growth promotion. Nitrogen limitation causes enhancement in lipid and/or carbohydrate content and reduction in protein, efficiency of photosynthesis, and microalgae growth rate (Jiang et al. 2012). During nitrogen starvation, the C fixation increases, and the extra carbon is converted into lipids and carbohydrates as storage compounds. As the organic nitrogen pool in cell starts to decrease, the lipid production is induced in algae. Nitrogen concentration has a significant quantitative and qualitative impact on the lipid production and is very crucial for the biofuel production. The overall major fat profile in algae remains same in control and nitrogen starved media, but there is a significant difference in proportion of fatty acids (saturated and unsaturated). Under nitrogen stress condition, the ratio of saturated fatty acids (i.e., C16:0 and C18:0) is decreased. In *Chlorella* and *Ankistrodesmus falcatus*, saturated fatty acid content of C23:0 and C24:0 is known to reduce under nitrogen stress condition (Singh et al. 2015). The difference in the fatty acid composition leads to increase in the cetane no. and stability of the produced biodiesel. Among polyunsaturated fatty acids, linolenic acid (C18:3) is a common component of algae which is known to lower the biofuel stability due to possible oxidation as per the report of European standards (EN14214). Under nutrient stress, the percentage of linolenic acid is reduced which increases the biodiesel quality produced by some algae, e.g., *A. falcatus* (Converti et al. 2009). Different nitrogen sources has different impact on the overall fatty acid composition in algae. For instance, urea utilization results in the generation of higher PUFA, e.g., C18:4, C20:5, C22:6; nitrate and nitrite as the nitrogen source increases the ratio of saturated (C14:0) and unsaturated composites (C16:1) (Fidalgo et al. 1998).

3.6.2 Phosphorus and Algal Lipid Content

Phosphorus has a key role in various metabolisms such as signal transduction, transfer of energy, and photosynthesis. When phosphorus supply is abundant, the algal cells assimilate the excess phosphorus inside as polyphosphate granules. When phosphorus is in limited amount, the algal membrane phospholipid components are enriched by nonphosphorus glycolipids and sulpholipids as a P conservation mechanism as reported in *Chlorella* sp. (Liang et al. 2013). During phosphorus depleted state, cell division is reduced, which results in the accumulation of carbon in the form of TGA rich in saturated and monounsaturated fatty acids. It causes alterations in the biosynthesis process and enhances the stored amount of lipid in *Dunaliella parva*, *Chlorella* sp., *Scenedesmus* sp., and many other species. Unsaturated fatty acids which make the highest proportion of lipids in green algae *Chlamydomonas acidophila* (*C. acidophila*), *Chlorella* sp. (Spijkerman and Wacker 2011), and *Phaeodactylum tricornutum* (*P. tricornutum*) significantly increase under P starving condition (El-Sheek and Rady 1995). Similar results have been reported in *Scenedesmus quadricauda* (Ahlgren et al. 1998) which showed a lower percentage of PUFA 18:3(9,12,15) under P-limited conditions.

3.6.3 Effects of Carbon

Almost 50% of the algal biomass has been made up of carbon, and CO_2 is the main supply of the carbon source. The effects of CO_2 levels on total lipid contents, biomass (dw), and lipid profile of the microalgae has been reported in a few studies as summarized in Table 3.2. Carbon has a very pronounced impact on the fatty acid composition of algae. It is known that a high carbon monoxide concentration

Table 3.2 The effect of metal ions on metabolism, biomass, and lipid content in algae

Metal	Metabolic role	Effect on biomass	Effect on lipid content
Fe concentrations up to almost 2×10^{-3} g/L	Fundamental enzymatic reactions of photosynthesis	Increased	Can't promote lipid accumulation
Mg^{2+} 2×10^{-3}–8×10^{-3} g/L	Promote acetyl-CoA carboxylase (ACCase) in vivo activity	Increased	Increased
Ca^{2+} 5×10^{-4}–5×10^{-3} g/L	Signal transduction of environmental stimuli	–	Increased
Heavy metals (cadmium, copper 31.4 mg/L—Approximately 0.49 mM and zinc)	Alter the lipid metabolism	–	Enhancement of lipid content
Silica deficiency	Most common stress in diatoms	–	Promoting storage lipid

enhances the ratio of C18:1 and C18:2, whereas it decreases the polyunsaturated fatty acids (C18:3). In contrast to CO effect, the studies report that low CO_2 concentration (less than 2%) increases the unsaturated fatty acids (C18:1, C18:2) and high carbon dioxide (2–10%) favours the biosynthesis of the saturated fatty acids (104). It can be concluded that the high CO_2 concentration tends to reduce the unsaturation.

3.6.4 *Effect of Metals*

Several metal ions such as iron, magnesium, calcium, chromium, and other heavy metals (e.g., Cd, Cu, Zn) have a very promising impact on the lipid content of algae. The metabolic effect of various metal ions and their subsequent impact on the algal biomass and lipid content have been summarized in Table 3.2. It is observed that an increase in iron concentration has a positive influence on increasing the saturated: unsaturated fatty acid in algae. Rocchetta et al. (2006) demonstrated the increased saturated fatty acids production in the treated algal cells with the higher metal (e.g., Cr) concentrations. Under increased metal concentrations, the algal cells increase the carbon assimilation which leads to the production of C14:0, C16:0, and C18: 0 enriched fatty acids with a decreased rate of production for PUFA. When iron is abundantly present at a higher CO_2 atmosphere, it increases the fatty acid production of longer carbon chain (Takagi et al. 2006; Ghasemi et al. 2012). Therefore, 2% CO_2 can be effectively used in combination with high metal ion concentrations for the production of long-chain saturated fatty acids containing C12:0 and C16:0.

3.7 Effective Process Conditions

Several studies have been carried out to recognize the effects of various parameters on the growth and productivity of algae. Light, temperature, nutrients, pH, salinity, and dissolved oxygen are examples of the important key process conditioning factors that can be optimized for maximizing the third-generation biofuel production.

3.7.1 *Light*

Light is the most important parameter for photosynthesis steps by algae. Microalgal cells experience three light zones based on the intensity of light (Fig. 3.3). According to Beer–Lamberts Law, the light intensity is continuously lessened as it penetrates any surface (Bernard 2011; Yuan et al. 2014; Blanken et al. 2016). Hydrodynamics of the culture, i.e., how cells move in media, their closeness towards light source, and also other factors, play a vital role in the study. Algae are photosynthetic organisms and utilize a particular suitable wavelength (400–700 nm) of light. High light intensity leads to the inhibition of pigment system II of photosynthetic apparatus by inhibiting the crucial electron transfer proteins during photosynthesis (Moheimani and Parlevliet 2013; Quaas et al. 2015). A moderate light (optimum range) is required for the optimal level of photosynthesis; high intensity leads to photoinhibition of early growth and low light intensity cannot support the cell growth for successive generations. Day and night length also plays a crucial role in algal growth by altering its biochemical composition. Wahidin et al. (2013) reported that the lipid content and growth rate of *Nannochloropsis* sp. was enhanced when the L–D cycle was changed from 12:12–18:6 h. It has been observed that the red light generally promotes increased biomass production, whereas the blue and far red-light wavelength positively impacts the lipid and carotenoids accumulation. Therefore, light needs to be optimized accordingly to obtain a balance between photoprotection, photosynthesis, and biochemical composition of algae.

3.7.2 Salinity

Biosynthesis of lipid is a known resistance mechanism toward salt stress. Environmental salt concentration has a sharp effect on the lipid productivity, fatty acid profile, and growth rate. It is reported that as the salt content is increases, the ratio of monounsaturated fatty acids between palmitic acid C16:1) and oleic acids (C18:1)) enhances along with decreased proportion of polyunsaturated FAs (PUFA), which in turn favors the production of good quality biofuels. These changes in the fatty acid profiling in *Chlamydomonas mexicana*, *Scenedesmus obliquus* (Salama et al. 2014), *Botryococcus braunii* (Rao et al. 2007), *Cladophora vagabunda* (Elenkov et al. 1996) play a vital role in the direction of keeping the membrane fluidity and its destruction. These changes in fatty acids are consistent until the algae are grown within the optimum salinity level. Cao et al. (2014) stated that the PUFA increases abruptly in fractions of lipid till the optimum concentration of sodium chloride is attained.

3.7.3 Effects of Temperature

The temperature fluctuation has substantial impact on lipid production, lipid profiling, and biomass yield in algae. Both high and low temperature severely affect the organism’s growth. High temperature has more severe negative impact on algal growth due to the denaturation of proteins and enzymes. The temperature effect on algal growth can be understood by a bell-shaped growth curve. Plasma membrane fluidity needs to be maintained at such temperature fluctuations. Therefore, higher and lower temperature alters the biosynthetic pathway of lipid resulting in variable expression of relevant lipids, so that the algae gets adjusted with the changing temperature. In several algae, i.e., *Chlorella vulgaris* (*C. vulgaris*), exposure to high temperature (38 °C) leads to the decolourization of algal biomass from green to brown, reduced biomass, and ultimately death (Converti et al. 2009). Temperature range of 15–30 °C is ideal for the growth and photosynthesis of most of the algal species.

To survive the imbalance between energy supply and consumption, algal cells might change their size and shape as it hampers the photosynthetic ability by modulating the RUBISCO enzyme efficiency (Atkinson et al. 2003). The relationship between environmental temperature fluctuation and alteration in lipid, carbohydrate, and protein biosynthesis varies among different groups of algae. However, it has been observed that, low temperature stress mostly enhances the lipid accumulation in algal cell with an increased biosynthesis of saturated fatty acids (Renaud et al. 2002). This effect can be attributed to the increase in the glycolysis intermediates which can be directed towards high pyruvic acid biosynthesis and therefore, an increase in the overall lipid biosynthesis (Wang et al. 2016). Increasing temperature increases the monounsaturated fatty acids while downregulating the PUFA biosynthesis. According to the report, the low temperature activates the fatty acid desaturase enzyme leading to the conversion of oleic acid (18:1) to linoleic acid (18:2) and linolenic acid (18:3). This shift in the expression of polyunsaturated fatty acids enables the algae to maintain its membrane fluidity (Renaud et al. 2002; Wang et al. 2016). However, the overall lipid profile response toward increased/decreased temperature fluctuation is highly dependent on algal species.

3.7.4 Effects of pH

pH affects the algal growth by altering the mineral absorption capacity, i.e., iron and carbon. Most of the algae grow well between pH 7 and 9, and the pH of the algal culture increases sharply during the day time because of photosynthesis, whereas at night, the respiration leads to decrease in pH. Algal culture can be maintained at an optimal pH range by supplementing CO_2 or mineral acids. Higher pH conditions inhibit the growth of algae, whereas low pH supports the algal growth and lipid accumulation (Cao et al. 2014). The lipid accumulation in algae also alters dramatically with pH alteration. pH range of 7.0–9.5 is known to promote significant lipid accumulation in the algal species. Under nitrogen limitation and increased medium pH, *Chlorella* produces higher triacylglycerol. Algae grown under pH stress, e.g., pH 7.6 and 9.5, show increased lipid accumulation, where the alkali pH stress favors higher saturated lipid production and reduced glycolipid and polar lipid biosynthesis.

3.8 Scope and Challenges of Bioethanol Production

Nowadays, algal biomass for biofuel production hasemerged as a solution to many problems that arises due to an increase in industrialization. Water ecosystem management for cultivation offers a comparatively cheap and environment friendly option. Sustainable, biofriendly practices are the main reasons that make biofuels beneficial. There are still many hurdles in the path for sustainable algal biomass production; the production cost is still much higher that needs to be reduced. Developing new and genetically modified strains that may lead to higher yields and thus lower the production cost is the need of the hour (Chia et al. 2018). Current techniques are still much expensive, and we need more focus and research on the development of more efficient and cost effective harvesting methods and reduction of production cost (Wu et al. 2005; Zhu et al. 2014; and Ambati et al. 2014). In addition to these, the biomass cultivation systems (i.e., PBR system and open pond system) should also be made more economical and of course much sustainable.

3.9 Biotechnological Engineering of Microalgae

Algal biofuels are known to be a sustainable alternative to traditional fuels, though they need to overcome several hindrances for competing in the fuel market. Studies on microalgal biotechnology have gained attention in the past few decades, and the industry is expanding into new areas. However, it is remarkable to comment here that the microalgae are still not a well-studied group if biotechnology is the parameter. Among thousands of species that are supposed to exist, only a few hundred are investigated at molecular and biochemical levels and some are cultured industrially. The major bottlenecks in making algal biofuels as an alternative source of energy are the lack of two characteristics—high content of lipid and faster growth rate in current microalgal species (Ghosh et al. 2016; Chen et al. 2017). Also, the lack of sufficient light harvesting capacity in natural growth environment is one of the major obstacles (Stephenson et al. 2011). Studying the molecular complexities of metabolism, biosynthesis of TAG, its regulation, and metabolic flux, possibly will assist in strain improvement, and hence, maximize the biofuel production in microalgae in a cost-effective manner. There are many promising ways in which these unicellular organisms may be engineered, and all these methods finally lead to increase in the valuable content of these algae. Augmenting synthesis of oil in microalgae mainly hinge on the enzymes manipulation which are convoluted in biosynthesis of lipid or other competitive pathway intended to sidetrack the carbon and equivalent fluxes in the direction of lipid biosynthesis. Manipulating the expression of enzymes such as PEP, pyruvate dehydrogenase, acetyl-CoA synthetase, NAD(H) kinase, etc. has been proved to considerably enhance the lipid content without adversely affecting the cell growth. The most commonly used technique is the manipulation of genes involved in the metabolic pathway; however, this strategy has seen mixed response (Bajhaiya et al. 2017). In recent times, the transcriptional regulation of biosynthesis of oil has become prevalent for controlling the expression of multiple constituents of a metabolic pathway at the same time (Courchesne et al. 2009). Moreover, efforts to manipulate numerous other targets like improving light using efficiency, cell dormancy control, refining carbon sequestration, etc. have gained attention; these approaches influence the lipid content indirectly. The enhanced biomass yield is crucial for the overall energy output (Barry et al. 2015). In microalgae, the biomass productivity is governed by CO_2 fixation rate, abiotic stress, and light utilization efficiency (Chu 2017). Genetic engineering in combination with omics analysis facilitates the recognition of the major transcription regulators, enzymes, and promoters for stress response. This could be beneficial and facilitates future molecular studies.

Engineering metabolic pathway for achieving enhanced productivity of the algal strains by gene editing propounds a robust mechanism to overcome genetic shortfalls (Ng et al. 2017). Recently, CRISPR/Cas9, a genome editing technique, has emerged. The utility of this technique in improving microalgae traits for biofuels and nutraceutical applications has a significant scope. Numerous advanced studies led to success in improving the microalgae species, which endorse the technology for its efficiency in producing targeted mutants. The main advantage of CRISPR technology in algae is it provides the ease of multiplexing and manipulating of metabolic pathways unlike traditional knock out approaches. One of the successful examples is engineering of lipid in oleaginous microalgal strains by blocking the metabolic routes such as generating starch, degrading lipid, etc., which are competitive to lipid production. There are certain drawbacks that has to be always kept in mind whenever genetic engineering is considered, e.g., they should always be environment friendly, the newly edited gene is not that easily thrown out of the transformants, etc. In short, the genetically engineered variants should be complementing the finding new ones and those already known and not substitute them (Pulz O and Gross W 2004).

3.10 Sustainable Economy and Industrial Growth

Macroalgae are promising source for the commercial utilization. This approach where the high value coproducts are formed from the algal biomass along with the substantial biofuel production is known as a "biorefinery approach". Many attractive valuable biochemicals are yet to be discovered from the micro and macroalgae for their maximum utilization in industries. In biorefinery approach, the collaborative simulation of bioprocessing and environment friendly technologies occurs in an economic manner. Micro and macroalgae have vast industrial applications including high cost bioactive compounds, health foods, and natural pigments (Fig. 3.4). Algal biomass has immense applications, including i. wastewater treatment and CO_2 mitigation, ii. food and nutrition, iii. Aanimal and aquatic feed, iv. cosmetics,

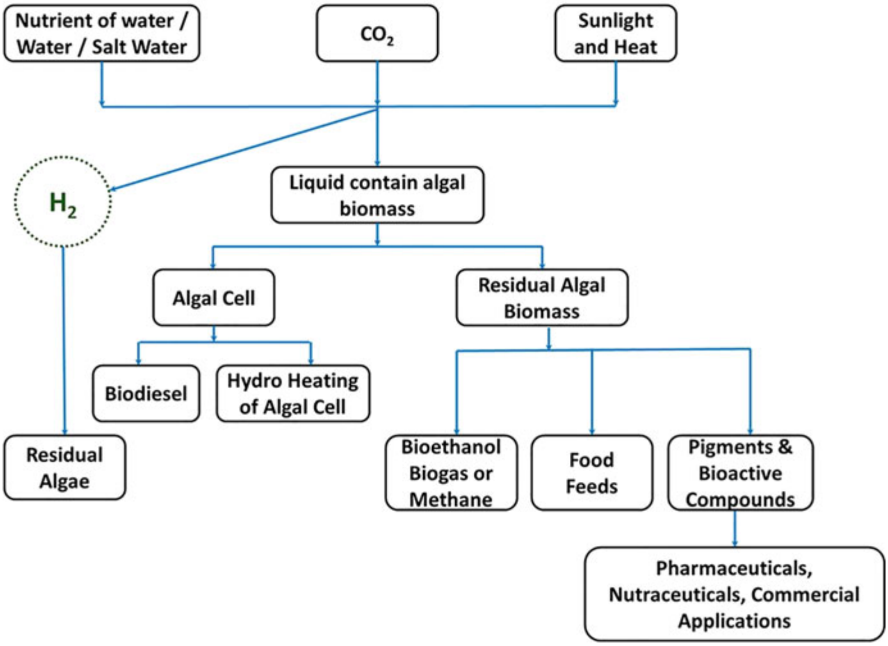

Fig. 3.4 Coproducts from algal biomass along with substantial biofuel production

v. fatty acid production, vi. synthesis of pigments, and vii. Bbiofertilizers. Therefore, the strategical bioproduction of biofuels from algae with maximizing the industrially important bioproducts should be carried out to utilize the algal biomass at its maximum ease. In this approach, the algae are cultivated in an algal farming facility, e.g., CO_2 mitigation and waste water treatment plant and algal biomass is harvested, following which, the bioactive products are effectively extracted, and finally, the biofuel can be produced by thermal processes (pyrolysis, liquefaction, or gasification).

3.11 Conclusion and Future Prospects

Regardless of the fascinating studies in the past few decades, microalgal cultivation is still challenging for industrialization. Despite the fact that the commercialization of third generation bioethanol is far from reality because of its high cost, microalgae could be a potential substitute of energy in the terms of biofuel and its production system. It does not raise the debate of food vs. fuel and set pressure on agricultural lands and forests. Various technologies such as metabolic engineering and gene editing could be promising for making this technology economically feasible. These techniques help in improving the strains and the microalgal biomass, which lead to decrease in the process cost. Biotechnology techniques possibly will reduce the production cost of microalagae produced biofuel by approximately 20% when compared with conventional approaches. Improvement at genetic levels has the capacity to inherit the traits such as rapid biomass production, elevated photosynthetic conversion rates during photosynthesis, alteration to its core structures, and adaptation to survive in varied climatic conditions could be a great opportunity in the field of sustainable biofuel production. The rapid studies in transcriptomics and whole genome sequencing facilitate the analysis of expression of newly modified metabolic pathways and the triggered gene expression for increased production of lipids by microalgae.

References

Ahlgren G, Zeipel K, Gustafsson I-B (1998) Phosphorus limitation effects on the fatty acid content and nutritional quality of a green alga and a diatom. Verh Int Ver Limnol 26:1659–1664. https://doi.org/10.1080/03680770.1995.11901011

Ambati R-R et al (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar Drugs 12(1):128–152. https://doi.org/10.3390/md12010128

Atkinson D, Ciotti BJ, Montagnes DJ (2003) Protists decrease in size linearly with temperature: ca. 2.5% degrees C(−1). Proc Biol Sci 270:2605–2611. https://doi.org/10.1098/rspb.2003.2538

Bajhaiya A-K, Moreira J-Z, Pittman J-K (2017) Transcriptional engineering of microalgae: prospects for high-value chemicals. Trends Biotechnol 35:95–99. https://doi.org/10.1016/j.tibtech.2016.06.001

Barry A-N, Starkenburg S-R, Sayre R-T (2015) Strategies for optimizing algal biology for enhanced biomass production. Front Energy Res 3:1. https://doi.org/10.3389/fenrg.2015.00001

Bernard O (2011) Hurdles and challenges for modelling and control of microalgae for CO_2 mitigation and biofuel production. J Process Control 21:1378–1389. https://doi.org/10.1016/j.jprocont.2011.07.012

Blanken W, Postma P-R, De Winter L, Wijffels R-H, Janssen M (2016) Predicting microalgae growth. Algal Res 14:28–38. https://doi.org/10.1016/j.algal.2015.12.020

Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev 14:557–577. https://doi.org/10.1016/j.rser.2009.10.009

Cao J, Yuan H, Li B, Yang J (2014) Significance evaluation of the effects of environmental factors on the lipid accumulation of *Chlorella minutissima* UTEX 2341 under lownutrition heterotrophic condition. Bioresour Technol 152:177–184. https://doi.org/10.1016/j.biortech.2013.10.084. Epub 2013 Nov 5

Chen B, Wan C, Mehmood M-A, Chang J-S, Bai F, Zhao X (2017) Manipulating environmental stresses and stress tolerance of microalgae for enhanced production of lipids and value-added products—a review. Bioresour Technol 244:1198–1206. https://doi.org/10.1016/j.biortech.2017.05.170

Chia S-R et al (2018) Sustainable approaches for algae utilization in bioenergy production. Renew Energy 129:838–852. https://doi.org/10.1016/j.renene.2017.04.001

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Chu W-L (2017) Strategies to enhance production of microalgal biomass and lipids for biofuel feedstock. Eur J Phycol 52:419–437. https://doi.org/10.1080/09670262.2017.1379100

Converti A, Casazza AA, Ortiz EY, Perego P, Del Borghi M (2009) 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 48:1146–1151. https://doi.org/10.1016/j.cep.2009.03.006

Courchesne N-M-D, Parisien A, Wang B, Lan C-Q (2009) Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. J Biotechnol 141:31–41. https://doi.org/10.1016/j.jbiotec.2009.02.018

Deng X, Li Y, Fei X (2009) Microalgae: a promising feedstock for biodiesel. Afr J Microbiol Res 3: 1008–1014

Elenkov I, Stefanov K, Dimitrova-Konaklieva S, Popov S (1996) Effect of salinity on lipid composition of *Cladophora vagabunda*. Phytochemistry 42:39–44. https://doi.org/10.1016/0031-9422(95)00857-8

El-Sheek MM, Rady AA (1995) Effect of phosphorus starvation on growth, photosynthesis and some metabolic processes in the unicellular green alga *Chlorella kessleri*. Phyton 35:139–151

Fidalgo J-P, Cid A, Torres E, Sukenik A, Herrero C (1998) Effects of nitrogen source and growth phase on proximate biochemical composition, lipid classes and fatty acid profile of the marine microalga *Isochrysis galbana*. Aquaculture 166:105–116. https://doi.org/10.1016/S0044-8486(98)00278-6

Ghasemi Y, Rasoul-Amini S, Naseri AT, Montazeri-Najafabady N, Mobasher MA, Dabbagh F (2012) Microalgae biofuel potentials. Appl Biochem Microbiol 48:126–144

Ghosh A, Khanra S, Mondal M, Halder G, Tiwari O-N, Saini S et al (2016) Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: a review. Energy Convers Manag 113:104–118. https://doi.org/10.1016/j.enconman.2016.01.050

Harun R et al (2010) Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sustain Energy Rev 14(3):1037–1047. https://doi.org/10.1016/j.rser.2009.11.004

Jiang Y, Yoshida T, Quigg A (2012) Photosynthetic performance, lipid production and biomass composition in response to nitrogen limitation in marine microalgae. Plant Physiol Biochem 54: 70–77. https://doi.org/10.1016/j.plaphy.2012.02.012

Liang K, Zhang Q, Gu M, Cong W (2013) Effect of phosphorus on lipid accumulation in freshwater microalga *Chlorella* sp. J Appl Phycol 25:311–318

McKendry P (2002) Energy production from biomass (part 2): conversion technologies. Bioresour Technol 83(1):47–54. https://doi.org/10.1016/S0960-8524(01)00119-5

Moheimani NR, Parlevliet D (2013) Sustainable solar energy conversion to chemical and electrical energy. Renew Sust Energ Rev 27:494–504. https://doi.org/10.1016/j.rser.2013.07.006

Ng I-S, Tan S-I, Kao P-H, Chang Y-K, Chang J-S (2017) Recent developments on genetic engineering of microalgae for biofuels and bio-based chemicals. Biotechnol J 12:1600644. https://doi.org/10.1002/biot.201600644

Nigam P-S, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37(1):52–68. https://doi.org/10.1016/j.pecs.2010.01.003

Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomol Eng 20(4–6):459–466. https://doi.org/10.1016/S1389-0344(03)00076-5

Prakash J et al (1997) Microalgal biomass drying by a simple solar device. Int J Solar Energy 18(4):303–311. https://doi.org/10.1080/01425919708914325

Quaas T, Berteotti S, Ballottari M, Flieger K, Bassi R, Wilhelm C et al (2015) Non-photochemical quenching and xanthophyll cycle activities in six green algal species suggest mechanistic differences in the process of excess energy dissipation. J Plant Physiol 172:92–103. https://doi.org/10.1016/j.jplph.2014.07.023. Epub 2014 Sep 3

Rajkumar R et al (2016) Potential of macro and microalgae for biofuel production. Bioresour Technol 9(1):606–1633. https://doi.org/10.15376/biores.9.1.1606-1633

Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA (2007) Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour Technol 98:560–564. https://doi.org/10.1016/j.biortech.2006.02.007. Epub 2006 Jun 19

Renaud SM, Thinh L-V, Lambrinidis G, Parry DL (2002) Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. Aquaculture 211:195–214

Rocchetta I, Mazzuca M, Conforti V, Ruiz L, Balzaretti V, del Carmen Ríos de Molina M (2006) Effect of chromium on the fatty acid composition of two strains of *Euglena gracilis*. Environ Pollut 141:353–358. https://doi.org/10.1016/j.envpol.2005.08.035. Epub 2005 Oct 4

Sajjadia B, Chena W-Y, Abdul A, Raman A, Ibrahim S (2018) Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. Renew Sust Energ Rev 97:200–232. https://doi.org/10.1016/j.rser.2018.07.050

Salama ES, Abou-Shanaba RA, Kim JR, Lee S, Kim SH, Oh SE et al (2014) The effects of salinity on the growth and biochemical properties of *Chlamydomonas mexicana* GU732420 cultivated in municipal wastewater. Environ Technol 35:1491–1498. https://doi.org/10.1080/09593330.2013.871350

Schenk P, Thomas-Hall S, Stephens E, Marx U, Mussgnug J, Posten C, Kruse O, Hankamer B (2008) Second generation biofuels: high efficiency microalgae for biodiesel production. Bioenergy Res 1:20–43. https://doi.org/10.1007/s12155-008-9008-8

Sharma K-K, Schuhmann H, Schenk P-M (2012) High lipid induction in microalgae for biodiesel production. Energies 5:1532. https://doi.org/10.3390/en5051532

Singh A et al (2011) Renewable fuels from algae: an answer to debatable land-based fuels. Bioresour Technol 102(1):10–16. https://doi.org/10.1016/j.biortech.2010.06.032

Singh P, Guldhe A, Kumari S, Rawat I, Bux F (2015) Investigation of combined effect of nitrogen, phosphorus and iron on lipid productivity of microalgae *Ankistrodesmus falcatus* KJ671624 using response surface methodology. Biochem Eng J 94:22–29. https://doi.org/10.1016/j.bej.2014.10.019

Spijkerman E, Wacker A (2011) Interactions between P-limitation and different C conditions on the fatty acid composition of an extremophile microalga. Extremophiles 15:597–609. https://doi.org/10.1007/s00792-011-0390-3

Stephenson P-G, Moore C-M, Terry M-J, Zubkov M-V, Bibby T-S (2011) Improving photosynthesis for algal biofuels: toward a green revolution. Trends Biotechnol 29:615–623. https://doi.org/10.1016/j.tibtech.2011.06.005

Takagi M, Karseno, Yoshida T (2006) Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae Dunaliella cells. J Biosci Bioeng 101:223–226. https://doi.org/10.1263/jbb.101.223

Um BH, Kim YS (2009) A chance for Korea to advance algal-biodiesel technology. J Ind Eng Chem 15(1):1–7. https://doi.org/10.1016/j.jiec.2008.08.002

Wahidin S, Idris A, Shaleh SRM (2013) The influence of light intensity and photoperiod on the growth and lipid content of microalgae Nannochloropsis sp. Bioresour Technol 129:7–11. https://doi.org/10.1016/j.biortech.2012.11.032. Epub 2012 Nov 16

Wang Y, He B, Sun Z, Chen Y-F (2016) Chemically enhanced lipid production from microalgae under low sub-optimal temperature. Algal Res 16:20–27

Williams P-J-l-B, Laurens L-M-L (2010) Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. Energy Environ Sci 3:554–590. https://doi.org/10.1039/B924978H

Wu L-C et al (2005) Antioxidant and antiproliferative activities of Spirulina and Chlorella water extracts. J Agric Food Chem 53(10):4207–4212. https://doi.org/10.1021/jf0479517

Yuan S, Zhou X, Chen R, Song B (2014) Study on modelling microalgae growth in nitrogen-limited culture system for estimating biomass productivity. Renew Sust Energ Rev 34:525–535. https://doi.org/10.1016/j.rser.2014.03.032

Zhu L-D et al (2014) Microalgal biofuels: flexible bioenergies for sustainable development. Renew Sust Energy Rev 30:1035–1046. https://doi.org/10.1016/j.rser.2013.11.003