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Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition

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ABSTRACT

Renewable energy sources e.g. biofuels, are the focus of this century. Economically and environmental friendly production of such energies are the challenges that limit their usages. Microalgae is one of the most promising renewable feedstocks. However, economical production of microalgae lipid in large scales is conditioned by increasing the lipid content of potential strains without losing their growth rate or by enhancing both simultaneously. Major effort and advances in this area can be made through the environmental stresses. However, such stresses not only affect the lipid content and species growth (biomass productivity) but also lipid composition. This study provides a comprehensive review on lipid enhancement strategies through environmental stresses and the synergistic or antagonistic effects of those parameters on biomass productivity and the lipid composition. This study contains two main parts. In the first part, the cellular structure, taxonomic groups, lipid accumulation and lipid compositions of the most potential species for lipid production are investigated. In the second part, the effects of nitrogen deprivation, phosphorus deprivation, salinity stress, carbon source, metal ions, pH, temperature as the most important and applicable environmental parameters on lipid content, biomass productivity/growth rate and lipid composition are investigated.

1. Introduction

The world population in 2050 is estimated to be 1.5 times the current population. Never before the necessity and challenge for sustainable production methods for food and energy was larger than in this century. On the other hand, about 85% of the current national energy needs are met by combustion of fossil fuels i.e oil, natural gas and coal–finite resources that increases concerns about the depletion of fossil fuel reserves and environmental pollution caused by carbon emissions. Therefore, the national energy strategy is changing towards using and getting more benefit from renewable and sustainable sources of energy to achieve energy security in an environmentally friendly manner. Biochar, biodiesel, bioethanol, biohydrogen are a few promising renewable energy sources, most of which are mainly produced from plant residues. As an example, more than 95% of current commercial production of biodiesel (Fatty Acid Alkyl Ester) is produced from edible vegetable oils at the cost of destruction of vital soil resources, deforestation, consumption of large amount of freshwater and much of the arable land. The increasing price of vegetable oil and the crisis of food versus fuel are the other problems associated with biofuel generation from plants, trees and oil crops. Accordingly, demand for other possible sources of biofuel has significantly increased. There is a wide range of raw materials for biofuel production. Based on their biomass feedstock, biofuels and biofuel production are classified into four different generations. First-generation biofuels are produced from mostly edible oil seeds, food crops, and animal fats. The main products of this generation include biodiesel, bioethanol, biobutanol. In contrast, second-generation biofuels use lower-value biomass residues such as Nonedible oilseeds, waste cooking oil and lignocellulosic feedstock materials (e.g. forest residues, sugarcane bagasse, cereal straw). In addition to the previous biofuels, syngas is also considered as the product of this generation. The third and fourth generations of biofuels are mainly produced from algae and other algae/microbes respectively. These feedstocks have potential to produce biodiesel, bioethanol, biobutanol, syngas as well as biohydrogen and methane [1]. Microalgae-based oil and biomass have several superiorities over terrestrial

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oleaginous crops [2].

- Algae grow quickly. They double every few hours and can be harvested daily. Generally, each unit area of land used for microalgae plant produces 10 times more oil compared to that of a typical terrestrial oleaginous crop.
- Algae use sunlight, consume carbon dioxide and release oxygen (O_2) as they grow. Generally, they are able to photosynthesize up to 2 kg of carbon dioxide per kg of biomass produced. It has been reported that microalgae generate approximately half of the atmospheric oxygen.
- Different from terrestrial oleaginous crops, microalgae do not require soil fertility and freshwater. They can grow in saline water medium and thus do not compete with other terrestrial crops [2].
- They can live and grow in wastewater and purify wastes while producing a biomass and oil suitable for biofuel production at the same time.
- The plant growth and the energy of terrestrial oleaginous crop depend on seasonal conditions, solar radiation and all other weather-based changes while algae tolerate extreme weather conditions.
- The photo-conversion efficiency of terrestrial crops versus incoming solar radiation for terrestrial crops is generally below 1% in temperate climates while the value can increase up to 5% for microalgae biomass.

The oil production efficiency of microalgae versus traditional vegetable oil crops was compared in term of land use in Table 1. As observed, although the oil content of microalgae is strain-dependent and similar to the oil content of terrestrial seeds, there is a significant difference in the overall biomass productivity and resulting oil yield. Besides, microalgae containing 30% oil by weight of dry biomass could yield almost 10 times more oil than palm, which is the most efficient vegetable oil crop. The value significantly increases for those strains that contain higher amount of oil. It is far in excess of what can be generated from palm, soybean and corn, which are currently the global sources of vegetable oil, respectively. Although microalgae-based oil and biomass yields are strain-dependent, significant economical and noneconomical advantages can be reached by using microalgae as biofuel feedstock. However, microalgae oil is estimated to be 3–4 times more expensive than plant oil [3]. Generally, the oil production from microalgae includes three steps: (1) microalgae cultivation (in open ponds or photobioreactors; (2) harvesting/concentration; and (3) oil extraction. Cultivation and harvesting steps are the limiting keys imposing 40% and 20–30% of the cost and energy in microalgal biofuel production, respectively [4,5]. Identifying an optimal balance among these stages is essential to reduce the environmental impacts and costs of the overall process. In this study, different potential organisms are reviewed in terms of biomass and lipid productivity/composition at first. Then, environmental factors e.g. nitrogen deprivation, phosphorus deprivation, NaCl stress, carbon, pH, temperature, and Fe(III) ion, as the most important and applicable strategies to enhance microalgae lipid content are investigated. The main objective of this study is to provide a comprehensive reference on the synergistic or antagonistic effects of environmental stresses on growth rate (biomass production), lipid productivity, lipid fatty acids compositions assisting the researchers to better understand the reactions of microalgae to the environmental factors. (Fig. 1)

Table 1
Comparison of microalgae oil potential with other biofuel feedstocks. Ref: [20,181–194].

Plant	Gal Oil/ Acre	Fat content in seed %
Oil Palm (*Elaeis guineensi)*	610	35.3
Macauba Palm(*Acrocomia aculeate)*	461	28.35
Pequi (*Caryocar brasiliense)*	383	45
Buriti Palm (*Mauritia flexuosa)*	335	19
Oiticica (*Licania rigida)*	307	80
Coconut (*Cocos nucifera)*	276	35.3
Avocado (*Persea Americana)*	270	8–32
Brazil Nut (*Bertholletia excelsa*)	245	66.9
Macadamia Nut (*Macadamia terniflora*)	230	71.6
Jatropha (*Jatropha curcas*)	194	28
Babassu Palm (*Orbignya martiana*)	188	60
Jojoba (*Simmondsia chinensis*)	186	48–56
Pecan (*Carya illinoensis*)	183	71.2
Bacuri (*Platonia insignis*)	146	13.5
Castor Bean (*Ricinus communis*)	145	48
Olive Tree (*Olea europaea*)	124	20
Rapeseed (*Brassica napus*), canola	122	30
Opium Poppy (*Papaver somniferum*)	119	45–50
Peanut (*Ariachis hypogaea*)	109	53–71
Sunflower (*Helianthus annuus*)	98	47.3
Tung Oil Tree (*Aleurites fordii*)	96	50–60
Rice (*Oriza sativa L.*)	85	10
Buffalo Gourd (*Cucurbita foetidissima*)	81	33
Safflower (*Carthamus tinctorius*)	80	30–40%
Crambe (*Crambe abyssinica*)	72	27.8–35.3
Sesame (*Sesamum indicum*)	72	49.1
Camelina (*Camelina sativa*)	60	40%
Mustard (*Brassica alba*)	59	35–46
Coriander (*Coriandrum sativum*)	55	13–20
Pumpkin Seed (*Cucurbita pepo*)	55	46.7
Euphorbia (*Euphorbia lagascae*)	54	48–52
Hazelnut (*Corylus avellana*)	49	50–70%
Linseed (*Linum usitatissimum*)	49	34%
Coffee (*Coffea arabica*)	47	10–20
Soybean (*Glycine max*)	46	17.7
Hemp (*Cannabis sativa*)	37	35
Cotton (*Gossypium hirsutum*)	33	40
Calendula (*Calendula officinalis*)	31	17–24%
Kenaf (*Hibiscus cannabinus L.*)	28	20%
Rubber Seed (*Hevea brasiliensis*)	26	24(seed) 40(kernel)
Lupine (*Lupinus albus*)	24	7.2–8.2%
Palm (*Erythea salvadorensis*)	23	20–45
Oat (*Avena sativa*)	22	3.1–11.6%
Cashew Nut (*Anacardium occidentale*)	18	41.7
Corn (*Zea mays*)	18	4
Microalgae (low oil content)	6275	30
Microalgae (medium oil content)	10,455	50
Microalgae (high oil content)	14,635	70

2. Basic information about microalgae

2.1. Microalgae cellular structure

Microalgae, named also as phytoplankton by biologists are very small single-cell plant-like organisms without leaves or roots. Green microalgae have a diameter between 1 and 50 micrometers, live in water systems such as streams, rivers, lakes and oceans and in fact are the first link in the oceanic food chain. These microorganisms convert carbon dioxide and water to oxygen and nutrient-rich biomass in the presence of sunlight through photosynthesis process. More than 50,000 microalga species are known to-date, which are categorized with respect to their ultrastructure, biochemical constituents, pigment composition and life cycle. Microalgae are classified into the microplankton (20–1000 μm), nanoplankton (2–100 μm), ultraplankton (0.5–15 μm) and the picoplancton (0.2–2 μm) with respect to their size.

Algae are made up of eukaryotic and prokaryatic cells, which are the cells with nuclei and organelles. However, most of the algae (some researchers say almost all) are eukaryotic. DNA in eukaryotic algae is localized within a minutely perforated nuclear membrane and their nuclei are similar to those of higher plants. All eukaryotic algae have intracellular organelle named as chloroplast, which contains photosynthetic lamellae with chlorophyll in which photosynthesis occurs. However, different types of algae have chloroplasts of different shapes with different combinations of chlorophyll molecules (Chlorophyll A, A

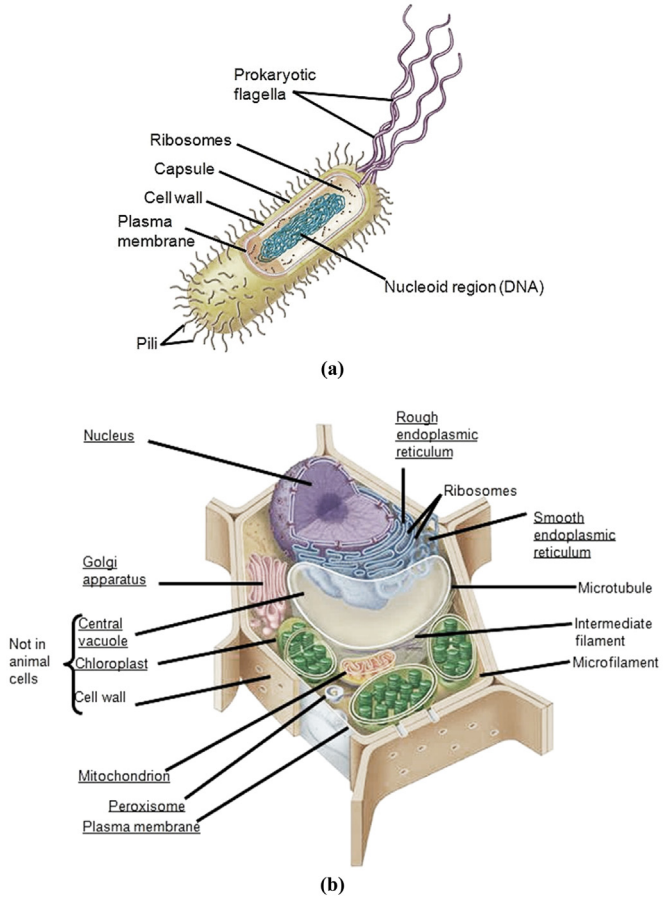

Fig. 1. A schematic diagram of **a)** prokaryotic cellular organization, B) eukaryotic cellular organization (Publishing as Benjamin Cummings PowerPoint Lectures for Biology, Seventh Edition Neil Campbell and Jane Reece). Pearson Education, Inc copy Right License (PE Ref. #: 206202).

& B or A & C). The color of microlage (i.e. green algae) relates to the Chlorophyll. Endoplasmic reticulum, mitochondria and Golgi bodies are the other intercellular organs of all eukaryotic algae. In many green, golden, brown and red algae, pyrenoids are present within the plastids. Pyrenoids are the centers for enzymatic condensation of glucose into starch. Vacuole is the other intercellular orange, which is predominantly found in the plant, fungal and algae cells, occupying 30–80% of the cell's volume. Lipids are mainly stored in vacuoles within the cell [6,7]. In prokaryotes cells, the nuclear materials are not confined by a nuclear membrane and are nearly dispersed throughout the cell. The membrane-bounded plastids, endoplasmic reticulum, mitochondria and Golgi apparatus are absent and the photosynthetic lamellae occur freely in the cytoplasm. Large aqueous vacuoles, are also absent from the structures of prokaryotes cells. Blue-green algae (*Cyanophyceae*) are the main group of algae which have prokaryotic cells and conduct photosynthesis directly within the cytoplasm, rather than in specialized organelles. The simplistic unicellular/multicellular structure of microalgae enhances their photosynthetic rates, enabling them for effective carbon sequestration and energy production due to rapid lipids accumulation in their biomass.

2.2. Microalgae biochemical composition

Biochemical composition of microalgae compromises four principal groups of molecules: proteins, carbohydrates, nucleic acids and lipids in varying proportions based on algae classes. The most energy-rich compound is lipid (37.6 kJ g^{-1}), followed by proteins (16.7 kJ g^{-1}) and carbohydrates (15.7 kJ g^{-1}). Microalgae contain primarily polar and nonpolar lipids. With respect to the metabolic rate, the proportion of these two types of lipids varies along different growth phases of algae. Polar lipids are structural lipids such as glycolipids and phospholipids. These lipids are bound to the organelle membranes such as the thylakoid membranes of the chloroplast [6,8]. Cell membrane that protects a cell and maintains its shape is mainly composed of glycolipids and phospholipids. The bilayer structure of phospholipids in cell membrane consists of a polar hydrophilic head and two hydrophobic fatty acid tails, which may be either off pure saturated tails or in combined with unsaturated tail [9]. Non-polar (neutral) are storage lipids i.e. triglycerides (TAGs), diglycerides (DAGs), monoglycerides (MAGs), free fatty acids (FFAs), hydrocarbons and pigments. Lipids are stored in algae in various ways depending on the algal species, growth phases and environmental growth conditions. Fatty acid compounds differ with algae. Predominantly, polyunsaturated fatty acids (PUFA) comprise the structural lipid fraction, while monounsaturated fatty acids (MUFA) and saturated fatty acids (SAFA) comprise the storage lipid fraction [6,9,10].

In addition to lipids, carbohydrates are the other products of microalgae derived from dark photosynthesis. In dark reactions, carbon dioxide is reduced to carbohydrates by the Calvin cycle [11]. Carbohydrates can be divided into structural components and storage components too. Structural components are found mainly in cell wall (e.g., pectin, cellulose, and sulfated polysaccharides) while storage components (e.g., starch) can accumulate inside or outside chloroplast. However, the metabolism and composition of carbohydrates is not similar in all groups of microalgae [12]. Microalgae carbohydrates can be used for fermentation into ethanol or anaerobic digestion for methane production [12].

Protein synthesis is the most difficult and complex mechanism in all cells. The main steps of the protein synthesis in a cell include amino acid synthesis, peptide chain condensation reaction and modification of the primary protein. Unicellular photosynthetic green algae are most commonly used for protein production as they only require inexpensive salt-based media, carbon dioxide and light for growth.

3. Microalgae taxonomic groups

Among over 50,000 algae species present in the world, either in only aquatic or terrestrial environments, about 4000 species have been identified. Algae are normally categorized based on i) presence or absence of distinct nucleus, ii) composition and relative amounts of different photosynthetic pigments, iii) types of food reserve, iv) cell wall chemistry, v) presence or absence of flagella, vi) number, type, place of origins and orientation of flagella in the motile cells and vii) mode of reproduction and life history. The modern tendency in classification is to use a combination of these characters. Accordingly, Chapman [13] classified the algae as follows:

Prokayotic	Cyanophyta (Blue-Green Algae)	Class	i) Cyanophyceae (Myxophyceae)
Division 1:	Rhodophyta (Red-Algae)	Class	i) Rhodohyceae
Division 2:	Chlorophyta (Green Algae)	Classes	i) Chorophyceae ii) Charophyceae ii) Prasinophyceae
Division 3:	Euglenophyta	Class	i) Euglenophyceae
Division 4:	Chloromonadophyta	Class	i) Chloromonadophyceae
Division 5:	Xanthophycta (Yellow/Green)	Class	ii) Xanthophyceae (Yellow/Green)
Division 6:	Bacillariophyta (Diatoms)	Class	iii) Bacillariophyceae

Division 7:	Chrysophyta (Yellow-Green Algae)	Classes	i) Chrysophyceae (Golden or Golden/ Brown) ii) Haptophyceae
Division 8:	Phaeophyta (Brown Algae)	Classes	i) Phaeophyceae
Division 9:	Pyrrophyta (Dinoflagellates)	Classes	i) Desmophyceae ii) Dinophyceae
Division 10:	Cryptophyta	Classes	i) Cryptophyceae

These species are not equally interesting for biodiesel production. Microalgae belonging to the five classes of *Bacillariophyceae*, *Chlorophyceae*, *Eustigmatophyte*, *Chrysophyceae*, *Haptophyceae* (*Prymnesiophyceae*) and *Cyanophyceas* are of primary importance for biofuel production (as highlighted). Amongst them, the green algae (*Chlorophyceae*) taxonomic group includes the most promising species for biodiesel production.

3.1. Bacillariophyceae

Bacillariphyceae, also known as diatoms, is the most widely distributed group of microalgae with about 100,000 species is known. They are mostly unicellular, although they can form colonies of various shapes (e.g. zigzag, ribbon, filament, fans and stars). Bacillariphyceae contains chlorophyll-a and chlorophyll-c. It has silicate cell walls. The cells of diatoms contain a high level of fuvoxanthin, a photpsynthetic accessory pigment that gives them golden-brown color. The main storage compounds of diatoms are triglycerides (TAGs) and carbohydrates.

3.2. Chrysophyceae

The Chrysophyceae involves 1100 species of unicellular algae and have the photosynthetic pigments containing chlorophylls a and c. Their chloroplasts contain a large amount of the pigment fucoxanthin, giving algae their brown color. They live in both marine and fresh water. Similar to the diatoms, the cell walls of Chrysophyceae algae are made of cellulose and pectin materials. Some species of this class also lack cell walls. The golden-brown algae reserve oil droplets and carbohydrates as storage compounds.

3.3. Eustigmatophyceae

Eustigmatophytes are a small group of yellow-green eukaryotic algae with species claimed to be between 20 and 35. In recent classifications, Eustigmatophceae is considered as a separate lingeage in Stramenopile, Heterokontophyta or Ochrophyta. For a long time, they had been considered members of Xanthophyceae. Hibberd and Leedale [14] discovered that these cells are different with Xanthophyceae in having an eyespot outside the chloroplast. They are in pale-green color due to the presence of chlorophyll-a. Eustigmatophytes are unicellular cells and microalgae belonging to this group are mostly small in cell size (2–4 micrometer). They have uni- or bi-flagellate, coccoil to spherical shaped cells containing polysaccharides in cell walls. Eustigmatophytes contain a high quantity of lipid, which is constituted of a large amount of essential polyunsaturated fatty acids. Therefore, some members of the class, especially the marine species of the genus *Nannochloropsis*, are very promising for biofuel production.

3.4. Chlorophylceae

Chlorophylceas forms a major class of green algae, which are made from eukaryotic cells. The grass green color of Chlorophylceas is due to the dominance of chlorophyll-a and chlorophyll-b light harvesting pigments in the cell. To-date, 2650 species of this class, which may be unicellular, colonial or filamentous has been known. Cells are eukaryotic, containing cellulose in the cell wall. Green algae usually have a rigid cell wall made of an outer layer of pectose and an inner layer of cellulose. Most of the members of this class have one or more storage bodies containing starch besides protein located in the chloroplasts. Some of them may store food in the form of oil droplets.

3.5. Prymnesiophyceae

Microalgae in the class of Prymnesiophyceae, known also as the Haptophyceae, consist of approximately 500 species. Haptophytes are primarily marine species. Chrysolaminarin, a carbohydrate food reserve, is the major storage product of these species. Similar to the chrysophytes and bacillariphyceae, fucoxanthin gives a yellow-brown to golden-brown color to the cell and lipids of this class.

3.6. Cyanophyceae

Microalgae belonging to Cyanophyceae are prokaryotic cells with mucilaginous cell walls or sheaths in blue-green color because of the presences of chlorophyll-a and phycocyanin. They possess unicellular, multicellular or colonial cell structures that contain no membrane enclosed nucleus or chloroplasts. More than 2000 species of this class known up-to-date have a different gene structure than the other microalgae. Some of them can absorb atmospheric nitrogen eliminating the need to provide fixed nitrogen for cell growth. This class of microalgae store less amount of lipids compared to other groups, causing them being less attractive for biofuel production. Nevertheless, they could be potential choices for carbon dioxide mitigation and production of novel bio-products.

4. Lipids in microalgae

Generally, microalgae generate a wide range of lipids including polar lipids, neutral lipids, wax esters, hydrocarbons, sterols and prenyl derivatives such as carotenoids, terpenes, tocopherols, quinines and pyrrole derivatives such as chlorophylls. Polar (structural) lipids mainly contain a high quantity of Poly Unsaturated Fatty Acids (PUFAs). Phospholipids and sterols are the key polar components that make cell membranes, which act as a selective permeable barrier for organelles and cells and participate directly in membrane fusion events. Moreover, some polar lipids may also behave as important intermediates in cell signaling pathways (e.g., sphingolipids, inositol lipids, oxidative products) and contribute in reacting to changes in the environmental parameters. Storage lipids are mainly in the form of triglycerides (TAGs), having a high content of saturated FAs and some unsaturated FAs. Most microalgae accumulate very little TAGs during exponential growth and the main amount of TAGs can accumulate during stationary phase [7,15,16]. Some oil-rich species have the potential to reach high level of long-chain polyunsaturated fatty acids as TAGs. TAGs can be easily catabolized to supply metabolic energy. They are mainly synthesized in the light, accumulated in cytosolic lipid bodies, and then reused for polar lipid synthesis in the absence of light [17]. Analysis on both accumulation of TAGs in *Parietochloris incisa* as a green microalgae and storage in *chloroplastic* lipids demonstrates that TAGs do not only play the role as an energy storage product but also have other roles. For example, in the reaction to an abrupt change in the environmental condition to speed up the adaptive membrane reorganization, PUFA-rich TAGs may endue specific acyl groups to Mono Galactosyl Diacyl Glycerol (MGDG) and other polar lipids [17]. A list of the most common fatty acids (lipids) along with their main specifications, which are normally included in microalgae and used in biodiesel synthesis are provided in Table 1 of our previous manuscript [18].

4.1. Microalgae lipid content

The oil content of the most common microalgae frequently used in

Table 2
Taxonomy and properties of the most suitable strains for lipid and biofuel production.

Taxonomy	Cellular Structure		Color	Diversity	Chloroplast Content	Storage
Bacillariophyceae	Eukaryotic	Marine & Freshwater & Brackish	Golden-brown	100,000	Chlorophyll-a Chlorophyll-c	Lipid & Chrysolaminarin
Chlorophylceae	Eukaryotic	Mostly Freshwater, A Few Marine	Green algae	8000	Chlorophyll-a Chlorophyll-b carotenes and xanthophylls	**Starch** Protein
Eustigmatophyceae	Eukaryotic	Mostly Freshwater	Pale-green or Yellow-green	< 35	Chlorophyll-a	Lipid & Chrysolaminarin (liquid β − 1,3-glucan)
Chrysophyceae	Eukaryotic	Freshwater & Marine	Golden-brown	1100	Chlorophyll-a Chlorophyll-c	Lipid Carbohydrate
Prymnesiophyceae	Eukaryotic	Mainly in Fresh Waters	Brown algae Due to fucoxanthin	500	Chlorophyll-c	Chrysolaminarin (known as α − 1, 3-linked carbohydrate)
Cyanophyceae Less attractive for biodiesel	Prokaryotic	Freshwater & Marine	blue-green	2000	chlorophyll-a & phycocyanin	

researches is summarized in Tables 2 and 3. As observed, oil content of 30% is quite common in most of the species while some have much higher oil content (56% in *Nannochloris sp.*, 53% in *Chlorella sp.* and 65% in *Neochloris oleoabundans*). However, higher oil strains grow slower than low oil strains [19]. Besides, there are algae (like *Schizochytrium sp.*) with more than 80% of their total mass is lipid. It is the oil (fatty acid) that can be extracted and converted into biofuels. However, it has been reported that microalgae containing 80% oil grow 30 times slower than those containing 30% oil [20]. As per Table 3, *Chlorophyceae* contains the most number of promising species for alga-oil production. The microalgae belonging to the *Labyrinthulomycetes* have been less studies; however, some of the members of this class (i.e. *Schizochytrium*) can store a high amount of oil. On the other hand, the species of *Cyanophyceae* and *Rhodophyceae* are more suitable for protein and carbohydrate production.

4.2. Micro algae lipid composition

The amount and ratio of saturated and unsaturated fatty acid is a key that determines the suitability of microalgae as a biofuel feedstock. The relevant details of the most widely used microalgae-oil are summarized in Table 4. Generally, unsaturated fatty acids, especially palmitolleic (16:1), oleic (18:1), linoleic (18:2), linolenic acid (18:3) and the saturated fatty acids of palmitic (16:0) are the main compositions of the oil generated by microalgae with only a small proportion made from saturated fatty acids of stearic (18:0). Some microalgae can also synthesize a large quantity of polyunsaturated fatty acids such as C22:6 (42%) in *Aurantiochytrium sp.*, C22:5 + C22:6 (39.4%) in *Schizochytrium limacinum*, C20:5 (25%) in *Porphyridium cruentum*. The fuel properties of biodiesel produced from microalgae significantly depend on the composition of miacroalgal fatty acids. For example, the biodiesel generated from the microalgae fat with lower saturated fatty acid content presents better cold temperature properties because long-chain saturated fatty esters dramatically increase the pour and cloud point of biodiesel. However, biodiesel containing a high amount of unsaturated compounds gets oxidized faster than conventional diesel, leading to insoluble sediments to interfere with engine performance. Therefore, the ability of microalgae in generating a high quantity of lipid and high quality of the fatty acid compositions should be considered while seeking efficient microalgae strains for biofuel production. Besides, most microalgae species are able to thrive under extreme conditions, in which their growth rate, lipid content and fatty acid composition are significantly affected. Understanding the effects of such situations on microlalgae behavior gives us a controlling parameter to achieve more favorable results.

5. Environmental effects on microalgae lipids

It is well known that the biological activities of microalgae highly depend on the culture conditions. Unfavorable conditions could change the cellular composition of microalgae (in phytoplankton cells as an example), their growth rate, lipid accumulation etc. For example, the culture conditions imposed onto the cells may increase the oil content of the *Chlorella vulgaris* by almost two or three times. Nutrient availability, e.g. nitrogen, phosphorous, and/or silicate (in diatoms) in the culture medium is the key to the growth and primary production of microalgae. The supply of carbon source, salinity, light intensity, and temperature are among the other effective factors. Overwhelmingly, compared to other nutrients, nitrogen and phosphorous manipulations have been found to be the most effective factor to increase lipid accumulation several folds in microalgae, respectively. Theoretically, the most common elements in the medium of alga culture should follow the stoichiometric formula of $C_{106}H_{181}O_{45}N_{16}P$ to reach the optimal growth of algae. Low ratio of nitrogen to phosphorus of about 5:1 suggests N-limitation and high ratio of about 30:1 suggests P-limitation.

The sensitivity of microalgae to nutrient manipulation is usually considered through three levels of limitation including starvation, limitation and depletion. During starvation, microalgae are grown in nutrient replete environment first and then the cells are separated and transferred into another media with the absence of a particular nutrient. The lack of nutrient causes a sharp biological shock resulting in generation and saving of high-energy compounds. Nutrient limited growth involves growing organisms in continuous cultures with a media in which all nutrients are available in excess except one particular nutrient that limits the maximum quantity of biomass that can be produced and causes physiological reaction to the limiting nutrient. The basic idea of this method is named as "law of the minimum" in microbiology. It assumes that there is always one particular nutrient that specifies the utmost amount of biomass that can be produced. Nutrient deficient growth is commonly performed in batch cultures. This method involves growing cells in an environment replete with required nutrients. The growth rate of the organisms rise along with cell density until the nutrient depletion is achieved. Then, the growth rate and photosynthesis potentially reduce while high-energy storage compounds increase due to some alterations in the metabolic processes in response to repletion of required nutrients.

5.1. Nitrogen factor

5.1.1. Effect of nitrogen on lipid content of microalgae

Protein is the major macromolecular pool of intracellular nitrogen and mainly affected by nitrogen rather than any other nutrients e.g. phosphorus [21]. Nitrogen is an essential constituent of cell structure

Table 3
Oil content of marine microalgae expressed on a dry matter.

Class	Edible Vegetable Oil	Oil Content (%)	Protein (%)	Carbohydrates (%)
Bacillariophyceae	Phaeodactylum tricornutum	18–57	30	8.4
	Thalassiosira weissflogii	5–20	43	12
	Skeletonema costatum[a]	13–51	25	4.6
	Chaetoceros muelleri	13–24	31–43	7–28
Chlorophyceae				
	Dunaliella primolecta	23	< 64%	11–23
	Dunaliella tertiolecta	11–16	20–29	12.2–14
	Dunaliella. salina	6–25	57	32
	Dunaliella Bioculata	8	49	4
	Nannochloris sp.	20–56	16.69	–
	Nannochloropsis oculata	22–29	35	7.8
	Scenedesmus obliquus	30–50	10–45	20–40
	Scenedesmus quadricauda	1.9	40–47	12
	Scenedesmus dimorphos	16–40	8–18	21–52
	Scenedesmus sp.	17–24	29–37	32.7–41
	Ankistrodesmus sp.	11.48–31	16.24–18.66	4.48–5.97
	Ankistrodesmus fusiformis			
	Chlamydomonas reinhardtii	21	48	17
	Chlamydomonas sp.	22.7	58.8	18.5
	Parietochloris incisa	62	–	–
	Tetraselmis tetrathele [b]	25–30	–	–
	Neochloris oleoabundans	35–65	10–27	17–27
	Scenedesmus falcatus	6.41–9.6	3.37–7.83	2.73–6.83
	Scenedesmus protuberans	17.53–29.30	25.4–45.05	20.95–29.21
Eustigmatophyceae				
	Chlorella sp.	28–53	25–45	24–30
	Chlorella vulgaris	41–58	51–58	12–17
	Chlorella pyrenoidosa	2	57	26
	Chlorella protothecoides	40–60	10–28	11–15
	Chlorella emersonii	23–63	36	41
	Chlorella sorokiana	22–24	40.5	26.8
	Chlorella minutissima	14–57	47.89	8.06
Cyanophyceae				
	Nostoc commune	22	20.3–43	34–56.4
	Synechocystis sp.[d]	11	63	15
	Spirulina Platensis	4–11	46–63	8–14
	Spirulina Maxima	6–7	60–71	13–16
	Spirulina sp	7.72	57.47	23.9
Haptophyceae				
	Pavlova lutheri[e]	35	29	9
	Pavlova salina[f]	12–30	26	7.4
Prymnesiophyceae				
	Emiliana huxleyi	43.8	39.2	17.2
Raphidophyceae				
	Heterosigma akashiwo	43	50	7
Cryptophyceae				
	Chroomonas salina	12–14.5	29–35.5	9–11
Rhodophyceae				
	Porphyridium cruentum[c]	9–14	28–39	40–57
Conjugatophyceae				
	Mesotaenium.sp.	19–35	53	27
Labyrinthulomycetes				
	Schizochytrium limacinum	43	39	5
	Schizochytrium sp.	50–77	15	12
	Aurantiochytrium sp.			

*Not belongs to this class but belongs to this phylum. a: Phylum: Bacillariophyceae, Class: Mediophyceae; b: Phylum: Cholorophyta, Class: Chlorodendrophyceae; c: Phylum:Rhodophyta, Class: Porphyridiophyceae; d: Phylum: Cyanobacteria; Class: Porphyridiophyceae; e: Phylum: Haptophyta; Class: Pavlovophyceae; f: Phylum: Haptophyta, Class: Pavlovophyceae, g: Phylum: Haptophyta, Class: Prymnesiophyceae.
Ref:[195–215].

and functional processes of microalgae since it is a key component of proteins, amino acids, nucleic acids, enzymes and photosynthetic pigments. It also affects the lipid content and growth of algae. Moreover, cellular nitrogen and lipid content are inversely related and so analysis of organic nitrogen is a representing key for nitrogen starvation and the beginning of lipid induction. Organic nitrogen per biomass of < 3% is usually considered as the nitrogen starvation. Under sufficient nitrogen concentration, the molar rate of photosynthetic carbon fixation is 7–10 folds the rate of nitrogen assimilation, which is an appropriate ratio for synthesis of essential nitrogen-containing cellular components. Different strain species develops different strategies to cope with variations in cellular N quotas, which can either make differences in rates of protein turnover or rearrangement in intracellular pools [22]. With respect to the temporal changes of N, some algal cells regulate N supply and some others regulate energy supply for N assimilation. The production of organic storage compounds is an example of the latter strategy. After transferring from N-sufficient culture to N-depleted culture, cell proteins decreased within the first few days (3–4 days depend on the species) due to continued cell division followed by dilution of cellular pools by new biomass production. Therefore, cell

Table 4
Fatty acid composition of pure edible vegetable oils.

Edible Vegetable Oil	C 10:0	C 12:0	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:1	C 18:0
Bacillariophyceae													
Phaeodactylum ricornutum	–	–	**7.2**	–	0.7	–	**18.6**	**35.8**	2.93	8.3	1.4	–	1.53
Thalassiosira weissflogii	–	–	**13.4**	–	–	–	**32.4**	**31.8**	–	–	7.4	–	1.5
Skeletonema costatum	–	–	**12.3**	–	2.7	–	7.25	**19.9**	3.75	**13.6**	7.8	–	1.15
Chaetoceros muelleri	–	–	**15.59**	–	1.14	–	**24.6**	**37.8**	3.06	3.4	–	–	2.06
Chlorophyceae													
Dunaliella primolecta			0.5		3.1		**23.9**	2.7	0.9	2.5	**12.3**		1.2
Dunaliella tertiolecta	–	–	0.77	–	0.63	–	**26.1**	2.59	1.72	3	17.4	–	4.05
Dunaliella. salina	2.43	6.9	0.97	–	2.51	–	**14.4**	3.53	1.3	3.8	**20.4**	–	2.77
Nannochloris sp.			4.95				**18**	**10.6**	1.02	2.9			1.84
Nannochloropsis oculata	0.5	0.8	9.1	5.94	0.97	–	**35.1**	**26.1**	–	–	–	0.4	2.53
Scenedesmus obliquus	0.13	0.04	0.413	2.65	1.92	0.36	**24.8**	4.13	0.62	3.63	11.2		1.31
Scenedesmus quadricauda	**15.2**	8.3	1.57	9.4	–	–	**28**	3.62	2.58	–	–	10.7	4.84
Scenedesmus dimorphos	–	–	0.5	–	0.4	2.3	**15.8**	5.2	2.1	2.1	**15.6**		0.6
Scenedesmus sp.	0.58	2.4	0.4	2.0	–	–	**17.9**	3.9	–	–	–	1.7	1.9
Franceia sp.	–	–	0.6		0.6	2.2	**12.9**	7.3	1.5	2	17		0.5
Ankistrodesmus sp.	–	–			–	–	**16.2**	3.06	–	–	–	–	7.18
Ankistrodesmus fusiformis	–	–	0.46	0.03	–	–	**25.3**	0.34	–	–	–	–	1.46
Chlamydomonas reinhardtii	–	–	3.07	–	–	–	**17.8**	5.77	–	–	–	–	6.85
Chlamydomonas sp.	–	–		–	–	–	**51.9**	1.2	–	–	–	–	**21.1**
Parietochloris incisa	–	–	–	–	–	–	13.5	2	3.1	1	–	–	6.2
Tetraselmis viridis	–	–	0.7	–	2.6	–	**16.1**	5.9	2.15	1.25	**19.1**	–	0.85
Eustigmatophyceae													
Chlorella sp.	–	3.6	2.8	1.6	1.56	0.7	**18.3**	3.48	3.51	8.76	0.3	–	2.46
Chlorella vulgaris	–	0.7	1.91	1.58	0.9	1.8	**15.3**	2.63	6.3	4.9	–	6.47	3.37
Chlorella pyrenoidosa	–	1.18	0.5	–	0.45	0.9	**18.6**	2.67	3.63	4.49	–	–	2.54
Chlorella protothecoides	–	–	0.8	0.8	–	–	**16.2**	5.07	5.8	–	–	–	2.89
Chlorella salina	–	–	3	–	–	–	**22.7**	6.9	–	–	–	–	8.69
Cyanophyceae													
Nostoc commune	–	–	0.3	–	–	–	**34.4**	**17.7**	0.4	–	–	–	1.5
Synechocystis sp.	–	–	**27.9**	–	–	–	**22.7**	**36.9**		–	–	–	3.5
Synechocystis sp. PNN 6803	–	–	2	–	–	–	**56**	4.05		–	–	–	2.4
Spirulina Platensis	–	1.0	0.9	–	1.12	1.96	**41.4**	3.44	2.84	0.05	–	–	2.32
Spirulina Maxima	–	0.5	1.5	–	–	–	**37.7**	3.44	–	–	–	–	3.42
Spirulina sp	–		3.28	3.8	–	–	**39.7**	6.68	–	–	–	–	3.66
Haptophyceae													
Pavlova lutheri	–	–	10.95	–	–	–	**17.4**	**27.3**		–	–	–	2
Pavlova salina	–	–	13.1	–	1.5	–	**15.1**	**30.4**	1	0.5	–	–	1
Emiliana huxleyi	–	–	**30.3**	–	–	–	14	5.5		–	–	–	6.45
Raphidophyceae													

(*continued on next page*)

Table 4 (*continued*)

Edible Vegetable Oil	C 10:0	C 12:0	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:1	C 18:0
Heterosigma akashiwo	–	–	6.4	–		–	**43.2**	**17**	4		0.4	–	0.5
Dinophyceae													
Gymnodinium kowalevskii	–	–	12.4	–	0.4	–	**26.7**	2		–	–	–	8.5
Cryptophyceae													
Chroomonas salina	–	–	5	–	2	–	**13.5**	2	0.2	–	–	–	3
Rhodophyceae													
Porphyridium cruentum	–	–	0.5	–	1.4	–	**34.4**	2.28	0.2	0.2	–	–	0.8
Chromalveolata													
Amphidinium sp.	–	–	–	–	–	–	**29.6**	**1**	–	–	–	–	3.5
Trebouxiophyceae													
Picochlorum sp.	0.1	–	0.5	–	0.3	–	**16.8**	1.2	5.1	3.5		–	3.4
Conjugatophyceae													
Mesotaenium.sp.	–	–	0.5	–	0.5	2.3	**13.4**	6.1	2.2	2.4	**16.4**	–	0.6
Labyrinthulomycetes													
Schizochytrium limacinum	–	–	3.94	–	5.1	–	**46.6**	–	–	–	–	3.5	3.6
Schizochytrium sp.	–	–	5.5	–	–	–	**14.8**	–	–	–	–	–	–
Aurantiochytrium sp.	–	–	3.03	–	–	–	**40**	0.66	–	–	–	–	1

Edible Vegetable Oil	C 18:1	C 18:2	C 18:3	C 18:4	C 20:0	C 20:1	C 20:2	C 20:4	C 20:5	C 22:1	C 22:6	Other	Ref
Bacillariophyceae													
Phaeodactylum ricornutum	5.64	2.13	1.33	1.28	–	–	0.2	–	**18.4**	–	1.33		[16,216–218]
Thalassiosira weissflogii	9.8		0.3		–	–	–	–	**14.1**	–	–		[216,219]
Skeletonema costatum	1.55	1.4	0.5	2.85	–	–	0.1	–	**20.8**	–	3.5		[217,220]
Chaetoceros muelleri	1.81	1.0	0.9	0.8	–	–	–	–	5.53	–	0.68		[217,221]
Chlorophyceae													
Dunaliella primolecta	11.4	6.6	**39.9**	2.35	–	–		–	–	–	1		[216]
Dunaliella tertiolecta	8.6	**22.9**	**41.4**	1.3	0.65	–	–	1.91	0.4	–	–		[208,217,222-,223]
Dunaliella. salina	11.6	11.5	**38.4**	1	–	1.02	0.26	0	0.05	9.44	5.12	3.7c	[207,209,217-,224]
Nannochloris sp.	**37.5**	**11.6**	**13.5**	3.2	0.21	1.61	2.56	–	0.03	0.01	0.4	9.97a	[216,225]
Nannochloropsis oculata	11.1	2.3	0.98	0.1	–	–	0.1	–	4.57	–	–		[217,226,227-]
Scenedesmus obliquus	**25.6**	11.3	14.4	4.28	2.32	7.85	4.42	–	–	2.72	–	0.48d 3.18 f	[203,228,229-]
Scenedesmus quadricauda	**23.9**	6.93	6.7		–	–		–	–	–	–	**36.4c**	[204,230]
Scenedesmus dimorphos	8.9	12.8	**26**	3.5	–	–	0.8	–	–	–	–		[227]
Scenedesmus sp.	10.2	**20.7**	**30.9**		–	–	–	–	–	–	–		[202]
Franceia sp.	6.7	8.5	**33.3**	3.7	–	–	–	–	0.9	–	–		[227]
Ankistrodesmus sp.	**17.7**	8.48	**28.7**	–		2.55	–	–		–	–		[202]
Ankistrodesmus fusiformis	**65.2**	2.24	3.84	–	0.12		–	–	0.17	–	0.01		[231]

(*continued on next page*)

Table 4 (*continued*)

Edible Vegetable Oil	C 18:1	C 18:2	C 18:3	C 18:4	C 20:0	C 20:1	C 20:2	C 20:4	C 20:5	C 22:1	C 22:6	Other	Ref
Chlamydomonas reinhardtii	**32.4**	6.58	**16.0**	–	–	3.8	–	–	–	–	–		[202,232]
Chlamydomonas sp.	**15.9**	10.4	9.03	–	–	–	–	–	–	–	–		[233]
Parietochloris incisa	**20.4**	16.3	5.07	–	–	–	–	30	2.65	–	–		[216,234]
Tetraselmis viridis	7.35	–	**15.7**	**12.7**	–	1.05	0.95	0.25	**6.15**	–	–		[217]
Eustigmatophyceae													
Chlorella sp.	12.6	**20.9**	13.2	0.1	3.43	0.26	0.4	1.69	1.3	–	–		[210,211,217-,235]
Chlorella vulgaris	**16.7**	**13.8**	**12.9**	–	3.12	1.85	1.05	1.06	0.47	–	–		[202–204,20-8,235,236]
Chlorella pyrenoidosa	**17.6**	**15.3**	**14.4**	–	–	–	–	–	0.24	–	–	2.2c	[208,236]
Chlorella protothecoides	10.3	**38.5**	**26.5**	–	–	2.35	–	–	–	–	–		[202,205,208-]
Chlorella salina	27.6	11.3	**29.7**	–	–	1.5	–	–	–	–	–		[202,208,212-,213]
Cyanophyceae													
Nostoc commune	6.9	**15.9**	**27.2**	–	–	–	–	–	–	–	–		[216]
Synechocystis sp.	8	0.2	9.45	–	–	–	–	–	–	–	–		[216]
Synechocystis sp. PNN 6803	6.6	16.	14.6	–	–	–	–	–	–	–	–		[214]
Spirulina Platensis	7.86	**15.5**	**17.5**	–	–	–	0.37	–	–	–	–	2.1c	[236,237]
Spirulina Maxima	9.01	13.8	**20.1**	–	2.52	–	–	–	–	–	9.97		[236–238]
Spirulina sp	6.17	**16.2**	**17.6**	–	3.33	–	–	–	–	–	–		[195,215,237-,239]
Haptophyceae													
Pavlova lutheri	8.8	0.6	0.5	9.1	–	–		0.3	**15.1**		9.7		[216]
Pavlova salina	3.8	–	2.2	4.2	–	–	0.3	3.7	**19.1**	–	–		[217]
Emiliana huxleyi	**32**	0.9	5.5	6.85	–	–				–	–		[216]
Raphidophyceae													
Heterosigma akashiwo	2.7	3.1	5.5	6.3	–	–		3.5	**11.8**	–	–		[216]
Dinophyceae													
Gymnodinium kowalevskii	6.5	–	7.2	**15.6**	–	0.2	3.7	–	0.1	–	9.5		[217]
Cryptophyceae													
Chroomonas salina	5.2	–	10.8	**30.3**	–	–	–	2.8	**12.9**	–	–		[217]
Rhodophyceae													
Porphyridium cruentum	1.33	0.25	0.55	–	–	0.1	1.9	**27.6**	**25**	–	–		[215]
Chromalveolata													
Amphidinium sp.	11	1.1	2.3	**19.1**	5.7	–	–	–	**14.5**		–		[227,239]
Trebouxiophyceae													
Picochlorum sp.	**15.5**	**35.8**	14.9	–	2.1	–	–	–	–	–	–		[227]
Conjugatophyceae													
Mesotaenium.sp.	7.6	11.8	**31.4**	3.1	–	–	–	–	0.8	–	–		[227]
Labyrinthulomycetes													
Schizochytrium limacinum	7.1	1	–	–	–	–	–	–	–	–	–	**39.4** *	[240,241]
Schizochytrium sp.	–	–	–	–	–	–	–	–	–	–	–		[242,243]
Aurantiochytrium sp.	–	–	–	–	0.4	–	–	0.6	0.6	–	**42.6**	10b	[244,245]

Total C22:5 + C22:6, a: C24:1, b: C22:5, *C22:5 + C22:6, c:C8:0, d: C22:0, f: C22:2.

divisions slow down while producing energy storage products is mobilized. In the initial phases of nitrogen deficiency, carbon fixation may exceed carbon demand for nitrogen assimilation and extra carbon may be converted into lipids and carbohydrates as the main storage compounds before photosynthetic capacity is dramatically exhausted. In other words, the ability of microalgae to use fixed carbon is switched from protein and polar lipids synthesis toward either carbohydrate or storage oil generation. Generally, nitrogen limitation imposes three changes to the species: decreasing the cellular content of thylakoid membrane, activation of acyl hydrolase and stimulation of the phospholipid hydrolysis. These changes may increase the intracellular content of fatty acid acyl-CoA. Meanwhile, nitrogen limitation could activate diacylglycerol acyltransferase, which converts acyl-CoA to triglyceride (TAG). Therefore nitrogen limitation could increase both lipid and TAG content in microalgal cells [23]. If N is resupplied, storage compounds supplies the energy and C for N assimilation until photosynthetic capacity is restored [24].

Nitrogen limitation results in a relative increase in carbohydrate and/or lipid storage of microalgae and a decrease in protein content, photosynthetic efficiency and growth rate [22]. However, these responses in microalgae are species-specific. For example, *Chlorella sp.* showed a great reduction in the protein content while no difference was observed in *Prorocentrum donghaiense* cultured under various nutrient limitations [21]. Generally, the lipid content of green algae multiplied up to 2- to 3-fold due to nitrogen deprivation for 4–9 days, whereas both increase and decrease have been reported in diatoms, depending on the species [25]. However, in an analysis on the effect of nitrogen starvation on lipid accumulation in *chlorella* Rathinasabapathi et al. [26] reported that lipid accumulation in algal cells began just hours after they were starved of nitrogen – not days. The oil content in some of the most important microalgae under nitrogen limitation is summarized in Table 5. As observed in some of the previous studies, low nitrogen medium induced lipid accumulation up to 30–70% within 7–20 days in most microalgae, which increased their calorific value [27]. *Monallantus salina,* reached the highest concentration of lipids (72%) well into 9 days of deficient nitrogen growth. *Botryococcus braunii, Chlorella* vulgaris and *Nannochloropsis sp.* have demonstrated the potential to reach 61.4%, 57.9% and 55% lipid production during their life frame, respectively. Some other microalgae, like *Scenedesmus sp.* and *Chlorella sorokiniana have* not reached a high content of lipid even during 7 and 14 days. However, as the average value, most of them reached to > 40% of lipid production under nitrogen deficient condition. As already mentioned, the increased oil content of algae due to nitrogen manipulation comes at the cost of diminished growth rate (see Table 5, in case of *Chlorella minutissima* for example). In other words, the overall rate of lipid generation is diminished during nutrient deficiency phase and so culturing either few cells with high lipid content or many cells with low lipid content will not produce an economically viable biofuel feedstock. Hence, there should be a favorable tradeoff between lipid accumulation and growth. With respect to this challenge, Adams et al [24], investigated this relationship in six species of green algae under high and low levels of N deficiency and reported the following trend for the productivity of those species: *Chlorella vulgaris* > *Chlorella oleofaciens* > *Neochloris oleoabundans* > *Scenedesmus dimorphus* > *S. naegleii* > *Chlorella sorokiniana*. They discussed that the most promising microalgae species as biofuel feedstock are the ones, which grow and accumulate lipid concurrently and respond to little stress that does not significantly damage the growth rate. In other words, *Chlorella vulgaris* and *Chlorella oleofaciens* significantly respond to low stress while *Chlorella sorokiniana*, *Neochloris oleoabundans*, *Scenedesmus dimorphus* and *S. naegleii* are more sensitive to high stress. Accordingly, the lipid productivity, which reflects the independent effects of N supply on both growth and lipid content should be the benchmarked.

Some researchers have also analyzed the effects of nitrogen manipulation combined with other cultural conditions on lipid accumulation in microalgae. For example, some authors have got benefit from temperature manipulation to reach proper balance of growth and lipid content in N-deficient conditions [28]. In an analysis on *Nannochloropsis*, Fakhry et al. [28] found that the combined effect of nitrogen and temperature stressors created an additional response by not only increasing cellular lipids and triglycerides but also enhancing lipid productivity in a shorter duration. However, this solution cannot be generalized to all algae because the lipid production and productivity are specific for each strain and subject to many factors. For example, although the lipid content in the *Nannochloropsis* [28], *eustigmatophyte N. salina* [29] and *chrysophytan Ochromonas danica* [30] increases with temperature, there is no significant response to the temperature factor in the lipid content of *Chlorella sorokiniana* [31]. Nitrogen also has a great interactive effect with other cultural parameters that are specific for each strain. In an investigation on the combined effect of nitrogen and phosphorus deprivation with iron, Singh et al. [32] found that nitrogen under the lowest concentration of iron ($3\ mg\ L^{-1}$) had a negative effect on both the lipid content and productivity of *Ankistrodesmus falcatus* whereas its effect changed positively as the iron concentration increased to its moderate value ($6\ mg\ L^{-1}$). In another analysis on *B. protuberans* and *Botryococcus braunii* in batch cultures, Singh et al [33] reported that *Botryococcus spp.* under anaerobiosis and nitrogen supplemented medium produced less amount of lipid than under anaerobiosis and nitrogen deficient conditions. Light and pH are the other factors that may have synergistic or antagonistic effects on lipid content of a species under nitrogen deprivation condition. Breuer et al. [34] found that the highest TAG content of *Scenedesmus obliquus* under nitrogen deficient conditions was independent of light intensity, but it varied from 18% to 40% of dry weight, depending on temperature and pH. It reached the maximum yield of fatty acids at the lowest light intensity, 27.5 °C and pH 7. Some researchers have also focused on the effect of cultivation duration under nutrient limitation. Shorter normal growth time may result in low oil accumulation, while long normal growth time may lead to cell disruption, followed by low oil production. Cao et al. [35] reported that the lipid content of *Chlorella minutissima* rose up to 29.19% of the dry weight of algae after 6-day normal condition and 3 days with limited nitrogen. Similar phenomenon was also observed during the cultivation of *Chlamydomonas reinhardtii*, which reached the maximum oil synthesis between 2 and 3 days following nitrogen depletion followed by a plateau around day 5 [36]. However, the proper length of normal nutrition culture causes variations in lipid content and should be analyzed as priority.

And the last but not the least point, algae are capable of utilizing nitrite, nitrate, ammonia and urea as the sources of nitrogen sources. As also observed in Table 6, most studies use nitrate in nitrogen depletion process, while urea has been more used in large-scale algal cultivation because of its relative low cost and universal availability compared to the others. However, the control of urea concentration during the cultivation is a basic challenge. Urea can release urease or be hydrolyzed to ammonia in alkaline conditions. As a result a portion of the nitrogen source is wasted, and the ammonia toxicity inhibits the growth at high levels [37]. Many reports have indicated that *Spirulina platensis* can use ammonium, nitrate and urea as nitrogen sources for growing [37–39]. While the use of nitrate as nitrogen source for *Neochloris oleoabundans* results in a higher growth rate and lipid content than that of using urea but the cell grow poorly in mediums with ammonium as the nitrogen source [40]. A nitrogen source test on *Chlorella sorokiniana* has also demonstrated that this strain can grow well with urea and nitrate, but not ammonium [41]. In the opposite, *Ellipsoidion sp.* with ammonium grow faster and accumulate higher lipid than that with urea and nitrate [42,43]. It has been reported that urea is a favorable nitrogen source for green algae growth [44]. *Arthrospira (Spirulina) platensis* [37] and *Chlorella* [43] demonstrate an excellent ability to uptake urea as nitrogen source for their growth. Danesi et al [39] reported that urea addition in an intermediate form had a favorable effect on the growth rate of *Spirulina platensis* at 27 °C while

Table 5
The effect of nitrogen limitation on microalgae lipid, lipid productivity and growth rate.

Nitrogen Source	N-stress	Microalgae Species	LC-NS (%)	LC-ND (%)	LP-ND mg/L d	LP-NS mg/L d	GR-ND g L^{-1}d	GR-NS g L^{-1} d	days	Ref
Nitrate	N-Deficiency	Dunalliela tertiolecta	12.9	20.3	-	-	1.21μ	−0.1 μ	15	[22]
-	-	Dunalliela. tertiolecta	36	42	-	-	-	-	-	[7]
$NaNO_3$	N-Deficiency	Dunalliela. tertiolecta	1x	2.3x					7	[66]
Nitrate	N-Deficiency 0.025–0.105 g/l	Nannochloropsis sp.	-	~50–53%	-	150	-	-	8	[246]
KNO_3	N-Deficiency	Nannochloropsis sp.	-	55%	-	-	-	-	10	[247]
$NaNO_3$	N-Deficiency 1.5–0.375 gL	Nannochloropsis oculata	-	-	10.01	16.41	0.13μ	0.1μ	14	[46]
$NaNO_3$ aerobic	N-Deficiency to	Nannochloropsis salina	30	52	230–25 °C	370–25 °C	0.61[B]	0.48[B]	12	[28]
$NaNO_3$ + KNO_3	N-Starvation 0.45 g/l	Nannochloropsis sp.	28.7	56%	90	-	2.5[B]	-	5	[248]
$NaNO_3$ anaerobic	N-Deficiency 10%X-1.25%X	Nannochloropsis sp.	35	59	80	35	-	-	7	[249]
-	N-Starvation 1.76 mM	Nannochloropsis oculata	22	31	-	-	0.07μ	0.03μ	10	[250]
KNO_3 anaerobic	N-Deficiency 0.15–0 g/l	Scenedesmus obliqus	12	33 [a] 58.3[b]	53	585.9[a] 2160[b]	0.22[B]	0.1[B]	10 8	[58]
KNO_3 aerobic	N-Starvation 10 mM	Scenedesmus obliquus	-	40%, > 250 h	-	-	-	-	10	[34]
NH_4Cl	N-Deficiency	Scenedesmus obliquus	0	34.6	-	-	0.186[B]	0.033[B]	-	[251]
KNO_3	0.1%	Scenedesmus obliquus	21.2	45.6			0.403[B]	0.018[B]		
$NaNO_3$ + KNO_3	N-Deficiency No-stress, Low stress:11 mM N High stress:4 mM N	Neochloris oleoabundans	13	29-58	-	91–131	2.4[B]	1.9[B]	12	[24]
$NaNO_3$ urea ammonium	N-Deficiency 3 mM(limited) 20 mM (rich)	Neochloris oleabundans	9 - -	37 16 17	40 - -	150 65 70	2.7[B] - -	1.85[B] - -	7	[40]
$NaNO_3$ + KNO_3	N-Starvation 0.45 g/l	Neochloris oleabundans	29	50%	90	-	-	-	5	[248]
$NaNO_3$ + KNO_3	N-Deficiency No-stress, Low stress:1mM N High stress:4mM N	Scenedesmus dimorphus S. naegleii	9 10	20-34 21-39	- -	86–111 83–83	5.3[B] 4.8[B]	4.0[B] 2.0[B]	12	[24]
$NaNO_3$ anaerobic	N-Deficiency 10%X-1.25%X	Scenedesmus sp.	20	22	-	-	-	-	7	[249]
NO_3–N	N-Deficiency + P-Deficiency 0.01 g/l	Scenedesmus sp	25	30	150	50	0.67[B]	0.18[B]	12	[53]
NH_4Cl	N-Deficiency	Chlorella vulgaris	0	52.8	-	-	0.205[B]	0.038[B]	-	[251]
KNO_3	0.1%	Chlorella vulgaris	22.6	57.9			0.287[B]	0.017[B]		
$NaNO_3$	N-Deficiency 0.247 g/l	Chlorella vulgaris	15.5	24.6	29	31.5	1.87[B]	1.28[B]	10	[252]
$NaNO_3$	N-Deficiency 1.5–0.375 gL	Chlorella vulgaris	-	-	8.16	20.30	0.14μ	0.13μ	14	[46]
$NaNO_3$	N-Deficiency	Chlorella vulgaris	29.53	40	12.77	8	-	-	17	[95]
NaNO3 aerobic	N-Deficiency 0.075 g/l	Chlorella vulgaris	18	40	41	37	0.99μ	0.77μ	14	[253]
		Chlorella emersonii	29	63	28	79	0.86μ	0.46μ	14	
		Chlorella protothecoides	11	23	2.5	23	0.33μ	0.27μ	14	
		Chlorella sorokiniana	20	22	2.7	4.8	0.58μ	0.19μ	14	
		Chlorella minutissima	31	57	32	16	0.43μ	0.43μ	14	
-	N-Deficiency	Chlorella luteoviridis	-	28.8					-	[253]
		Chlorella capsulate	-	11.4						
		Chlorella pyrenoidosa	-	29.2						
KNO_3	N-Deficiency,0.4 g/l	Chlorella pyrenoidosa	11%	26%	-	-	0.315	0.127	12+12	[254]
Nitrate	N-Starvation	Chlorella minutissima	12.5	29.19	-	-	-	-	6+3	[35]
NO_3	N-Deficiency	Chlorella sp.		30-50%					7-12	[255]
$NaNO_3$ anaerobic	N-Deficiency 10%X-1.25%X	Chlorella sp.	23	21	-	-	-	-	7	[249]
$NaNO_3$ + KNO_3	N-Deficiency No-stress,	Chlorella sorokiniana	15	21-47	-	68–85[c]	4.1[B]	1.8[B]	12	[24]
	Low stress:11 mM N	Chlorella vulgaris	10	40-48	-	146–94	4.3[B]	2.1[B]		
	High stress:4 mM N	Chlorella oleofaciens	12	35-48	-	127–86	4.3[B]	2.0[B]		
$NaNO_3$ anaerobic	N-Deficiency 10%X-1.25%X	Tetraselmis suecica	25	35	-	-	-	-	7	[249]
-	N-Starvation	Tetraselmis subcordiformis	13.5	25	-	-	0.04μ	0.012μ	10	[250]
	1.76 mM	Pavlova viridis	25	26			0.065μ	0.015μ		

(*continued on next page*)

Table 5 (*continued*)

Nitrogen Source	N-stress	Microalgae Species	LC-NS (%)	LC-ND (%)	LP-ND mg/L d	LP-NS mg/L d	GR-ND g L^{-1}d	GR-NS g L^{-1} d	days	Ref
Nitrate	N-Deficiency	Thalassiosira pseudonana	11.5	25.9	-	-	0.84μ	0.1μ	15	[22]
		Thalassiosira pseudonana	21	31						[7]
KNO_3	N-Starvation	Botryococcus braunii	38.6	61.4	-	-	-	-	10	[33]
aerobic	0.4–0 g/l	Botryococcus protuberans	35.2	52.2	-	-	-	-	10	
KNO_3	N-Deficiency	Botryococcus (TRG)	25.8	35.9 [d]	46.9	-	0.182μ	−0.07μ	7[C]	[70]
		Botryococcus (KB)	17.8	30.2	39.7	-	0.223μ	−0.11μ	14[S]	
		Botryococcus (SK)	15.8	28.4	21.3	-	0.135μ	−0.05μ		
		Botryococcus (PSU)	5.7	14.7	3.5	-	0.061μ	−0.1μ		
NO_3	N-deficiency to N-Free for 10 days	Green Alga	17.1	30-50	-	-	-	-	4–9	[25]
		Diatom	24.5	∼						
NO_3	N-deficiency to N-Free for 10days	Monallantus salina	-	72%	-	-	-	-	9	[25]
NO_3 aerobic	N-Deficiency 0.037 g/l	Isochrysis zhangjiangensis	-	2.32fold	-	-	-	-	4	[256]
KNO_3	N-Deficiency	Anacystis nidulans	14.8	14.3	-	-	0.453[B]	0.008[B]	-	[251]
KNO_3	0.1%	Microcystis aeruginosa	23.4	17.7			0.136[B]	0.011[B]		
KNO_3		Oscillatoria rubescens	12.8	0			0.289[B]	0.05[B]		
KNO_3		Spirulina platensis	21.8	0			0.235[B]	0.065[B]		
$NaNO_3$	N-Deficiency, 0.75 g/l	Ankistrodesmus falcatus	23.33	59.6	58.99	74.07	3.54[B]	1.74[B]	14	[32]
N-free	-	Ellipsoidion sp.	-	7.99	-	-	0.17μ	-		[42]
$NaNO_3$			-	27.6	-	-	0.29μ	-		
NH4Cl			-	33.3	-	-	0.31μ	-		
urea			-	21.5	-	-	0.28μ	-		

P: Productivity [mg L^{-1} day^{-1}], C: Control Condition, S: Stress Condition, dcw: of dry cell weight. [a]: 1.5% glucose-supplemented condition, [b]: under optimized condition: N:0.04 g/l, P:0.03 g/l, [c]: The values of productivity correspond to the low and high stress, [d]: A combination of nitrogen deficiency, moderately high light intensity (82.5 μE m^{-2} s^{-1}) and high level of iron (0.74 mM), ∼: both decrease and increase observed
NS: Nitrogen Sufficient, **ND:** Nitrogen deficient, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate

continuous mode presented more benefit to both growth and lipid content at higher temperature (30 °C). Comparing the effects of nitrate, nitrite and urea on the lipid content of *Isochrysis galbana*, higher lipid content can be yielded by urea if it is added in logarithmic manner or during early stationary phase while nitrite and nitrate are more favorable if they are added at the late stationary phase. Besides, although nitrate is widely used in nitrogen depletion process, microalgae grown in wastewater first metabolize ammonia before nitrate [45]. Accordingly, research combining ammonia depletion with the reclamation of water would have been beneficial not only to oil and biofuel synthesis but wastewater treatment industries.

5.1.2. Effect of nitrogen on lipid composition

Aside from the quantitative effect of nitrogen on lipid content, its qualitative effect is of importance for biofuel production (Table 7). Although the major fatty acid compositions in microalgae are similar in both control and nutrient-stress medium, there is a considerable difference in their percentage composition.

Nitrogen stress has been found to decrease the saturated fatty acid of C16:0 while increase C18:0. Accordingly, the final effect of nitrogen depends on the tradeoff between this increase and decrease. However, in some microalgae, the reduction of other saturated compounds is of importance; (C23:0 and C24:0 in *Chlorella sp.*). Nitrogen stress has been found to cause a reduction in the saturated fatty acids of *Ankistrodesmus. falcatus* [32], which could improve the Cetane number and oxidative stability of the biodiesel produced. On the other hand, no significant change in the amount of saturated fatty acids of *C. vulgaris* but a slight decrease in those compounds of *N. Oculata* have been observed [46]. The same completion is observed in terms of monounsaturated values between C16:1 and C18:1 compounds. The balance between these compounds causes the increment of MUFA in *Chlorella. Vulgaris* and *Scenedesmus obliquus*, but a slight reduction in *Chlorella* sp.

Linolenic acid is the main poly-unsaturated compound in most microalgae lipids. The presence of linolenic acid methyl ester (C18:3) in high amount, above 12% based on European standards (EN14214) leads to lower stability of biodiesel due to oxidation. However, the percentage composition of linolenic acid is slightly reduced in some microalgae e.g. *A. falcatus*, grown with nutrient stress, making it suitable for biodiesel production. While no significant changes were observed in C18:3 of *C. vulgaris* and *N. Oculata* [46].

Very few studies can be found in case of nitrogen sources rather nitrite and nitrate. Based on Fidalgo's work [47], the urea usage as the

Table 6
The effect of urea limitation on microalgae lipid, lipid productivity and growth rate.

Nitrogen Sources	Worst Conc.	Best Conc.	No* or Neg	Microalgae Species	L-LC %	H-LC %	L-LP g/Ld	H-LP g/Ld	L-GR g L^{-1}	H-GR g L^{-1}	day	Ref
Urea, 0.1–0.3g l^{-1}	0.15	0.25	< 0.25 <	Desmodesmus abundans	16.64	18.25	38.3	49.29	–	–	12	[44]
Urea, 0.025–0.2g l^{-1}	0.025	0.1	< 0.1 <	Chlorella sp.	0.66	0.52	0.051	0.124	0.036	0.058	6	[43]
Urea, 0.56–1.7 mM	0.56	1.1	< 1.1 <	Spirulina platensis	–	–	–	–	870 dcw	1270 mg l^{-1}		[38]
Urea, 0–5.6 g	5.6	2.5	–	Spirulina platensis	20.7	18.6	–	–	0.47	0.61	18	[39]
Urea, 4 mg atom Nl^{-1}	–	–	–	Isochrysis galbana	–	42.05	–	–	–	–	14	[47]

L: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate.
Worst and best concentrations versus Lipid content and Lipid Productivity.

nitrogen source resulted in generation of lipid with higher content of polyunsaturated fatty acids (C18:4, C20:5, C22:6), while nitrite and nitrate increased the proportion of saturated (C14:0) and monounsaturated compounds (C16:1). The addition mode of urea also demonstrated a great effect on fatty acid composition of the intercellular microalgae. The least amount of saturated and monounsaturated but highest amount of polyunsaturated compounds obtained by adding the urea at the early stationary phase.

5.2. Phosphorous effects

5.2.1. Effect of phosphorous on lipid content of microalgae

Phosphorus is the other essential nutrient for the growth of microalgae as it plays a key role in cellular metabolic processes, which are related to signal transduction, energy transfer and photosynthesis. Phosphatases convert the organic phosphates to orthophosphates at the cell surface and microalgae take up the phosphorous as inorganic orthophosphate (PO_4^{3-}). This process requires energy and occurs especially when inorganic phosphate is in short supply. In high supply, microalgae assimilate excess phosphorus in their cells in the form of polyphosphate granules so they can prolong the growth process in the absence of phosphorus. Accordingly, the growth of microalgae does not immediately respond to the environmental changes of phosphorus concentration, in contrast to the instant responses of microalgae to light and temperature. It has been reported that the phosphorus concentration in cells varied with supply concentration, from a minimum of 10 mg dry mass per mg P at supply concentration of 5 mg $P.l^{-1}$ to a maximum of 1170 mg dry mass per mg P at a supply concentration of 0.1 mg $P.l^{-1}$ [48] (Table 8).

Under phosphorus starvation, a drastic reduction in membrane phospholipids happens and these compounds are replaced by nonphosphorus glycolipids and sulfolipids, offering an effective phosphorus-conserving mechanism as it has been reported for *Chlorella sp.* [21]. Li et al. [49] discussed that galactolipid, which is a type of glycolipids, was accumulated to rectify the loss of phospholipids under phosphorus-deprived conditions. These changes represent an effective phosphate-conserving mechanism. Besides, cell division rates are reduced under P-limited condition, though photosynthetic rates slightly decreased. This may result in accumulation of carbon, which might be stored in the form of triacylglycerols that are rich in saturated fatty acids and monounsaturated fatty acids [50]. Spijkerman et al. [51] reported that the total FA content increased over twofold, increasing cellular C content while total FA content decreased with increasing cellular P content over a factor of ten. However, it has also been reported that a decrease in the cellular P content results in an enhanced FA accumulation, independent of cellular C status.

In addition to lipids, carbohydrates are considered as a long-term store under nutrient limitation. In other words, depending on the species, microorganism could accumulate either lipid or carbohydrate or both. In phosphorus deficient environments, more lipids, mainly TAG, can be stored in *Dunaliella parva* [52], *Chlorella sp.* [21] *Scenedesmus* sp. [53], *Monodus subterraneus* [54], *Chaetoceros* sp. (Badllariophyceae), *Pavlova lutheri* (Prymnesiophyceae) [55] while carbohydrates have higher storage proportion in *Arthrospira* (*Spirulina*) *platens* [56] *Nannochloris atomus* (Chlorophyceae), *Tetraselmis* sp. (Prasinophyceae) [55]. In contrast, protein as the major macromolecular pool of intracellular nitrogen, has been found to be mainly affected by nitrogen rather than phosphorus. In an effort to identify the optimum condition between phosphorus and nitrogen as the two most determining nutrient compounds, Xin et al. [53] reported that N/P should be controlled in a proper range of 5:1–8:1 to increase the lipid content of *Scenedesmus sp*. Although they could reach higher amount of lipid but lipid productivity and accumulation rate were not at their highest. Similar value of N/P (8:1) was reported by Kapdan and Aslan [57] for *Chlorella vulgaris* too. It has also been reported that *Scenedesmus obliqus* can reach to the lipid content of 58% at the nitrate, phosphate and thiosulphate

Table 7
The effect of nitrogen stress (compared to normal growth condition) on fatty acid composition of different microalgae.

Edible Vegetable Oil	g/l	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	SFA	MUFA	PUFA	Ref
Chlorella sp.[a]	Starvation	~	~	↓	~	~	~	–	–	–	–	–	↑↑	↓	↑	↑	↑↑	↑	~	~	↓	↑	[65]
Chlorella. vulgaris	1.5–0.37	–	–	–	–	~	–	–	–	–	–	–	~	←	↓	~↓	–	–	–	~	←	~↓	[46]
	Limitation																						
Chlorella vulgaris	NH_4Cl	–	–	–	–	↓	↓	↓↓	↑↑	–	–	–	~↑	↑↑	↓↓	↓↓	–	–	–	~↓	↑↑	↓↓	[251]
Chlorella vulgaris	KNO_3	–	–	–	–	~↑	↓	↓↓	↑↑	–	–	–	~↑	↑↑	↓↓	↓↓	–	–		~↑	↑↑	↓↓	[251]
Nannochloropsis.oculata	0.3–0.07	–	–	–	–	↓	–	–	–	–	–	–	↓	↓	↑↑	~	–	–	–	↓	↓	←	[46]
	Limitation																						
Dunaliella tertiolecta	$NaNO_3$ deficiency	–	–	–	–	↓	–	~	–	–	–	–	~	→	→	←	–	–	–	→	→	←	[66]
Ankistrodesmus falcatus	deficiency	–	–	–	–	←	–	–	–	–	–	–	←	→	→	→	–	–	←	←	→	→	[32]
Scenedesmus obliquus[b]	NH_4Cl deficiency	–	–	–	–	←	–	~	↑↓	↑↓	–	–	←	↑↑	→	↓↓	–	–	–	←	↑↑	↓↓	[251]
Scenedesmus obliquus	KNO_3 deficiency	–	–	–	–	↑↑	↓↓	→	↓↓	↑↓	–	–	~↑	↑↑	→	↓↓	–	–	–	↑↑	↑↑	→	[251]
Anacystis nidulans	KNO_3 deficiency	–	–	–	–	↑↑	↓↓	–	–	–	–	–	~↑	↑↑	–	–	–	–	–	↑↑	↓↓	–	[251]
Microcystis aeruginosa	KNO_3 deficiency	–	–	–	–	~	~↓	–	–	–	–	–	~↑	~↑	↑↓	~	–	–	–	~	~	~	[251]
Oscillatoria rubescens	KNO_3 deficiency	–	–	–	–	~↑	~	–	–	–	–	–	–	~↓	~↓	~	–	–	–	~↑	~↓	~↓	[251]
Spirulina platensis	KNO_3 deficiency	–	–	–	–	~↑	↑↓	–	–	–	–	–	–	~↓	~↓	←	–	–	–	~	~↓	→	[251]
Tetraselmis subcordiformis	deficiency	~	–	–	–	→	~	~	←	–	–	–	~	→	→	←	←	~	–	~↓	→	←	[250]
Nannochloropsis oculata	deficiency	~↑	←	–	–	←	←	←	~	–	–	–	–	~↑	–	–	~↑	–	–	←	→	→	[250]
Pavlova viridis[c]	deficiency	~↑	–	–	–	←	←	~↓	–				–	–	←	←	←	–	–	←	←	→	[250]

a: C23:0: ↓, C24:0: ↓↓, b: C18:4: ↓, C: C22:5: ~↓.

concentrations of 0.04, 0.03 and 1 g/l, respectively [58].

5.2.2. Effect of phosphorous on lipid composition

Phosphorus limitation causes dramatic changes in the biosynthesis process and hence the composition of the lipid. As per Table 9, the fatty acid composition of algae is consistent with their taxonomy. Unsaturated fatty acid, which makes the highest proportion of lipids in green algae *Chlorella kessleri*, significantly increases under phosphorus starving conditions [59]. The same result has also been reported for *Chlamydomonas acidophila* [51] and *Chlorella sp.* [60], *P. tricornutum, Chlorella sp., I golbaria* [61]. It was because although the fatty acid composition of lipids is species-dependent, the membrane lipid classes in the green alga are mainly constructed by unsaturated fats such as 16:3, 16:4, 18:1, 18:2, 18:3 and 18:4 and an enhanced level of unsaturated fatty acids cause greater membrane "fluidity" [51]. However, it has been reported that the concentration of 16:4(3,7,10,13) and 18:3(9,12,15) is more sensitive to carbon source (their concentration in low CO2 cells is higher) and less to P-limitation [61].

However, opposite results have been observed for *Ankistrodesmus falcatus* under the combined effects of nitrogen, phosphorus and iron. Singh et al. [32] discussed that decrease in percentage of polyunsaturated fatty acid and simultaneous increase in the composition of saturated fatty acid under nutrient stress condition could be possible because of the oxidative damage of unsaturated fatty acids. The same results has also been reported in case of the green alga *Scenedesmus quadricauda*, which has demonstrated a lower percentage of polyunsaturated fatty acids at P-limited than at P-replete conditions, mainly due to the lower content of 18:3(9,12,15) [62].

5.3. Metal effects

5.3.1. Effect of metals on lipid content of microalgae

The trace metals have a significant effect in the growth rate, lipids and carbohydrates content in numerous microalgae. However, based on the analysis in different cases, it can be concluded that the effectiveness of these compounds depends on their concentration in the media, their interactive synergy or antagonistic effects with other environmental factors and species type (Table 10). Iron is one of the most effective trace metals because ferric ion is involved in fundamental enzymatic reactions of photosynthesis. Iron is crucial to regulate the gene expression and metabolism in algae. Presence of Fe^{3+} in the culture media prolongs the period of the exponential growth phase and increases the final cell density although it cannot promote lipid accumulation in exponential growth phase. It has been reported that the exponential growth phase in the presence of Fe^{3+} is at least 4 days longer than those in the absence of Fe^{3+} [63,64] while the change in cultures with $NaNO_3$ supplement was opposite to this [64]. Accordingly, iron deprivation expects to reduce the biomass growth. For example, Praveenkumar [65] analyzed the effect of iron deprivation on *Chlorella sp.,* and stated that under this situation, there was a slight decrease in biomass with no significant increase in lipid content. The same results have been reported in the case of *Dunaliella tertiolecta* but with a significant reduction in the cell growth [66]. Iron limitation could also increase the content of glucose, as reported in *Agmenellum quadruplicatum* from 5% to 45% [67]. In some microalgae, increasing the bio-available iron concentration in the culture medium could result in a simultaneous increase of both growth rate and lipid content [68]. However, excessively high or low Fe^{3+} in the culture medium has an inhibitory effect on the growth and lipid production (Table 10).

Table 8
The effect of phosphorus limitation on microalgae lipid, lipid productivity and growth rate.

Phosphate Source	P-Stress	Microalgae Species	Taxonomy	C-LC (%)	S-LC (%)	C-LP mg/Ld	S-LP mg/Ld	C-GR g L^{-1}d	S-GR g L^{-1}d	days	Ref
Na_2HPO_4	P-Deficiency	Dunaliella tertiolecta	G	1x	0.9x	-	-	-	~↓	7	[66]
Na_2HPO_4	P-Deficiency	Scenedesmus obliqus	G	7	24	-	-	0.1^B	0.8^B	10	[58]
PO_4–P	P-Deficiency	Scenedesmus sp	G	22	53	90	75	0.35^B	0.14^B	12	[53]
-	P-concentration 4–0.5 mM C^{-1}	Chlamydomonas acidophila	G	8	30	-	-	-	-	1.6	[51]
K_2HPO_4	P-concentration 40–0 mg L^{-1}	Ankistrodesmus falcatus	G	31.31	59.6	54.79	74.07	0.175^B	0.124^B	14	[32]
BG11 medium	P-Starvation	Choricystis minor	G	25.2%	34.2%	-	-	1.45^B	1.3^B	2	[257]
	N-Starvation			27%	45.3%	-	-	1.89^B	1.86^B	5	
				26%	59.5%	-	-	1.85^B	1.73^B	10	
PO_4^{3-}	P-concentration	Coccomyxa mucigena	G		↓↓		TAG↓	-	-	14	[258] and
	Normal:1.7 mM	Coccomyxa peltigera	G		↓↓		TAG↑				[55]
	Limited:0.017 mM	variolosae Trebouxia aggregata	G		↓↓		TAG↓				
		Trebouxia erici	G		↓		TAG↓↓				
NaH_2PO_4	P-Deficiency	Nannochloris atomus	G	-	11-9	-	-	1.66	0.83–0.078	4–5	[61]
	Low stress:	Tetraselmis sp.	G		15-8			0.82	0.38–0.046		
	50%μ_{max}	Phaeodactylum tricornutum	GB^b		12-20			1.06	0.53–0.047		
	High stress:	Chaetoceros sp.	GB^b		11-21			1.29	0.66–0.006		
	5%μ_{max}	Isochrysis galbana	Br		22-30			1.11	0.54–0.051		
		Pavlova lutheri	Br^H		21-23			1.19	0.53–0.061		
		Gymnodinum sp.	Di		13-12.5			0.55	0.30–0.15		
K_2HPO_4	P-Starvation 175–0 μM	Monodus subterraneus	YG^x	6.5^a	39.3^a	-	-	3.4x	1x	4 + 4	[54]
K_2HPO_4	P-Deficiency	Chlorella sp.	YG	31.2	31.9	40.27	39.35	2.59^B	2.45^B	16 + 4b	[65]
K_2HPO_4	P-concentration 240–32 μM	Chlorella sp.	YG	14	23.6	6	15.67	1.9^B	2.2^B	22	[21]
Na_2HPO_4, NaH_2PO_4	P-Starvation	Chlorella kessleri	YG	7.4	9.5	-	-	-	-	6	[59]
KH_2PO_4	P-concentration 22–444 mg L^{-1}	Botryococcus braunii	P^C	22% at 444 mg L^{-1}	54% at 222 mg L^{-1}	-	-	0.8^B	1.9^B	30	[68]

a: TAG, b: Normal Condition: 16 days, deprivation condition: 4 days, P-concentration: Analyzing different concentration of P, GB^b: Golden-Brown Bacillariophyceae, YG^x: yellow Green Xanthophyceae, Br^H: Brown, Phylum Hyptophyta, P^C: Phylum: Chlorophyta, **C:** Control Condition, **S:** Starvation Condition, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate **B:** Biomass Production **and rest:** Specific Growth rate,

Table 9
The effect of phosphorus stress (by reduction) on Fatty acid composition of different microalgae.

Edible Vegetable Oil	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	SFA	MUFA	PUFA	Ref
Ankistrodesmus falcatus[a]	–	–	–	–	↑↑	~	–	–	–	–	–	↑↑	↓↓	→	→		←	←	↑↑	↓↓	→	[32]
Coccomyxa mucigena	–	–	–	–	N	→	–	–	–	–	–	~	~→	←	–	–	–	–	~↑	→	←	[258]
Coccomyxa peltigera variolosae	–	–	–	–	←	→	–	–	–	–	–	~	←	→	–	–	–	–	~	~	→	[258]
Chlamydomonas acidophila	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	←	←	←	[51]
Chlorella sp.	↑↑	→	←	~	~	~	–	–	–	↑↑	→	↓↓	←	→	←	–	–	←	↓↓	←	~	[21]
Chlorella sp.	←	–	~	–	←	←	–	–	–	–	–	~	↑↑	~↑	–	–	–	g	←	↑↑	←	[61]
Chlorella sp.	~	~	←	←	~	↑↑	–	–	–	–	–	↓↓	←	→	~	→		D	→	↑↑	→	[65]
Chlorella kessleri	~	–	–	–	→	→	↑↑	~	–	–	–	←	←	←	→	–	–	–	~	→	→	[59]
Monodus subterraneus	~	–	–	–	~	↑↓b	–	–	–	–	–	↑↑	↑↑	→	~	–	–	C	↑↑	←	↓↓	[54]
Phaeodactylum tricornutum	←	–	~	–	←	←	–	–	–	–	–	~	↑↑	~↑	–	–	–	e	←	↑↑	←	[61]
Isochrysis galbana	←	–	~	–	←	~	–	–	–	–	–	~	↑↑	~→	–	–	–	f	←	↑↑	←	[61]

a: Nitrogen and phosphorus starvation, N: not assessable due to the interactive role of copper and lead.
b: ↑: C16:1 ω7, ↓: C16:1 ω9.
c: ↑: C20:3 ω6, ↓: C20:4 ω6, ↓↓: C20:5 ω3.
d: ↑: C24:0.
e: ↑: C20:5.
g: ↑: C20:4, ↑: C20:5.
f: ~↑: C18:4, ↑: C22:6.

Generally, most studies have reported that Fe^{3+} concentrations up to almost 2×10^{-3} g/L have a positive effect on the microalgal growth while Fe^{3+} concentrations beyond that concentration (up to almost 4×10^{-2} g/L) affect microalgal growth negatively. However, the lipid content increases with a greater amount of Fe^{3+} up to 1×10^{-3} while further increase in Fe^{3+} (1×10^{-2} -1×10^{-1}) concentration does not improve the lipid accumulation or reduce it as reported for in *Scenedesmus sp.* [69], *Botryococcus* [70], *Scenedesmus dimorphus* [68], *C. vulgaris* [64]. In case of *Chlorella vulgaris*, it has been reported that the total lipid content in cultures supplemented with $1.2 \cdot 10^{-5}$ mol L^{-1} $FeCl_3$ is up to almost 3–7 folds than that in other media supplemented with lower iron concentration [64]. This result has also been confirmed by Baky et al. [71] in the case of *Scenedesmus obliquus*. The authors reported that the maximum biomass production was achieved at 10 mg/L Fe^{3+} and beyond that a decreasing rate was observed. However, the highest lipid content and lipid productivity were observed at 20 mg/L Fe^{3+} [71]. The highest lipid content in *Chlorella sorokinian* was observed with 10^{-4} mol l^{-1} of iron which was 2.8-fold than that without iron [63].

In addition to iron, appropriate concentration of calcium and magnesium [72], cadmium, copper and zinc can also be favorable for the algal biomass and lipid production. Magnesium has demonstrated an important role in the growth of microalgae so that Mg starvation hinders cell division [73]. It is discovered that the increase of Mg^{2+} could promote Acetyl-CoA carboxylase (ACCase) in vivo activity. ACCase can catalyze and increase the production of microalgal cells [69]. The lipid content and productivity of microalgae are much enhanced when Mg^{2+} concentration is in the range of 2×10^{-3}-8×10^{-3} g/L. The role of calcium is more critical in the signal transduction of environmental stimuli. Neutral lipid synthesis can be regulated based on cytosolic Ca^{2+} level through Ca^{2+} signals in microalgae [74]. Moreover, Ca^{2+} concentration in the level of 5×10^{-4}–5×10^{-3} g/L is also associated with the rise in lipid content in hetrotrophic cultivation of *Scenedesmus sp.* [69] and the photoautotrophic cultivation of *C. vulgaris* [72]. Compared to iron, magnesium and calcium demonstrate a stronger effect on lipid content and productivity of *Scenedesmus* in different concentrations [69]. It has been found that the lipid productivity of *Scenedesmus* under Mg^{2+} concentration of 7.3×10^{-3} g/L is about 390% higher than that of the control.

Heavy metals such as cadmium, copper and zinc are also known to alter the lipid metabolism of algal resulting in enhancement of lipid content in some microalgae e.g. *Euglena gracilis* [83,84] and algal lichen [85]. Higher concentration of fatty acids has also been observed in *C. vulgaris* (0.2 mg/g), *C. protothecoides* (0.16 mg/g) and *Chlorella pyrenoidosa* (0.11 mg/g) at copper levels of 4 mg/L [75]. Copper stress (31.4 mg/L~approximately 0.49 mM) can yield lipid accumulation of 5.78 g/L in *Chlorella protothecoides* [76].

Silica deficiency is the most common stress in diatoms promoting storage lipid accumulation in this taxonomy. Silica effect is more rapid and severe than N or P deficiencies in these organisms and can provide a controllable means to induce lipid synthesis in a two-stage production process. As an example silicon limitation increases the lipid content in diatoms of *Chaetocerosmuelleri*, *Cyclotellacryptica*, and *Navicula saprophila*, up to almost 89%, 110%, and 104%, respectively [77].

Although most studies have focused on the effect of individual metals in microalgae behavior, few studies have investigated the combined impacts of metals with other parameters on improving biomass and lipid productivities of microalgae. It has been found that *Ankistrodesmus falcatus* can reach its maximum lipid content and lipid productivity under moderate nitrogen (750 mg/L) and high iron supplementation (9 mg L^{-1}), while *hosphorus* show no significant influence on lipid productivity [32]. Generally, a greater improvement in the lipid content is demonstrated when the Fe^{3+} is sufficiently supplied in the medium under nitrogen-deficient condition [70]. Combined influence of iron/nitrogen/NaCl and iron/nitrogen/potassium-phosphate positively affects the lipid accumulation in *Chlorella minutissima* [35]

Table 10
The effect of metal availability on microalgae lipid, lipid productivity and growth rate.

Iron Source	Worst Conc.	Best Conc.		Algal Species	L-LC(%)	H-LC(%)	L-LP mg/ld	H-LP mg/ld	L-GR mg/ld	H-GR mg/ld	day	Ref
$FeCl_3$ 0–20 mg L^{-1}	0	20	< 20	Scenedesmus obliquus	5.75	28.13	20.1	95.35	0.891[B] mg/l	1.25[B] mg/l	18	[71]
$FeCl_3{\cdot}6H_2O$ 0–0.4 mM	0	0.05–0.10 mM	< 0.05–0.10 <	Chlorella minutissima	11.25	16.73	0.120 gld	0.140 gld	–	–	7	[35]
$FeCl_3{\cdot}6H_2O$ 0–1.2e^{-5} M	0	1.2e^{-5}	–	Chlorella vulgaris	7.8	56.6	–	–	13e^6 Cell/ml	18e^6 Cell/ml	25	[64]
$FeCl_3{\cdot}6H_2O$,0–1e^{-4}M	0	1e^{-4}	< 1e^{-4}	Chlorella sorokiniana	12.5	32.5	–	–	0.4[B]	0.46[B]	23	[63]
Iron deprivation	0	normal	–	Chlorella sp.,	31.2	31.4	39.96	40.27	2.54[B]	2.58[B]	16	[65]
FeCl_3-$6H_2O$,0.86 ppm	0	0.93	–	Dunaliella tertiolecta	1x	0.52x	–	–	–	–	7	[66]
MnCl_2-$4H_2O$	0	927 ppm	–	Dunaliella tertiolecta	1x	0.42x	–	–	–	–	7	[66]
Na_2**Mo**O_4-$2H_2O$	0	1.45 ppm	–	Dunaliella tertiolecta	1x	0.42x	–	–	–	–	7	[66]
ZnCl_2	0	0.028 ppm	–	Dunaliella tertiolecta	1x	0.42x	–	–	–	–	7	[66]
Fe^{3+}, 0–1.2e^{-4} g/l	0	1.2e^{-3}	< 1.2e^{-3} <	Scenedesmus sp.	9	42	0.03	0.245	1.3[B]	3.4[B]	6	[69]
Mg^{3+},0–7.3e^{-4} g/l	0	7.3e^{-3}	< 7.3e^{-3} <	Scenedesmus sp.	35	42	0.04	0.245	0.7[B]	3.5[B]	6	[69]
Ca^{3+},0–9.8e^{-4}g/l	0	9.8e^{-4}	< 9.8e^{-4} <	Scenedesmus sp.	11	48	0.065	0.280	3.5[B]	3.5[B]	6	[69]
EDTA,0–1 g/l	1	1e^{-3}	< 1e^{-3} <	Scenedesmus sp.	26	45	0.04	0.280	0.7[B]	3.5[B]	6	[69]
Mg,0–0.1 g L^{-1}	0	0.1	–	C. vulgaris	15.1	27.1	–	–	0.6[B]	1.4[B]	18	[72]
Ca, 0–0.02 g L^{-1}	0.02	0			20.8	40.3			1.4[B]	0.9[B]	18	
NaCl (0–0.05 g L^{-1})	0.05	0	–		11.9	39.1			1.2[B]	1.02[B]	6	
Mg 0–0.1 g L^{-1}	0	0.1	–	S. obliquus	14.9	26.4	–	–	0.5[B]	1.4[B]	18	[72]
Ca 0–0.02 g L^{-1}	0.02	0			20.0	37.0			1.2[B]	0.8[B]	18	
NaCl (0–0.05 g L^{-1})	0.05	0	–		11.3	38.7			1.1[B]	0.98[B]	6	
$FeSO_4$,9–45mgL^{-1}	45	27	< 27 <	Botryococcus braunii	11	35	–	–	0.25[B]	0.22[B]	–	[68]
$CuCl_2$ 0.05–0.22 mM	control	0.22	–	Euglena gracilis	0.8 μg/10^5cell	1.7 μg/10^5cell	–	–	–	–	3	[259]
$ZnCl_2$ 0.22–1.76 mM	control	0.88	–	Euglena gracilis	0.8 μg/10^5cell	2.1 μg/10^5cell	–	–	–	–	3	[260]
Chromium 0–96.4 μM	96.4 μM	48.2		Euglena gracilis UTEX	2.2 μg/mg	4.2 μg/mg	–	–	–	–	6	[81]
Chromium 0–96.4 μM	96.4 μM	48.2		Euglena gracilis MAT	2.7 μg/mg	4.5 μg/mg	–	–	–	–	6	[81]
Combined effect												
Iron, Ni, Pi $(NH_4)_5Fe(C_6H_4O_7)_2$ 3–9 mgL^{-1}		9		Ankistrodesmus falcatus	47.96	59.6	25.35	74.07	0.053[B] gld	0.124[B] gld	14	[32]
Fe^{3+}, 0–0.74 mM	Fe: 0	Fe: 0.74		Botryococcus (SK)	11	29	–	–	–	–	20	[70]
Ni	Ni-rich	Ni-deficient		Botryococcus (TRG)	13	36	–	–	–	–		
Rich-deficient				Botryococcus (PSU)	5	15	–	–	–	–		
				Botryococcus (KB)	11.5	30	–	–	–	–		
Fe^{3+}, Acetate 0.1–20 μM	0.1	20	–	Chlamydomonas reinhardtii	–	–	–	–	0.96 μ (day) 4e6[B]	1.68 μ (day) 0.5e7[B]	9	[261]
Fe^{3+},CO_2 0.1–20 μM	0.1	20	–	Chlamydomonas reinhardtii	–	–	–	–	0.56 μ (day) 1e6[B]	0.74 μ (day) 7e6[B]	9	[261]
Fe^{3+}, 2.4e^{-5},4.8e^{-5} M CO_2:0.036%	4.8e^{-5}	2.4e^{-5}	–	Chlorella vulgaris	17	20	–	–	0.25[B]	0.22[B]	32	[80]
Fe^{3+}, 2.4e^{-5},4.8e^{-5} M CO_2:1%	2.4e^{-5}	4.8e^{-5}	–	Chlorella vulgaris	23	26	–	–	0.41[B]	0.44[B]	32	[80]
Fe^{3+}, 2.4e^{-5},4.8e^{-5} M CO_2:2%	4.8e^{-5}	2.4e^{-5}	–	Chlorella vulgaris	22.5	27	–	–	0.44[B]	0.41[B]	32	[80]

L: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate, **B:** Biomass Production,**B:** Biomass Production, **μ:** Specific Growth rate.

and *Chlorella sp.* [65], respectively. However, the enhanced lipid accumulation under the combination of nitrogen, potassium-phosphate, and iron deprivation in case of *Chlorella sp.* is mainly due to the lack of nitrogen, rather than the absence of potassium-phosphate or iron [65].

It is worth noting that, the responses of microalgae to metal ions are mostly under photoautotrophic condition in which microalgae are cultured by using light as energy for photosynthesis. Heterotrophic culture, in which microalgae can utilize organic carbon to accumulate biomass and lipids in the absence of light, has been rarely investigated in the presence of metal ions. The microalgal growth and lipid production are higher under the latter condition. A few researchers have focused on the lipid content and productivity of microalgae when CO_2 or organic carbon source supply energy and carbon to microalgae. It has been found that Fe augmentation with ambient or moderately high-CO_2 aeration is associated with an increase in lipid content; as has been reported for *C. vulgaris* [78,79] and *S. obliquus* [71]. Ren et al. [69] discussed that the biomass and lipid production of heterotrophic microalgae exhibited a significant increasing trend with increased concentration of Fe^{3+}, Mg^{2+} and Ca^{2+} in dark environment. EDTA addition (1.0×10^{-3} g/L) could increase the availability of iron and calcium under heterotrophic condition, thereby further enhancing the lipid production performance. However, the interactive effects of metal ions

Table 11
The effect of metal ion on fatty acid composition of different microalgae.

Fe	metal	C 12:0	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 17:1	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	Other	SFA	MUFA	PUFA	Ref
Chlorella sp.[a] Fe deprivation	Fe↑		–	–	–	–	↓	↑	~	~	~	~	~	↓	↓	↓	↓	↓↓	↑	~	a	↑	↓	↓	[65]
Chlorella v. $2.4e^{-5}$,$4.8e^{-5}$ M CO_2:0.036%	Fe↑	↑	–	–	–	–	↑	–	–	–	–	–	–	–	↓	~	↓	–	–	–		↑↑	↓	↓	[80]
Chlorella v. $2.4e^{-5}$,$4.8e^{-5}$ M CO_2:1%	Fe↑	↑	–	–	–	–	~	–	–	–	–	–	–	–	↓	↑	↓	–	–	–		~↑	~↓	~	[80]
Chlorella v. $2.4e^{-5}$,$4.8e^{-5}$ M CO_2:2%	Fe↑	↑	–	–	–	–	↓↓	–	–	–	–	–	–	–	↑↑	↑↑	↑↑	–	–	–		↓↓	↑↑	↑↑	[80]
Euglena gracilis Utex	Cr^{+6}↑	–	↑	–	–	–	~	–	–	–	–	–	–	↑	↑	↓	↓	–	–	–	b	↑	~	↓	[81]
Euglena gracilis MAT	Cr^{+6}↑	–	~	–	–	–	↑	–	–	–	–	–	–	↑	↑	↓	↓	–	–	–	c	↑	~	↓	[81]
C. vulgaris	Ca↑[d]	–	–	–	–	–	↓	–	–	–	–	–	–	~	↓↓	↑↑	↑	–	–	–	–	↓	↓↓	↑↑	[72]
C. vulgaris	Mg↑[d]	–	–	–	–	–	↑↑	–	–	–	–	–	–	↑↑	↓	↓↓	↓↓	–	–	–	–	↑↑	↑	↓↓	[72]
S. obliquus	Ca↑[d]	–	–	–	–	–	↓↓	–	–	–	–	–	–	↓↓	↑↑	↓↓	↑↑	–	–	–	–	↓↓	↑	↑	[72]
S. obliquus	Mg↑[d]	–	–	–	–	–	↑↑	–	–	–	–	–	–	~	↑↑	↓↓	↓↓	–	–	–	–	↑↑	↑↑	↓↓	[72]
C. mucigena	Pb↑	–	–	–	–	–	~↓	~	–	–	–	–	–	~	↑	~	–	–	–	–	–	~↓	↑	~	[258]
C. mucigena	Cu↑	–	–	–	–	–	~↓	~↑	–	–	–	–	–	~↑	↓	~	–	–	–	–	–	~↓	~↓	~	[258]
C. peltigera variolosae	Pb↑	–	–	–	–	–	~↓	~↓	–	–	–	–	–	~	~	~↑	–	–	–	–	–	~↓	~↓	~↑	[258]
C. peltigera variolosae	**Cu**↑	–	–	–	–	–	~↓	~	–	–	–	–	–	~	~	~↑	–	–	–	–	–	~↓	~	~↑	[258]
C. pyrenoidosa	**Cu**↓	–	–	–	–	–	~↓	~↓	–	–	–	–	–	~	↓↓	↓	–	–	–	–	–	~↓	↓↓	↓	[75]
C. protothecoides	**Cu**↓	–	–	–	–	–	↑	↓↓	–	–	–	–	–	↓	↑	↓↓	–	–	–	–	–	~	↓↓	↓↓	[75]
C. vulgaris	**Cu**↓	–	–	–	–	–	↑↑	–	–	–	–	–	–	↑	↓↓	↑↑	–	–	–	–	–	↑↑	↓	↑↑	[75]

a: C21:0: ↓↓, C24:0: ↑↑↑, b: C20:4: ↓, c: C20:4, C20:5, ↓.

with carbon source highly depend on their concentration. For example, Caprio et al [80] reported negative interactive effects of high-Fe with very low or very high CO_2 concentrations on lipid content. However, in either low or high Fe ions, the biomass production increased with CO_2 up to 1% in their case.

5.3.2. Effect of metal ions on lipid composition

The fatty acid content is clearly affected by metal ions. As observed in Table 11, the fatty acid saturation increases with iron concentration while the portion of unsaturated compounds decreases. This result has also been obtained while analyzing the effect of hexavalent chromium on the fatty acid composition of *E. gracilis*. Rocchetta et al. [81] found that the saturated fatty acids increased in the treated cells with the higher metal concentration. It might be due to the ability of the cells to incorporate carbon from the medium to form lipids storage rich in C14:0, C16:0 and C18:0 in spite of the significant decrease observed in PUFA content. However, the effect of iron concentration on FA composition of microalgae can be affected by the other factors as well. For example, the interactive effect of iron with low-CO_2 concentration is similar to the individual effect of iron on lipid composition of *Chlorella v.* but at higher CO_2 concentration, the intensity of iron effects moderated in favor of the synthesis of fatty acids with longer carbon chain. It is attributed to the high CO_2 fixation and consumption, which might affect the enzymatic desaturation and elongation reactions in lipid synthesis [80]. Similar results have been observed for *S. obliquus* [3] and *C. vulgaris* [23,80]. Based on these studies, 2% CO_2 can make a proper condition in synergetic effect with metal ions for accumulation of high amount of SFAs such as C12:0 and C16:0.

5.4. Carbon effects

5.4.1. Effect of carbon on lipid content of microalgae

Generally, 50% of microalgae biomass weight is made of carbon (on a dry weight basis) which is mainly supplied from carbon dioxide. Accordingly, around 180 tons of CO_2 can be disposed by generation of 100 tons of microalgae biomass, under either artificial or natural light [46].

Microalgae are efficient biological factories capable of taking zero-energy form of carbon, synthesizing and then storing it in the form of natural oils or as a polymer of carbohydrates. It is well known that different sources and amounts of carbon have significant effects on the growth kinetics, content and composition of lipids in microalgae cells. An acceptable theory to justify the positive effect of CO_2 presence is that as CO_2 increases, unutilized CO_2 is converted to H_2CO_3, which reduces the pH of the culture medium and affects the algal growth in consequence. pH value as high as 11 in high-density algae production systems is quite common where no additional CO_2 is supplied. Abu-Rezq et al. [82] could control the pH of algae cultures in the range of 6.75–7.25 with CO2 injections, while the pH levels reached as high as pH 8.28 by day 15 in untreated cultures. However, the growth rate of some algae is inhibited in some specific concentration of CO_2, which is likely due to overloading H_2CO_3 in the media, e.g. *Chlorella* and *Nannochloropsis oculata* at 10 > [83,84]. By contrast, algal growth can also be inhibited by the low carbon source if the CO_2 level is low [85]. In term of microalgae growth, it should be mentioned that the low-nutrition condition combined with the optimized value of carbon source greatly shortens the microalgae cultivation cycle and improves the production efficiency. In an analysis on *Chlorella minutissima* using the optimum amount of carbon source, it has been observed that the maximum biomass productivity is obtained at the second day under low nutrition condition [35] and the sixth day under normal condition [86]. Besides, low nutrition has a negative effect on lipid productivity. On the other hand, increment of CO_2 concentration under nitrogen depletion conditions increases the lipid content. Accordingly, in competition of nitrogen depletion and CO_2 increment, the latter is the winner.

The effect of CO_2 levels on total lipid contents, biomass (dw.) and total lipid productivity of different microalgae cells are shown in Table 12. As observed, increasing the CO_2 concentration (up to ~5–7% (v/v)) significantly increases the growth rate of most algal species grown planktonically. However, higher concentration negatively affects the growth. Much research has demonstrated that *Chlorella sp., Nannochloropsis oculata*, *Dunaliella terticlecta*, *C. kessleri, Spirulina sp.* and *C. vulgaris* [87,88] had optimal growth potential in the lower range of 2–6% CO_2, and the concentrations higher than that caused a decrement in the growth rate [89,90]. In contrast, the best growth potential of *Chlorococcum littorate* [91], *Chlorella ZY-1* [92] and *Scenedesmus obliquus* [71], [87,93] has been observed under high CO_2 level (10–15%). Aside from cell growth, enhanced lipid production in cell at various CO_2 concentrations has also been reported [84,93]. For example, *S. obliquus* SJTU-3 culture has shown great abilities of CO_2 biofixation under the high CO_2 level. The total lipid productivity (25.1–95.35 $mgL^{-1}d^{-1}$) and total lipid contents (4.21–33.14%, w/dw.) in *S. obliquus* cultures exhibited an increasing trend with the increase in CO_2 levels (0.3–12%) and produced the best growth potential at 10% CO_2 [71]. As another example, *Chlorella vulgaris* has produced the maximum lipid productivity of 30 $mg\,l^{-1}\,day^{-1}$ at 1.0 mM KNO_3, 1.0% CO_2 and 60 mmol photons $m^{-2}\,s^{-1}$ at 25 °C [78]. In another study, the highest lipid productivity of 40 $mg\,l^{-1}\,day^{-1}$ was obtained at 8% (v/v) CO_2 in which higher saturated fatty acids were obtained [94]. Enhanced growth and lipid productivity of *Chlorella vulgaris* with CO_2 concentration have also been confirmed by other researchers as well [95]. However, increasing CO_2 concentration above the optimal level results in reduction of microalgae lipid content. It has been reported that the fatty acid content of *Dunaliella salina* decreases with the increase of the CO_2 concentrations from 2% to 10% [96]. The lipid reduction for *Thalassiosira weissflogii* happens in CO_2 range of 5–20% [97].

Generally, the most common process for cultivation of microalgae is autotrophic growth under which the cells harvest light energy and use CO_2 as a carbon source. However, some microalgae are also able to use organic carbon instead of CO_2 as the carbon source for heterotrophic growth. Compared to heterotrophic culture, photoheterotrophic culture uses light as a stimulant and could yield higher lipid content along with higher microalgae growth rate. In other words, light is required to use organic compounds as carbon source in photoheterotrophic metabolism. Mixotrophic culture is different from photoheterotrophic culture only in the need of CO_2. Mixotrophic species can use organic carbon or sunlight, whatever they can get. In an analysis on the effect of carbon source on *Chlorella minutissima*, glucose is identified as the best carbon source, followed by glycerin, mannitol, and glycine to reach the maximum biomass, lipid content and lipid yield respectively [35]. Methanol has also been tested as another alternative. Faster growth and higher cell densities of *Chlorella minutissima* have also been observed by using methanol as alternative carbon source with daily administration of 0.005% and 0.1% (v/v) methanol [98].

The *Chlorella* cells can grow heterotrophically with 10 g/L glucose as the carbon source and accumulates about 280% more lipids and 45% more carbohydrates than autotrophically grown cells [99]. Heterotrophical cultivation of *Chlorella sorokinian* strain with high concentration of glucose as the organic carbon source yields lipid content as high as 56% (w/w) dry weight after 7 days against 19% lipids achieved in 30 days of photoautotrophic culture [41]. In case of *Chlorella protothecoides,* supplementation of glucose to the growth medium under nitrate limitation has been proven to raise the crude lipid content up to 55% (of dcw) compared to 15% under control condition [58].

Vazhapilly et al. [100] tested the growth of twenty different microalgae under two different carbon sources, glucose and acetate. The results showed that all microalgae except *Nannochloropsis Oculata* could grow under heterotrophic conditions using 5 g/l glucose. Among them, excellent growth was observed in *Crypthecodinium cohnii* using either glucose or acetate as carbon source. Besides, *A. carterae, Phaeodactylum tricornutum, Schizochytrium aggregatum, Thraustochytrium aureum* and

Table 12
The effect of carbon source availability on microalgae lipid, lipid productivity and growth rate.

Carbon Sources	CO_2 aeration%	Worst Conc.	Best Conc.	No* or Neg	Algal Species	L-LC%	H-LC%	L-LP g/Ld	H-LP g/Ld	L-GR g L^{-1}	H-GR g L^{-1}	day	Ref
CO_2	0.03–50	50	10	> 10	Sc. obliquus	–	–	–	–	0.82^B	1.84^B	14	[89]
CO_2	0.3–12	0.3	12		Sc. obliquus	4.2%	33.14%	25.1	69.23	0.5^Bcdw	0.4^Bcdw	18	[71]
CO_2	5–15	0	10–15	< 15%	Sc. obliquus	–	–	–	–	1.7^B	2.3^B	–	[85]
CO_2	5–70	70%	10	< 10 <	Sc. obliquus	–	38.9%	–	–	0.2^B	3.5^B	12	[93]
CO_2	0–12	0	12	–	Sc. obliquus	–	–	–	0.22	0.3^B	1.8^B	10	[87]
CO_2	0.04–18	18	6		Sc. obliquus	–	–	–	–	0.28 μ	0.33 μ	20	[88]
CO_2	0.03–15	15	3	< 3 <	Chlorella sp.	–	–	0.089	0.161	0.21^Bcdw	1.48^Bcdw	7	[90]
CO_2	2–15	15	2	> 2	Chlorella sp.	–	–	0.097	0.143	0.009^B cdw	1.2^Bcdw	8	[83]
CO_2	0–16	0	8	< 8 <	*Ch. vulgaris*	–	–	–	–	1.4^Bcdw	6.8^Bcdw	27	[94]
CO_2	0.5–12	12	1	–	*Ch. vulgaris*	–	–	–	–	0.6^B	0.78^B	4	[78]
CO_2	0–50 ml/min	0	50	< 50	*Ch. vulgaris*	20%	25%	0.004	0.013	–	–	17	[95]
CO_2	0.04–12	0.04	4	–	Ch. vulgaris	–	–	–	–	0.34^B	3.32^B	9–10	[262]
CO_2	0.04–18	0.04	6	–	Ch. vulgaris	–	–	–	–	0.19 μ	0.26 μ	20	[88]
CO_2	0.04–18	0.04	18	–	Ch. kessleri	–	–	–	–	0.19 μ	0.39 μ	20	[88]
CO_2	0.03–50	50	5–10	> 10	Ch. pyrenoidosa	–	–	–	–	0.69^B	1.55^B	14	[89]
CO_2	10–70	70	10–20	< 10, > 20	Chlorella ZY − 1	–	–	–	–	0.77^B	5.5^B	6	[92]
CO_2	2–15	15	2	> 2	N. oculata	22.7	29.7	0.084	0.142	$1x^B$	$1.3x^B$	8	[84]
CO_2	0–12	0	6	–	Spirulina sp.	–	–	–	–	0.8^B	3.5^B	10	[87]
CO_2	0.04–18	18	0.04		Spirulina sp.	–	–	–	–	0.26 μ	0.39 μ	20	[88]
CO_2	0.04–12	0.04	4		D. tertiolecta	–	–	–	–	0.4^B	3.32^B	6–7	[262]
CO_2	5–50	50	5	> 5	Chlorococcum littorale	–	–	–	–	$1x^B$	$1.6x^B$	14.5	[263]
methanol	0–5%	0	0.05	> 0.05	Ch. minutissima	–	–	–	–	0.25 μ	1.45 μ	5	[98]
glucose	10–17.5 g/L	10	17.5	> 17.5 *	Ch. minutissima	6%	9.1%	–	–	5.5^B	8.2^B	10	[35]
glycerin	33.73 g/L	–	–	–	Ch. minutissima	–	10.2%	–	–	–	7.5^B	10	[35]
glucose	32.96 g/L	–	–	–	Ch. minutissima	–	10.2%	–	–	–	9^B	10	[35]
glycine	41.20 g/L	–	–	–	Ch. minutissima	–	17.2%	–	–	–	2.44^B	10	[35]
Mannitol	33.36 g/L	–	–	–	Ch. minutissima	–	19.7%	–	–	–	3.36^B	10	[35]
sodium acetate	74.76 g/L	–	–	–	Ch. minutissima	–	17.6%	–	–	–	0.5^B	10	[35]
Sodium bicarbonate	92.30 g/L	–	–	–	Ch. minutissima	–	14.5%	–	–	–	0.7^B	10	[35]
glycerin	9–25 g/L	25	9	–	Ch. minutissima	0.7 g/l	0.75 g/l	–	–	0.2^B	0.55^B	15	[86]
glucose	10 g/L	–	–	–	Ch.protothecoides	14.6b	55%a	–	–	–	–	–	[99]
dextrose	1 g/l	BM	–	–	Ch. minutissima	1x	3.1x	–	–	$1x^B$	$2.7x^B$	7	[264]
oxalic acid	1.46 g/l	BM	–	–	Ch. minutissima	1x	3.33x	–	–	$1x^B$	$1x^B$	7	[264]
starch	0.88 g/l	BM	–	–	Ch. minutissima	1x	2.8x	–	–	$1x^B$	$1.1x^B$	7	[264]
sucrose	0.93 g/l	BM	–	–	Ch. minutissima	1x	4x	–	–	$1x^B$	$1.13x^B$	7	[264]
glycine	1.22 g/l	BM	–	–	Ch. minutissima	1x	3.7x	–	–	$1x^B$	$2.15x^B$	7	[264]
sodium acetate	1.33 g/l	BM	–	–	Ch. minutissima	1x	2.6x	–	–	$1x^B$	$1.99x^B$	7	[264]
glycerin	1 g/l	BM	–	–	Ch. minutissima	1x	5.7x	–	–	$1x^B$	$3.6x^B$	7	[264]

a: heterotrophical, b: photoautotrophic, c: mixotrophic culture, B: Biomass Production, BM: Basic Medium [264], CDW: Cell Dry weight, **L**: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate, **B:** Biomass Production, **μ:** Specific Growth rate, Worst and best concentrations in terms of Lipid content and Lipid Productivity.
Ch. vulgaris: Chlorella vulgaris, Ch. minutissima Chlorella minutissima, Ch. protothecoides: Chlorella. Protothecoides, Ch. Kessleri: Chlorella kessleri, Ch. Pyrenoidosa: Chlorella pyrenoidosa, N. oculata: Nannochloropsis oculata, N. sp: Nannochloropsis sp., D. tertiolecta: Dunaliella tertiolecta.

Amphidinium sp. demonstrated very high growth using glucose. The last three showed moderate growth under acetate (1 g/L). However, *A. carterae, Chröomonas salin, Cryptomonas sp. Nannochloropsis oculata, Pavlova lutheri, P. lutheri, Por. Purpureum, Prorocentrum minimum* could not use acetate as the carbon source and grow [100]. Some authors have suggested that Sodium bicarbonate and HCl do not only control pH but also form carbonic acid, which can be broken down into CO_2 and water [101]. Many microalgae can also directly use Bicarbonate as for their carbon source for photosynthesis [102,103].

5.4.2. Effect of carbon on lipid composition

The lipid profile of the microalgae has been reported in a few studies as summarized in Table 13. It is observed that by increasing carbon monoxide, the portion of C18:1 and C18:2 increases in all compounds while the content of C18:3 dramatically decreases. However, the intensity of this increment or reduction is strain-specific. Accordingly, it is difficult to judge the effects of CO_2 on the total amount of saturated and unsaturated compounds. However, as a general idea, it has been reported that very low concentration of CO_2 (< 2%) facilitates the production of unsaturated fatty acids (C18:1, C18:2) whereas high concentration of carbon dioxide (2–10%) induces the accumulation of saturated fatty acids. This observation has been confirmed by many researchers [104]. However, production of saturated fatty acids outpaces the unsaturated compounds at very high CO_2 concentration (> 10%). In other words, it can be stated that, the degree of unsaturation is reduced by increasing the CO2 concentration.

5.5. Salinity effects

5.5.1. Effect of salinity on lipid content of microalgae

Salinity is the other environmental factor which not only affects the growth and productivity of algae but also limits contaminants and competing microorganisms. High salinity causes high extracellular osmotic pressure, resulting in stress generation inside the alga cell, which is reacted in physiological and biochemical mechanisms. Restoration of turgor pressure, regulation of the uptake/export of ions through the cell membrane, and accumulation of osmo-protecting solutes and stress proteins become active when microalgae cells are exposed to salinity. High salinity stress mainly affects membrane fluidity and permeability [35]. In very high concentration, salinity can damage the microalgae

cell, but an optimal stress can increase lipid production. Various sodium salts (NaCl, $Na_2S_2O_3$, $NaHCO_3$, $C_2H_3NaO_2$) have been used to induce lipid accumulation of different microalgae (in two phase mode) and NaCl has been found to increase the lipid content dramatically while demonstrating a slight reduction in biomass production [44]. In order to cope with the contradiction between lipid production and biomass production yield, a culture mode that allows an optimum growth rate and permits a lipid enhancement was recommended by some researchers [105]. Toward this objective, optimal conditions (free or moderate concentration of salt) to highly concentrate biomass production are provided in the first phase, while stressful conditions are employed to induce lipid biosynthesis in the second phase.

Most microalgae regulate lipid biosynthesis as a physiological resistance strategy to salt stress. However, salinity tolerance capability of every strain is different. Table 14 lists the effect of salt stress on lipid content, growth rate and lipid productivity of many microalgae. As per Table 14, *C. nivalis* has been found to be extremely resistant to NaCl stress and can survive under the lethal NaCl concentration of 70.13 g/L (1.2 M) [106]. *Dunaliella sp.* is also one of the most resistant microalgae to high salt concentration. Their ability to get benefit from salinity stress in order to increase the biomass growth and lipid content makes them one of the most suitable strains to analyze the effect of salinity. It has been reported that with an initial NaCl concentration of 1.0 M, an addition of 0.5 or 1.0 M NaCl at mid-log phase or the end of log phase during cultivation, *Dunaliella* can reach the lipid content of 70% but at the cost of inhibited cell growth [35]. As per Table 14, higher lipid contents are observed in most microalgae that are subjected to salt stress e.g. *Scenedesmus* species [85,107] *Chlorella. vulgaris* [108], *Chlamydomonas Mexicana*, [109], *Scenedesmus sp.* [110]. In the case of *Dunaliella tertiolecta*, salinity increment (0.5 M) does not only increase the lipid content from 60% to 68% but also increase the TG in lipid by 17% [111]. This concentration of salt is also found in favor of the total carotenoid production in *Dunaliella tertiolecta* [112]. Low concentration of salt (0.43 mM) induces carotenoid accumulation in *Haematococcus* too, while high levels of salinity is lethal for it [113]. On the other sides, *Chlorella minutissima* reluctantly grow in the medium with 20 g/L NaCl and its growth is inhibited under higher NaCl concentration.

Generally, most microalgae reach the maximum lipid content at an optimum salt concentration after which the lipid content sharply decreases. This concentration has been reported to be between 20 and 40 g/L (0.34–0.68 mol/L) in most microalgae. For examples, lipid accumulation and biomass synthesis of *Chlorella minutissima* [35] and *Nitzschia Laevis* (*Bacillariophyceae*) [114] under salinity stress till an optimum values of 40 and 20 g L^{-1} respectively, have significantly increased while negative effect has been observed by further increasing salinity. In case of *Nitzschia laevis*, NaCl optimum concentration is about 10 g L^{-1} beyond which polar lipids increase but storage lipid (i.e. TAG) decrease [114]. In some cases, elevated salinity conditions beyond the optimal value leads to increased storage lipid content and decrease in membrane lipid content [115]. These variations in lipid and fatty acids confirm a decrease in membrane fluidity and permeability under salinity stress, which can help the algae bear and acclimate to these conditions. Increment of protein and carbohydrate by salinity have been observed in other microalgae i.e *Ankistrodesmus falcatus* [116]. Some microalgae produce carbohydrates with low molecular weight in response to salt stresses, mainly in cyanobacteria [104,117–120]. Increment of carbohydrate and lipid simultaneous with reduction of protein due to salinity stress was also reported in case of *Scenedesmus sp.* [110]. Some micro algae are also less sensitive to salinity [35]. Different authors have reported that salinity stress does not have any effect on lipid and carbohydrate content of *Botryococcus braunii* alga strain [68,70,120], but protein content decreases [121]. Ben-Amotz et al. [122] found similar results in terms of protein and carbohydrate of *Botryococcus braunii* but higher lipid accumulation under the salinity of 0.5 M. Rao et al. [120] reported that salt concentration of 34 mM induces significant higher biomass and carbohydrate and carotenoids

Table 13
The effect of carbon availability on fatty acid composition of different microalgae.

Edible Oil	CO_2	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	other	SFA	MUFA	PUFA	Ref
Ch. vulgris	0.03–2	~	–	–	–	~↑	~	↑	↓	–	–	~↑	↑↑	↑↑	↓↓	–	–	–	–	~↑	↑	↓	[265]
Air-Air CO_2 riched																							
Ch. vulgris	1–10	–	–	–	→	↑↑	~↑	–	–	–	↑	↓↓	↑	↑↑	↓	↓↓	–	–	–	→	↑	↑	[266]
Ch. vulgris	0–8	~	–	–	–	↑	–	–	–	–	–	↓↓	↑	↑	↓↓	–	–	–	–	~	↑	→	[94]
S. obliquus	0.03–50	~	–	–	–	→	~	↑↓	↓↑	–	–	~	~	↑↓	↓↑	~	~		a	→	~	↑	[89]
								10	10					5–10	10								
Ch. pyrenoidosa	0.03–50	~	–	–	–	↓↑	~	↑↓	↑	–	↓↑	~	↑↓	↑↓	↓↑	–	–	–		↓↑	↑↓	↑↓	[89]
						10		10–20					10	10	10					10	10	10	
Emiliania huxleyi	1–53 μM/l	↑	–	–	–	↑↓		–	–	–	–	↑↓	↑	↑	↓↑	–	–	–	b	↑↓	↑↓	↓↑	[267]
Chlorococcum littorale	5–50		–	–	–	→	~	–	–	↓↓	–	–	~	→	↑	–	–	–	c	→	~	→	[263]

a: C20:5, ↑, b: C18:4 ↓↑, C18:5 ↓, C22:6 ↓, c: all compounds have not been analyzed, < 10%.

Table 14
The effect of salinity on microalgae lipid, lipid productivity and growth rate.

Salinity M	Worst Conc.	Best Conc.	Negative effect	Algal Species		L-LC %	H-LC %	L-LP gld	H-LP gld	L-B ^{B}:g/l	H-B ^{B}:g/l	day	Ref
0–1.4 M	1.4	0.7	< 0.34–0.7 <	Ch. minutissima	G	15%	31.82%			8^{B}	7.5^{B}	$4^{D}+3$	[35]
0.5,1 M	0.5	1	–	Du. tertiolecta	G	60.6%	67.8%	–	–	1^{B}	1.03^{B}	10^{I}	[111]
0.1,0.2,0.3 M^{I}	0.1	0.3	–	Sc. obliquus	G	15%	30%	–	–	–	–	15	[85]
0–0.1 M	0	0.025	< 0.025 <	Sc. obliquus	G	18	34			0.25^{B}	0.65^{B}	20^{I}	[123]
0–0.001 M	0.0002	0.001	< 1	Sc. quadricauda	G	6.2% dcw	6.9% dcw	–	–	1.5^{B}	1.35^{B}	15^{I}	[107]
0–0.4 M	0	0.4	< 0.4	Sc. sp.	G	18.98	33.1%	–	–	0.4^{B} dcw	0.12^{B} dcw	15^{I}	[110]
0.2–1 M	0.2–0.4	0.6	< 0.6 <	N. salina	G	< 15%	36%			$1x^{B}$	$7x^{B}$	$35^{I} - 40^{I}$	[115]
0–1.2 M	0	0.6	< 0.6	N. oculata	G	35	44.5	0.231gld	0.324gld	2.15^{B}	1.91^{B}	$5^{D} + 4$	[105]
0–0.7^{b}M	0.17	0	< 0	N. oculata	G	17	29	–	–	–	0.5^{aB}	$14^{a}+3^{b}$	[268]
0–0.5^{d}M NaCl	0^{d}	0.34^{d}	< 0.34 <	De. abundans	G	23	35.5	0.045	0.058	0.19^{BP}	0.16^{BP}	$8^{c}+6^{d}$	[44]
0–0.3 M $NaHCO_3$	0	0.3	–	De. abundans	G	23	33.44	0.045	0.054	0.19^{BP}	0.16^{BP}	$8^{c}+6^{d}$	[44]
0.04–0.34 M	0.04	0.17	< 0.17 <	An. falcatus	G	43	56.1	–	–	–	–	20	[116]
0–0.7^{b} M	0	0.17	< 0.17 <	Du. salina	G	22	43	–	–	–	0.25^{aB}	$10^{a}+1^{b}$	[268]
0–0.7^{b} M	0.17	0	< 0	Du. tertiolecta	G	23	40	–	–	–	0.28^{aB}	$10^{a}+2^{b}$	[268]
0–0.1 M	0	0.025	< 0.025 <	Chl. mexicana	G	15	37			< 0.1^{B}	0.8^{B}	20^{I}	[123]
0–0.34 M	0	0.34	< 0.34	Sc. obtusus	G	35.3	47.7	0.051	0.061	0.15^{BP}	0.13^{BP}	$12^{c}+8^{d}$	[269]
0–0.085 M	0	0.02–0.034	< 0.017, > 0.034	Bo. braunii	P^{C}	–	–	–	–	0.9^{B}	1.5^{B}	18	[120]
											1.35^{B}	18	
0–0.34 M	0.34	0, 0.08	< 0.08	Bo. braunii	P^{C}	26	31, 22	–	–	0.8^{B}	0.9^{B} 1.1^{B}	20	[68]
0.017,0.034,0.5 M^{I}	0.5	0.17	> 0.17	Nitzschia laevis	GB^{b}	–	–	–	–	10.1^{B}	5.67^{B}	–	[114]
0–0.14 M	0	0.1	–	Ch. vulgaris	YG	47.7%	53.93%	–	–	5.47^{B}	5.47^{B}	5	[108]
0–0.7^{b}M	0.7	0.17	< 0.17 <	Is. galbana	B	30	47	–	–	–	0.8^{aB}	$12^{a}+2^{b}$	[268]

L: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate, **B**: Biomass Production, Worst and best concentrations versus Lipid content and Lipid Productivity, D: Initial Days without salt (2 phase grwoth), I: initial salt, *: 2phase culture, a: initial condition salt:30psu (0.5 M)b: second phase, d: second phase P^{C}: Phylum: Chlorophyta, BP: g/lday, c/d: phase I(c) without salt, Phase II(d) different salt concentration.
De. abundans: Desmodesmus abundans, Is. galbana: Isochrysis galbana, An. falcatus: Ankistrodesmus falcatus, Chl. Mexicana: Chlamydomonas Mexicana.

production in *Botryococcus braunii* compared to the control conditions. Accordingly, it can be concluded that salinity contributes to different effects (i.e. levels of storage type or content) for different algae species.

5.5.2. Effect of salinity on lipid composition of microalgae

Environmental salinity as any other physiological state of the culture affects the fatty acid profile of the intracellular lipid of microalgae (Table 15).

The fatty acid profile of the intercellular lipids confirms that the relative content of monounsaturated FAs remarkably (i.e. palmitic (C16:1) and oleic acids (C18:1)) increases under high salinity and high alkalinity compared to the control without any salt addition. These compounds mainly make up the neutral lipids which in turn favors biodiesel production [44]. On the other side, the proportion of polyunsaturated FAs (PUFA), especially linolenic acid (C18:3), decreases substantially. These changes are in the direction to keep the membrane fluid and prevent its destruction. These results are consistent in the cases of *Chlamydomonas Mexicana* [123], *Scenedesmus obliquus* [123], *Desmodesmus abundans* [44], *Dunaliella sp* [124], *Botryococcus braunii* [120], *Cladophora Vagabunda* [125]. In term of saturation, some authors have reported that the fatty acids of membrane lipids are desaturated, leading to increment in the proportion of unsaturated fatty acids, e. g. as in *Boekelovia hooglandii* [126]. The same trend has also been observed in the fatty acid composition of polar lipid in *Dunaliella salina Teodoresco* by the change in NaCl concentration [127]. However, increment in the saturated fatty acids under high-salt stress has been reported by a few cases of *Dunaliella sp* and *Nitzschia laevis* [114,124]. In the case of *Botryococcus braunii*, the saturated compounds have not decreased significantly but they change remarkably. In the intercellular of this microalga, C18:0 reduces significantly while C16:0 increases. There is also a remarkable content of C22:0 and C24:0, which are not generated under control condition [120]. In *B. braunii*, the fatty acids with 18 carbon atoms in their chain have been reported as the precursor in the formation of very long chain fatty acid derivatives through chain elongation [77]. However, it should be noted that, this trend was consistent till the optimum concentration of salt or the concentration with which the strain could adapt itself and after that the trends sometime changed. For example, Cao et al [35] reported that the degree of fatty acid unsaturation in both polar and neutral lipid fractions increased sharply till the optimum concentration of NaCl, but decreased at higher NaCl concentration.

5.6. pH effects

5.6.1. Effect of pH on lipid content of microalgae

Acidity or basicity of culture medium is another factor that directly affects the microalga growth rate and species composition. The pH value affects the availability and absorption of nutrients such as iron and carbon, which indirectly affects the growth rate [104]. pH in microalga cultures rises steadily during the day due to photosynthesis and the use of CO_2. During the night (dark), respiration reverses this process and lowers pH levels again. pH is normally controlled by injection of CO_2 (also used as carbon source) into the culture, using buffer or injecting mineral acids into the culture medium [128]. pH value may reach 10 when no CO_2 is supplied and the values of 11 or above is not uncommon if CO_2 is limiting and bicarbonate is used as a carbon source. This range of pH is quite inappropriate for most microalgae. It has been reported that the pH of *Nannochloropsis*, *Tetraselmis*, and *Isochrysis* can be maintained within the range of 6.75–7.25 with CO_2 injections, while untreated cultures reached the pH of 8.25 within 15 days [82]. In recent studies addition of HCl and Sodium bicarbonate has also been reported to control pH as in *Tetraselmis suecica* and *Chlorella sp* cultural medium, [101]. These compounds can form carbonic acid followed by decomposing into CO_2 and water consequently. Many microalgae also use bicarbonate as an inorganic carbon source for photosynthesis [129].

Table 15
The effect of salinity on fatty acid composition of different microalgae.

Edible Oil	best	Optimum salt Concentration	C 14: 0	C 14: 1	C 15:0	C 15: 1	C 16: 0	C 16: 1	C 16: 2	C 16: 3	C 16: 4	C 18: 0	C 18:1	C 18:2	C 18: 3	C 20:0	C 20: 1	C 20: 2	other	SFA	MUFA	PUFA	Ref
0.4–4 M	-	Dunaliella sp	~	~	~	-	-	↑	~	-	↓	-	↑	↑	↓↑	-	-	-		↑	↑	↓	[124]
↑10,20,30 g/l	10	Nitzschia laevis[TAG]	↓	~	-	-	↑↓	↓	-	-	-	~	~	~	~	-	-	-	a	~	↓	↑	[114]
0–85 mM	85	Botryococcus braunii	–	–	–	–	↑↑	↑↓↓	–	–	–	↓↓	↑↑	↓↓	–	–	–	–	c,de	~↓	↑↓	↓	[120]
0, 20 g/l NaCl	20	Desmodesmus abundans	↓	–	–	–	↓	↑	–	–	–	↑	↑↑	↓	↓	~	–	–		↓	↑↑	↓↓	[44]
0, 25 g/l $NaHCO_3$	25	Desmodesmus abundans	↓	–	–	–	↓	↑	–	–	–	↑	↑↑	↓	↓	~	–	–	e	↓	↑↑	↓	[44]
↑0.85–3.4		Dunaliella salina	–	–	–	–	↓	–	–	–	–	–	↓	↑	↓	–	–	–	f	↓	↓↑	↑	[127]
		polar lipids																					
0–100 mM	25	Chlamydomonas mexicana	–	–	–	–	↓↑ 25	↑	–	–	–	~	↑↓ 25	↓↑ 25	↓↑ 25	–	–	–	–	↓↑	↑↓	↓↑	[123]
0–100 mM	25	Scenedesmus obliquus	–	–	–	–	~	~	–	–	–	~	↑↓ 25	↑↓ 25	↓↑ 50	–	–	–	–	~	↑↓	↓	[123]
0–0.8 M		Boekelovia hooglandii	↓↑	–	–	–	↓	↓	–	–	–	–	↑	–	–	~	–	–	g	↓	↓	↑	[126]
50% – 200%	–	Cladophora Vagabunda	~				~	↓	~			↑	↑↑	↑	↓				i	~↓	↑↑	↓	[125]
$NaNO_3$ 150–3000 μM		Nannochloropsis sp.	~				↓	↓				~	↓	↑			↑	–	j	↓↓	↓↓	↑↑	[270]
NaH_2PO_4, 6–120 μM		Nannochloropsis sp.	~				↓	~				~	↓	↑			↑	–	j	↓	↓↓	↑↑	[270]
NaCl 0.2–1 M		Nannochloropsis sp.	↑				↑	↑↓ 0.7 M				–	↑↑	~			↓		k	↑	↑↑	↓↓	[270]

TAG: Fatty acid profile of TAG, A: C20:5: ↑. B: C13:0↑↑, C: C22:1 ↓↓ D: C24:0 ↑↑ E: C22:0, ↑, e: C22:0 ↓, f: C14:2 ↑↑, g: C18:4, ↑, C20:5 ↑↓, C22:5↑↑, C22:6↑↑, i: C18:4 ↓, C20:4 ↓, C20:5 ↓, C22:5 ↓, C22:6 ↓, j: C20:4 ↑, C20:5↑↑, k: C20:4 ↓, C20:5↓↓.

Table 16
The effect of culture pH on microalgae lipid, lipid productivity and growth rate.

pH	Worst pH	Best pH	Negative or No effect	Algal Species	L-LC	H-LC	L-LP	H-LP	L-BY gL^{-1}	H-BY gL^{-1}	GR-W	GR-B	day	Ref
4–11	9	6–7	> 8	Ch. minutissima	1%	14%	–	–	–	–	1.2^{B}	8.5^{B}	10	[35]
5–8	–	7	< 7	Chlorella sp.	–	–	–	–	0.11gl	0.15gl	0.85^{B}	1.3^{B}	15	[137]
5–11c	–	7	< 7, > 7	Chlorella sp.	15%	33%	–	–	50 mg L^{-1}	167.5 mg L^{-1}	0.39^{B}	0.47^{B}	30	[138]
< 10- > 11	–	< 10–11	> 11	Chlorella.	–	–	103^{a} g/dw	171^{a} g/dw	–	–	–	–	10	[140]
7.2,9.5	7.2	9.5	–	Chlorella FGP5	10%	20%	–	–	–	–	1.175 g/ld	0.382 g/ld	7	[139]
7.2,9.5	7.2	9.5	–	Chlorella Rb1a	14%	17%	–	–	–	–			7	[139]
7.2,9.5	7.2	9.5	–	Chlorella RBD8	5%	23%	–	–	–	–			7	[139]
7.6,10	10	7.6	–	Chlorella OS1–3	37%	44%	–	–	–	–			7	[139]
7.6,10	10	7.6	–	Chlorella OS4–2	15%	36%	–	–	–	–	1.62 g/ld	1.71 g/ld	7	[139]
5.5–8	5.5	7	< 7 > 7	Chlorella	–	–	10mgld	90mgld	200mgl	1600mgl	0.1 g/ld	0.4 g/ld	50	[101]
3–12c	3 &12	6–11	< 6, > 11	Sc. sp R− 16	17%	42 > %					0.7^{B}	3.3^{B}	6	[271]
7.5–11.1	pH: 7.5	8.4–11.1	–	Sc. sp w	$0.6\%^{b}$	$16.8\%^{b}$	–	–	–	–	0.97^{B}	0.83^{B}	28	[135]
7.5, 8.5, 9.5	9.5	7.5	–	Sc. obliquus	–	–	–	–	–	–	0.95^{B}	1.6^{B}	6	[272]
7.5, 9.5	9.5	7.5	–	Ch. vulgaris	–	–	–	–	–	–	0.7^{B}	1.35^{B}	6	[272]
6.5–10	pH: 7.5	9.7–10	–	Coelastrella sp. p	$0.1\%^{b}$	$0.6\%^{b}$	–	–	–	–	0.86^{B}	0.71^{B}	14	[135]
7–9	–	8	7&9	Nannochloropsis	17%	25%	–	–	–	–	–	–	21	[129]
6.5–8	6.5	7.5	< 7.5 > 7.5	Tetraselmis suecica	–	–	0.030 gld	0.09 gld	400 mgl	950 mgl	0.1 g/ld	0.3 g/ld	50	[101]
5–10	5,10	8	< 8, > 10	Pavlova lutheri	24%, 23%	35%	–	–	–	–	0.2^{B}	0.4^{B}	~6	[273]
4–8	8	4–6	> 6	Nannochloropsis sp.	25%	37%	–	–	–	–	–	–	–	[274]

L: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **BY:** Biomass Yield **GR**: Growth Rate, **B:** Biomass Production **and rest:** Specific Growth rate, Worst and best conditions in terms of Lipid content and Lipid Productivity, **a:** μmol g^{-1}dry wt, **b:** % TAG, **c:** initial pH, D: growth experiment within 3 days.

Although different species might have different growth responses to the changes of pH values, the acceptable pH range for most microalgae species is 7–9. However, the optimal pH range for microalgae growth is strain-specific and narrow. It is between 8.2 and 8.7 for most of them [104]. For example, the optimal productivity of *cyanobacterium Anabaena variabilis* is in a medium with pH range of 8.2–8.4, being slightly lower at 7.4–7.8. The productivity decreases dramatically above pH 9, and the cells are unable to thrive at pH 9.7–9.9 [130]. The maximum growth rate of *Nannochloropsis* is also observed in pH range of 8–9. It is significantly lower at nutrient medium culture (pH 7) and it is stopped at pH lower than 6 and higher than 10 [129]. The pH value of 8 for keeping the optimal growth rate of *Nannochloropsis* has also been confirmed in other work [131]. Consistent decline of growth rate and photosynthesis of *Thalassiosira pseudonana* and *Thalassiosira oceanica* has also been reported at high pH levels (> pH 8.8) [132]. By analyzing the growth rate of *Skeletonema costatum* under different pH conditions ranging from 6.5 to 9.4, the maximum growth rate was observed at pH 7.5 while it significantly reduced at pH > 8.5 [133]. The maximum biomass and lipid productivity of *T. suecica* have been reported to be in pH range of 7–7.5 [101]. However, some species are acclimated to high alkali or high acidic environments. As reported, the cells of *Chlorella* protothecoides grow rapidly at the pH value of 5.0, (compared to the pH of 4.0, 4.5, 5.5, 6.0, 6.5, 7.0) and its growth is inhibited under alkaline pH [134]. In contrast, *Scenedesmus sp* [135] grow better under alkali conditions (pH > 9) and have higher lipid productivity in these conditions. Generally, high alkali conditions seriously inhibit the normal growth of most microalgae while acidic conditions are conducive for microalgae growth and oil accumulation [35,114]. It is known that high level of pH is often associated with harmful microalgae blooms or red tides [135,136].

Lipid content is also dramatically affected by pH value. As per Table 16, the pH value ranging from 7 to 9.5 can also yield a higher lipid accumulation in most microalgae species. Under nitrogen limitation, *Chlorella* could yield higher triacylglycerol (TAG) production with a rise in the culture medium pH [135]. Rai et al [137] also showed that the lipid content of *Chlorella sp.* increased to 23% at pH 8 though the highest biomass production was observed at lower pH (=7). The same trend was exactly reported by Zhang et al [138]. The authors observed the maximum algal lipid yield at initial pH of 7.0. The triacylglycerols content was significantly enhanced to 63.0% at initial pH of 5.0 and biomass accumulation was at its maximum level at pH 9. Based on that work, although the lipid content dramatically changed by pH, the total TAG and biomass production did not change significantly at pH range of 5–9. Skrupski et al [139] analyzed five different strains belonging to the genus *Chlorella* but three of them (FGP5, Rb1a, RBD8) originated from a medium culture with pH 7.2 and two of them (OS1-3 OS4-2) from an environment with pH 10. Accordingly, the researchers considered the pH values of 9.5 and 7.6 in order to put the microalgae under stress and found the lipid increment in both groups. However, in the first group, lipid increment came at the cost of lower mass productivity but in the second, mass productivity increased.

5.6.2. Effect of pH on lipid composition of microalgae

pH variation in the culture medium also affects the lipid composition accumulated in the microalgae, as summarized in Table 17. Generally, alkali pH stress results in generation of lipid with higher saturated compounds in most microalgae originated from acidic medium. For example, alkaline pH stress significantly reduces the glycolipid and polar lipid content of *Chlorella* while increasing the TAG accumulation, which is not dependent on nitrogen or carbon limitation levels. However, lower total lipid with higher amount of saturated (C16:0) and monounsaturated (C18:1) is the resultant outcome [140]. Consistent with this, Analysis of pH effect on an unidentified strain (*Chlamydomonas* sp.) originated from an acidic lake shows that FAs of polar lipids are more saturated compared with that microalga (*C. reinhardtii.*) isolated from a medium with higher pH. In other words, the increase in saturated fatty acids in membrane lipids of *microalgae* at under pH stresses (a pH value higher or lower than their originality) represents an adaptive reaction to decrease membrane lipid fluidity [141]. Therefore, changes of pH do not induce any stress unless the change is opposite of the originality of microalgae. For example, in the case of *Nannochloropsis*, the maximum lipid content has been obtained at pH 8 while accumulation of unsaturated, saturated, and mono/polyunsaturated lipid does not change by reducing or increasing the pH value to 7 and 9 respectively [129]. Skrupski et al [139] also reported that, the saturated and polyunsaturated content of both phospholipid and TAG increased while the monounsaturated decreased at high pH values. However, high pH values do not mean pH stress for all *Chlorella* strains since some of them originate from alkali medium culture [139].

5.7. Temperature effects

5.7.1. Effect of temperature on lipid content of microalgae

Seasonal temperature fluctuations, daily temperature variations, and abrupt temperature variations due to any reason can modify the growth conditions of microalgae and thus its production efficiency or vice versa. Generally, outdoor photo-bioreactors induce the temperature fluctuations of 10 and 45 °C (depends on the region) the microalgae. Inconsistent with this assumption that the lipid metabolic pathways of microalgae may change in a way resulting in higher lipid accumulation and higher ability to resist external temperature stress, both low and high temperatures may conceal lipid accumulation. Moreover, such situation severely inhibits microalgae growth [142]. Among them, the effect of elevated temperatures is more deleterious than low temperatures. In the case of *C. vulgaris*, if the temperature reaches 38 °C, an abrupt interruption happens and later the cells die. These changes are easily visible because of the change in cells color from green to brown, and the growth rate, which divert to negative values [46].

Though most microalgae species are capable of carrying out cellular division and photosynthesis over a wide range of temperature (15 and 30 °C), the temperature optimal range differs for various microalgae. It is about 32 °C for *Dunaliella sp.* [143], 25–30 °C for of *C. Vulgaris* [46] and 15–20 °C for *Chlorella Minutissima* [35]. Generally, *Chlorella* species have the optimal growth rate over a wide range of temperatures. Studies of 17 different *Chlorella* strains have demonstrated that this group of microalgae can grow successfully between 26 (i.e. *C. Prothotecoides, C. Vulgaris*) and 36 °C (i.e *C. Kessleri, C. Fusca,*) [144]. Accordingly, microalgae are divided into three categories based on their optimal growth rate at different temperatures, which are 20 and 25 °C for *meso*philic species; 17 °C for psychrophilic strains (*Asterionella Formosa*) or increase to 40 °C for thermophilic strains (*Anacystis Nidulans*, *Chaetoceros*). Microalgal growth under above-optimal temperature is species-dependent and except for the thermophilic species, it has been rarely reported for *meso*philic and psychrophilic strains. Moreover, the optimal growth temperature may change slightly by the medium culture condition. For example, the optimal growth temperature of *Dunaliella tertiolecta* increase by 6 °C by increasing the sodium chloride content from 0.125 to 1.5 M [145].

It should be noted that, a balance must be maintained between the energy consumption inside the cell and the photosynthetic energy supply in order to grow microalgae. An imbalance between energy supply and consumption, caused by environmental factors results in a change of the photosynthetic apparatus (Rubisco activity, unit size). Some microalgae shrink their cells in order to cope with the imbalance between anabolic and catabolic processes due to temperature rise. In other words, microalgae reduce their volume in order to reduce metabolic costs and enhance uptake rates [146]. Such acclimation has been observed in the cases of *Microcystis aeruginosa*, *Scenedesmus acutus* and *Dunaliella* species by reduction of photosynthesis rates, respiration rates and cell size [147,148]. Aside from rapid physiological adjustments, slow generational adaptation is another solution in response to

Table 17
The effect of pH on fatty acid composition of different microalgae.

Edible Vegetable Oil	pH	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 17:1
Chlorella.	< 10 -> 11	~	–	–	–	↑	~	~	~	~	–	–
Nannochloropsis	↑7–9	~	–	~	–	~	~	–	–	–	~	–
Chlorella FGP5[a]	↑7.2,9.5	~				↑	↑	↓	~	~		
Chlorella FGP5[b]	↑7.2,9.5	~				↑	↓↓	↑	↑	~	–	–
Chlorella OS4–2[a]	↑7.6,10	~				↓	~	~↓	↑	~	–	–
Chlorella OS4–2[b]	↑7.6,10	~				~	~	~	~	~	–	–
Chlorella FGP5[a]	7.2, 7.2–9.5					↑	↓↓	↓↓	↑			
Chlorella FGP5[b]	7.2–9.5, 9.5					↓↓	↓↓	↑	↑			
Chlorella OS4–2[a]	10, 7.6–10					~	~	~↑	↑			
Chlorella OS4–2[b]	7.6–10, 7.6					~↓	~	~	~			
N. oleoabundans	8.1–10↑					↑	~↑					

Edible Vegetable Oil	pH	C 18:0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	SFA	MUFA	PUFA	Ref
Chlorella.	< 10 -> 11	↓	↑	~	↓	–	–	–	↑	↑	↓	[140]
Nannochloropsis	↑7–9	~	~↑	~	~	–	–	–	~	~	~	[129]
Chlorella FGP5[a]	↑7.2,9.5	~	↓	~	↑				~↑	~	~↓	[139]
Chlorella FGP5[b]	↑7.2,9.5	↑	↓↓	↑	↑↑				↑	↓↓	↑	[139]
Chlorella OS4–2[a]	↑7.6,10	~	↑	↓	~				~↓	↑	↑	[139]
Chlorella OS4–2[b]	↑7.6,10	~	↑	↓↓	~				~	↑	↓↓	[139]
Chlorella FGP5[a]	7.2, 7.2–9.5	~	↓↓	↑↑	↑				↑	↓↓	~	[139]
Chlorella FGP5[b]	7.2–9.5, 9.5	↑	↓↓	↑↑	↑↑				↓	↓↓	↑↑	[139]
Chlorella OS4–2[a]	10, 7.6–10	~	↑	↓	↓				~	↑	↓	[139]
Chlorella OS4–2[b]	7.6–10, 7.6	~	↑	↓	~				~↓	↑	↓	[139]
N. oleoabundans	8.1–10↑	↑	↑	↑	↓				↑	↑	↑↑	[275]

a: Phospholipid, b: TAG.

temperature elevation, which is the case for *Scenedesmus*. It has been observed that this microalga could adapt to 30 °C after 15 generations, to 35 °C after 30 generations and to a maximum of 40 °C after 135 generations [149]. Converti et al. [46] found that increasing the temperature from 20 to 25 °C doubled the lipid content of *Nannochloropsis oculata* (from 7.9% to 14.9%) at the cost of reduced growth rate. In some cases (i.e. *Isochrysis galbana* [150]), high growth temperature has been associated with decreases in carbohydrate and lipid and increase in protein content. In case of *Isochrysis galbana,* lipid classes and fatty acid profiles of the cells changed with the specific growth rate of the culture. Neutral lipids decreased while glycol and phospholipids increased with the dilution rate [150].

The effect of temperature fluctuation, especially reduction, on total lipid content of microalgae has been widely studied, as summarized in Table 18. The temperature effects on lipid yield vary depending on different species. As observed, in most strains, low temperature causes stress in favor of higher lipid accumulation. It has been found that low temperature stress has slight effects on carbohydrate production while reducing carbon incorporation into proteins. A possible conception to justify this observation is that the biosynthesis of amino acid and putative osmolyte biosynthesis is inhibited as the temperate reduces. Therefore, glycolysis intermediates, which can play as the role of precursors of acetyl-CoA increases, leading to the synthesis of relatively more fatty acids [151].

A decrease in cultivation temperature from 25 to 20 °C increases the lipid content of *Scenedesmus sp* by 2.5 folds with only a slight effect on the growth rate (8% loss) [152]. The same trend has been observed in the case of *Chlorella vulgaris*, for which a temperature drop from 30 to 25 °C increases the lipid content by 2.5 folds without any effects on the growth rate [46]. However, in some cases i.e. *Chlorella sorokiniana*, although low temperature (18 °C) increases the lipid content, algal growth is inhibited at 18 °C and so the lipid productivity only reaches 76% of that at 26 °C [151]. In order to alleviate the adverse effects of low suboptimal temperatures on lipid productivity, Wang et al. [151] added Glycine betaine (GBT), a quaternary ammonium compound, to the media at the initial phase of culturing. The authors reported that GBT could enhance the biomass, lipid content and lipid productivity of *C. sorokiniana* by enhancing carbon fixation at both the optimal temperature of 26 °C and a low sub-optimal temperature of 18 °C without any effects on its composition. Moreover, low temperatures generally decrease the carboxylase activity and so the energy supply is overproduced if the light condition does not change, creating light saturation conditions. *Chlorella vulgaris* can manage this imbalance and grow successfully at 5 °C with low chlorophyll content [153]. *Dunalliella tertiolecta* [154] and *Dunaliella salina* [155] have also demonstrated similar acclimation. In the case of *Skeletonema costatum*, the response is different and the chlorophyll content increases with lower temperature.

Arrhenius equation is the most popular model to explain the relation between below-optimal temperatures and growth rate. According to this model, cell division, growth and photosynthesis in most microalgae are expected to double for each 10 °C increase until unfavorable temperature is achieved. In other words, the temperature coefficient Q10 is expected to present a value near 2 by the Arrhenius model. Microalgae growth rate significantly decreases as temperature exceeds the optimal temperature, due to the unfavorable effect of heat stress on the functionalities of enzymes and proteins. The response of microalgae growth to the temperature enhancement is often described by a bell-shaped growth curve. The temperature in which the growth rate reaches zero value is named as lethal temperature, which is from 30 to 35 °C onwards for mesophilic microalgae [156,157].

5.7.2. Effect of temperature on lipid content of microalgae

Although in most research, the focus is on total lipid, some researchers have also examined the effect of temperature on different classes of lipids. However, simultaneous analysis of lipid and fatty acid compositions to both temperature and growth phase has been rarely investigated.

Temperature has a major effect on the composition of fatty acids produced by microalgae. Most microalgal species respond to increased growth temperature by decreasing the ratio of unsaturated to saturated fatty acids [158]. As per Table 19, in all cases, monounsaturated fatty acids increased while polyunsaturated fatty acids were down-regulated when the culture temperature increased beyond the optimal temperature. Meanwhile, in most cases, the content of saturated fatty acids increased with temperature and vice versa. Suga et al [159] discussed that low temperatures induced the expression of genes encoding fatty acid desaturates, including those that were able to desaturate oleic acid (18:1) to linoleic acid (18:2) or linoleic acid (18:2) to linolenic acid (18:3). In other words, lower growth temperature causes microalgae to generate higher content of lipid with a high percentage of unsaturated fatty acids, which helps the strain to maintain membrane fluidity under low temperatures [151]. This is however inconsistent with the observations of some researchers in the cases of *Nannochloropsis oculata* and *Chlorella vulgaris*, which found that the percentage of saturated fatty acid decreased when the culture temperature increased beyond the optimal temperature [46]. However, The response of microalga chemical composition to low and high growth temperatures is species-dependent with no overall consistent relationship between temperature

Table 18
The effect of culture temperature on microalgae lipid, lipid productivity and growth rate.

Temp °C	Worst Temp	Best Temp	Negative or No effect	Algal Species	L-LC	H-LC	L-LP	H-LP	L-B g L^{-1}	H-B g L^{-1}	day	Ref
10–45	35–45	20	> 30 < 15	Ch. minutissima	17.5%	14%	–	–	1.5^{B}	9^{B}	–	[35]
25–38	38	25	> 25	Ch. vulgaris	1x	1.3x	− 2.72	20.22	–	–	14	[46]
18 &26	26	18	–	Ch. sorokiniana	1x	1.2x	0.030 gld	0.023 gld	–	–	10^{26C} – 15^{18C}	[151]
15–25	15	25	< 25	Nannochloropsis. oculata	1x	1.07x	9.11	10.10	–	–	14	[46]
10–30	10	20	> 20	Scenedesmus sp.	$1x^{TAG}$	$1.83x^{TAG}$	–	–	1x	1.56x	15	[152]
25–35	33	27	> 27	Isochrysis sp.	20.2%	21.7%	–	–	–	–	–	[158]
25–35	30	25	> 25	Chaetoceros sp.	12.2	16.8	–	–	–	–	–	[158]
25–35	33	27	> 27	Rhodomonas sp.	8.0	12.7	–	–	–	–	–	[158]
25–35	30	25	> 25	Cryptomonas sp	19.6	21.4	–	–	–	–	–	[158]
25–35	33	25	> 25	prymnesiophyte	13.8	14.7	–	–	–	–	–	[158]
15&30	15	30	–	Isochrysis galbana	19%	24%			0.1^{B}	0.5^{B}	6	[150]
15–35	15	30	< 25–30 <	Nannochloropsis salina	45%	57%	0.31 gld	0.43 gld	0.4^{B}	0.5^{B}	10	[28]

L: Lowest, **H**: Highest, **LC**: Lipid Content, **LP**: Lipid productivity, **GR**: Growth Rate, **B:** Biomass Production, Worst and best conditions in terms of Lipid content and Lipid Productivity.

Table 19
The effect of temperature on fatty acid composition of different microalgae.

Edible Vegetable Oil	T	C 14:0	C 14:1	C 15:0	C 15:1	C 16:0	C 16:1	C 16:2	C 16:3	C 16:4	C 17:0	C 17:1	C 18: 0	C 18:1	C 18:2	C 18:3	C 20:0	C 20:1	C 20:2	SFA	MUFA	PUFA	Ref
Chlorella. vulgaris	↑25–38 25	–	–	–	–	⇅	–	–	–	–	–	–	⇅	↑↑	→	→	–	–	–	⇅	↑↑	→	[46]
Nannochloropsis. oculata	↑15–25 25	–	–	–	–	→	–	–	–	–	–	–	⇅	↑↑	→	→	–	–	–	→	↑↑	→	[46]
Chlorella sorokiniana	↑18–26 26	←	–	–	–	←	~	←	→	–	–	–	→	~	←	→	–	–	–	←	←	→	[151]
Scenedesmus sp.	↑10–30 20	–	–	–	–	~	–	–	–	–	–	–	–	↑↑	←	⇅	–	–	a	–	↑↑	→	[152]
Isochrysis sp.	↑25–30 20	⇅	–	–	–	←	→	→	–	–	–	–	←	⇅	⇅	⇅	–	–	–	→	←	~	[158]
Chaetoceros sp.	↑25–35 27	←	–	–	–	←	→	←	~↑	–	–	–	⇅	~	←	~	–	–		←	⇅	~	[158]
Rhodomonas sp.	↑25–33 30	←	–	←	–	←	→	~	←	~	→	–	↑↑	←	←	⇅	–	–	–	⇅	←	→	[158]
Cryptomonas sp	↑25–30 25	~	–	~	–	←	~	~	~	~	←	–	←	←	~	–	–	–	–	←	←	→	[158]
prymnesiophyte	↑25–30 25–27	←	–	–	–	←	←	⇅	⇅	–	~	–	←	~	⇅	–	–	–	b	←	←	→	[158]
Isochrysis galbana	↑15–30	←	–	~	–	←	←	–	–	–	–	~	←	←	←	←	~	–	c	←	←	→	[150]
Selenastrum capricornutum	10–25	–	–	–	–	–	–	–	–	–	–	–	–	←	←	–	–	–	–	–	–	–	[276]
Phaeodactylum tricornutum	10–25	⇅	–	–	–	←	←	–	–	–	–	–	→	←	←	~	–	–	b	→	←	→	[277]
Chlorella vulgaris*	20–30	~	~	~	–	~	←	⇅	⇅	–	–	–	⇅	←	⇅	⇅	–	–	–	~	←	→	[278]
Botryococcus braunii*	18–32	←	~	←	–	←	←	←	→	–	–	–	←	←	←	→	–	–	–	←	→	→	[278]
Spirulina platensis* *	30&40	~					→						←	~	→					←	→	→	[278]
Spirulina platensis*	30&40	→	~	→	–	→	←	–	–	–	–	–	←	←			–	–	–	→	←	~	[278]
Chlorella vulgaris*	20&30	~	~	~	–	←	→	–	–	–	–	–	→	~	~		–	–	–	←	~	~	[278]
Botryococcus braunii*	18&32	~	~	~	–	→	→	–	–	–	–	–	→	←	→	~	–	–	–	→	~	~	[257]

A: C22:3, ↑↓,b: C20:5: ↓, c C22:6: ↓↓, bC20:5: ↓↓, *eukaryoticalgae, **.

and fatty acid unsaturation [158].

5.8. Light effects

Earth receives approximately 3.9×10^6 MJ of solar energy each year, however, only a small portion of this radiation is converted to biomass and chemical energy. Algae are photosynthetic organisms and hence light is a vital source for their photosynthetic activity and autotrophic growth. However, microalgae are only capable of using narrow wavelengths (400–700 nm ~ 43% of the total solar energy) of the natural light spectrum. Hence, utilization of suitable light wavelengths and light intensity are the other key factors that can affect or even control the biomass and lipid production in algae. Generally, photosynthesis incorporates light-dependent reactions and dark reactions. In the former phase, light energy is captured and converted to energy carriers and oxygen is released as a by-product of photolysis. The electrons provided by the oxygen during light reactions are transferred to two large proteins including PSI and photosystems II (PSII) [160–162]. Dark reactions, also referred to as the Calvin-Benson (CB) cycle, occur concurrently with the light-dependent reactions and do not require light. The three phase of CB cycle include CO_2 fixation, reduction, and regeneration which are associated with thirteen different enzymatic reactions. Excess light intensities causes photo-oxidation to PSII components and the subsequent photo-inhibition. This phenomenon damages the essential electron transferor proteins during photosynthesis and finally reduces microalgal productivity [163,164]. Generally, high light intensities results in photoinhibition at the initial growth stages, while low light intensities are insufficient for cell growth at the next stages of cultivation (due to self-shading effect). Hence, gradual increase of light intensity till its optimum value improves growth rate.

The light-dark (L-D) cycle is the other important factor, which affects the growth rate of microalgae. The biochemical composition of algae is altered by change of light/dark cycle. As a result, enhanced frequencies of the light-dark cycles can dramatically increase the photosynthetic efficiency and microalgal biomass productivity and vice versa [165]. Wahidin et al. [166] found that the growth rate and lipid content of *Nannochloropsis sp.* in batch cultivation increased by changing the L-D cycle from 12:12–18:06 h. The authors reported that longer light irradiation was better at high light intensities (200 μmol m^{-2} s^{-1}) while shorter light irradiation was better at moderate light intensities (100 μmol m^{-2} s^{-1}).

Generally, microalgal biomass production can increase under red light irradiation while the optimal light wavelength for lipid and carotenoid accumulations are blue and far-red light wavelengths. Chlorophyll a and b are the major light harvesting pigments. These pigments and the algae containing these pigments are sensitive to wavelengths of blue and red light. As an example, green algae, which contain chlorophyll a and b, grow better in blue and red light. In order to enhance microalgal productivity, light wavelength, intensity and light-dark cycles should be adjusted to achieve an optimal balance between photoprotection and photosynthesis. This strategy would also allow for a maximum utilization of natural light irradiation by microalgae. There are two comprehensive review articles on the effect of light on the growth of different algae species [167] and the strategies to improve microalgae productivity by using light irradiation [162].

6. Simulation and modeling of microalgae behavior

The increasing rate of microalgae-based technologies and applications has encouraged the development of new mathematical models to study the simultaneous effect of different factors and variables that influence microalgae growth and allow forecasting of algal production. Studies on modeling of microalgae growth kinetics were initiated with the work by Droop [168,169]. After that, a number of models have been developed based on single factors such as nitrogen [170], temperature

[171], light intensity [172], and photosynthesis and photoinhibition effects [173]. Contrary to other factors (e.g. pH, temperature and nutrient levels) which can be adjusted and maintained to avoid limiting or inhibitory effects, the light intensity cannot be easily controlled at its optimum level. Hence, many researchers have focused on modeling the behavior of biomass as a function of light intensity. Moreover, these models are mainly steady-state, which keep the factors values constant over time. In most recent works, the studies have been focused on more complicated dynamic models that can predict the interactive effect of multi-parameters (e.g. light intensity and nitrogen [174], temperature, light intensity and pH [175]), following a structure according to Monod's or Droop's kinetics models. In contrast to steady-state models, factor values change over time in dynamic models.

On the other side, the interactions between microalgae and bacteria have also been extensively studied and modeled. Research on the growth of microalgae and bacteria in HRAPs started with the pioneering work by Buhr and Miller [176]. Since then more complicated models have been developed such as River Water Quality Model 1 (RWQM1) [177] or Sah Model [178]. RWQM1 models the growth of microalgae and bacteria on nitrogen (N) and phosphorous (P), but it does not include inorganic carbon (C) limiting growth. In Sah model, the effects of nutrients, environmental factors (i.e. temperature, solar radiation, and wind) and physical processes (such as re-aeration) are taken into account. These mechanistic models are mainly based on Monod kinetics. It should also be noted that most of the integrated microalgae-bacteria models do not combine the overall biochemical processes and the simultaneous effects of pH, temperature, light intensity, or high dissolved oxygen (DO) concentration on biomass growth. This weakness has been well-addressed in one of the new integral mechanistic model BIO-ALGAE [179].

7. Biotechnological engineering of microalgae

Biotechnological engineering of microalgae aims at enhancing the lipid content of microalgae which can be achieved by either conventional, genetic engineering and metabolic engineering approaches. Conventional methods include nutrient deprivation, physical stress like temperature, salt stress, and heavy metal stresses etc., as already discussed. In recent years, there has been an increasing interest and expectation on the genetic engineering of microalgae for the generation of strains with high productivity of either fermentable carbohydrates or fatty acids. Improving photosynthetic efficiency by increasing light penetration and decreasing cell shading, engineering and improving different enzymes toward lipid biogenesis and identifying rate-limiting enzymes/committed step are among the most important strategies that have been studied to reach the full potential of microalgae. However, most of such studies have been limited to a few species. Instability and low efficiency of transgenes expression, as well as the lack of suitable promoters, are the main difficulties preventing nuclear transformation of new microalgal strains. Moreover, the use of microalgae as platforms to produce recombinant proteins and the efficient engineering of metabolic pathways has been hampered in some cases due to low expression of exogenous genesis. Hence, it is necessary to develop new tools, which ensure high expression levels and stability of the transgene [1,180].

8. Conclusion

Microalgae-based lipid and biomass are one of the most promising alternatives to biofuel feedstocks, due to their promising sustainable advantages along with the productivity potential compared to traditional terrestrial feedstocks. However, high biomass density (growth) is necessary to increase yield per unit culture area and high lipid content is necessary to reduce the processing costs per unit of biomass product. The proper balance of maximum growth and maximum lipid content against the use of minimum nutrient additions, as much as it can be achieved and supplied, will be the ultimate goal of biofuel production from microalgae.

This work presented a comprehensive review on the mutual and individual effects of nutrients (e.g. nitrogen, phosphorus, iron) and environmental stress (e.g. pH, temperature, NaCl, CO_2) on microalgae lipid content, productivity, growth rate and lipid composition. It summarizes the lipid compositions of the most suitable and widely studied strains as well as the effect of environmental factors on their lipid compositions. Although most researchers have focused on individual effects of the aforementioned parameters, getting benefits from synergistic or antagonistic effects of those factors can lead us toward the best economic strategies to reach the highest lipid productivity. It has been found that nitrogen deprivation with iron supporting can be one of the most effective strategies. Temperature, though is very vital for microalgal life, does not have significant effects on lipid increment. Increment in temperature can even be lethal for microalgae. The other useful strategy is to support the microalgae to reach their maximum density (growth) and apply the environmental stress at the stationary phase. On the other hand, addition of organic carbon sources greatly increases the growth rate of microalgae and the lipid content of some microalgae. Optimized value of carbon source combined with low-nutrition condition greatly improves the microalgae production efficiency and shortens the cultivation cycle.

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