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Bioprocess engineering of microalgae to optimize lipid production through nutrient management

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Abstract Microalgae have been used commercially as a feedstock for the production of high-value compounds, pigments, cosmetics, and nutritional supplements. In addition, because of their rapid growth rates, high photosynthetic efficiency, and high lipid and protein content, commodity products including biodiesel, feed supplements, and polyunsaturated fatty acids derived from algal biomass are of current interest. Since microalgae lack non-photosynthetic structures and float in water, they do not need massive amounts of structural cellulose found in land plants. Thus, under optimal culture conditions, some oleaginous species can allocate up to 70 % of their biomass to lipids. Lipid production and its regulation in microalgae are species-specific and influenced by environmental conditions. Various strategies have been developed to improve lipid productivity and fatty acid composition to meet specific production goals. Manipulation of physiochemical parameters, trophic modes, and nutrient levels, known as process engineering, is a simple approach that leads to desired alterations in the biochemical composition of algal biomass, including lipid quantity and quality. In this paper, we review the effects of manipulating biochemical parameters such as necessary nutrients (C, N, P, S, Fe, and Si), NaCl concentration, and pH of culture medium to optimize lipid content and profile in some algae strains with commercial potential.

Keywords Microalgae · Lipid profile · Growth media · Bioprocess engineering · Micronutrients · Macronutrients

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Introduction

Microalgae are a phylogenetically diverse group of aquatic photosynthetic organisms that vary greatly in their metabolic capabilities, environmental adaptations, and morphologies. Significant characteristics of microalgae with respect to their biotechnological potential include high productivity and autotrophy (i.e., they fix carbon dioxide to produce organic carbon compounds in sunlight). Some species are heterotrophic and/or mixotrophic (i.e., they can assimilate a variety of organic compounds in the dark and in the light, respectively), and they produce variable amounts of storage lipids, primarily as triglycerides. Some algae can accumulate biomass faster than terrestrial plants, and most species store excess carbon as lipids rather than carbohydrates. Significant improvements in several key technologies, including strain selection, best cultivation practices, maintaining selected species in ponding operations, harvesting, and oil extraction, are needed to advance the economics of algae-based commodities. In addition, integrated technologies coupling algal cultivation for lipid production to other applications are gaining interest (Lee et al. 1998). For example, the synergistic combination of wastewater treatment or CO_2 reclamation with lipid production improves the economics of biofuel production (Benemann and Oswald 1996; Woertz et al. 2009; Craggs et al. 2011; Lyon et al. 2015; Taziki et al. 2015).

Microalgae produce a variety of lipids, tri- and di-glycerides, phospholipids, and glycolipids. Algal neutral storage lipids are

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similar in structure and molecular weight (carbon chains ranging from 12 to 22 atoms) to the oils extracted from terrestrial seed plants. Some species, such as the chlorophyte *Botryococcus braunii*, synthesize high levels of hydrocarbon mixtures with carbon chains of up to 38 atoms (Banerjee et al. 2002). Microalgae can have oil contents that vary from 15 to 77 % of their dry weight (Chisti 2007) although the highest reported value has only been achieved after a long period in stationary phase (Borowitzka 2013a). In most algae, lipid biosynthesis is regulated by environmental variables (Flynn and Butler 1986; Roessler 1990; Guschina and Harwood 2013). Lipids are synthesized from photosynthate to serve as membrane components (phospholipids), as metabolites, and as storage products, primarily triacylglycerols (TAGs). Many algal lipids have lower oxygen content and higher H/C ratio and are more calorific than plant oils, suitable traits for biodiesel application (Knothe 2013; Ogbonna and Moheimani 2015). Triglycerides and fatty acids can be converted to biodiesel through transesterification, producing fatty acid methyl esters (FAMEs). FAME composition directly influences the quality of biodiesel. Major characteristics of biodiesel, including viscosity, flash point, and oxidation stability, are affected by the composition and saturation level of FAMEs (Atabani et al. 2012; Knothe 2013). Thus, manipulation of algal lipid profiles during production could have a significant impact on biodiesel quality influencing the economics of the industry (Ogbonna and Moheimani 2015).

Currently, viable commercial algae production systems focus on high-value products for consumption including feed and nutritional supplements such as essential fatty acids rather than on biofuels (Borowitzka 2013b). Microalgae are essential food sources in nature and are used in aquaculture operations in the rearing of molluscs, crustaceans, and small fish and more recently as animal feed supplements (Becker 2007). Some algae species accumulate high levels of long-chain polyunsaturated fatty acids (PUFAs) as TAGs (Sharma et al. 2012). Nutritionally important PUFAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA), have been commercialized for mariculture, pharmaceutical, and therapeutic applications (Milledge 2011). In addition, some of the potentially useful agricultural and pharmaceutical secondary metabolites improve food and feed products for consumption. The proximal composition of algae, however, is species-specific (Brown 1991) and strongly influenced by environmental parameters, including light (Singh and Singh 2015), temperature, and nutrient levels (Herrero et al. 1991; Gatenby et al. 2003). Therefore, increasing lipid content of microalgae and altering their lipid profile for the purpose of optimizing specific lipids used for biodiesel or other commodities is of great importance (Koller et al. 2012). While different strategies such as genetic and transcription factor engineering are being developed for improving lipid quality and quantity (Rasala et al. 2013; Chungjatupornchai et al. 2016; Iskandarov et al. 2016), regulatory concerns and the need to minimize production costs for low-cost commodities dictate the use of highly productive strains that can be stably cultivated in outdoor ponds (Benemann 2013). Large-scale biomass production operations must be concerned with maintaining species composition, biochemical composition, and the environmental influences on both (Borowitzka 2016). One of the simplest strategies is to change environmental parameters and key nutrients, known as bioprocess engineering, which is the focus of this review (Roessler 1988). A brief outline of the topics discussed in this review article is depicted in Fig. 1.

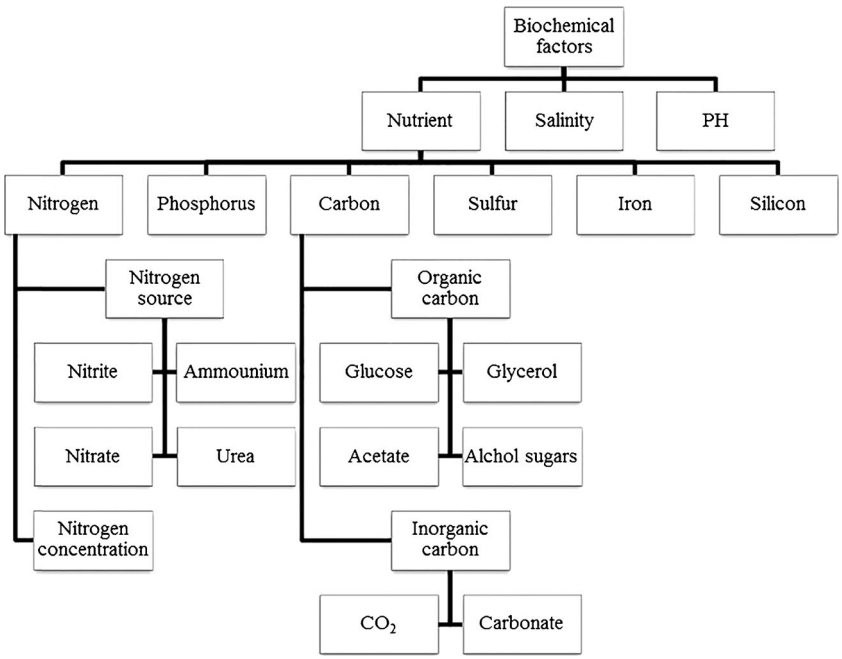

Fig. 1 Biochemical parameters affecting the lipid content and composition of microalgae

Bioprocess engineering

For cost-efficient and sustainable microalgae biomass production schemes, it is important to understand how proximal composition can be optimized for specific applications by controlling environmental parameters. While lipid profile is characteristic of some organisms, microalgae show great inter-specific and intra-specific variation in fatty acid profiles, which can be affected by environmental parameters (Guschina and Harwood 2013). Manipulating the nutritional composition of microalgae culture media to channel metabolic flux generated in photosynthesis into a specific end-product biosynthesis is considered a bioprocess engineering approach. It may include addition, depletion, or changing some components of the cultivation medium (e.g., Roessler 1988; White et al. 2013). This strategy can lead to hyper-accumulation of lipids as well as influencing lipid profiles for specific production goals.

Key parameters determining the economic feasibility of both algae-based biofuels and other products include biomass productivity, lipid content, and lipid productivity. Physiological responses in lipid biosynthesis due to physiochemical culture conditions have a strong influence on lipid content (Roessler 1988, 1990). However, lipid content is usually inversely correlated with overall lipid productivity (Lyon et al. 2015). Griffiths and Harrison (2009) surveyed the literature and found a stronger correlation between biomass and lipid productivity rather than simply lipid content. In addition to lipid productivity, a critical factor from the production perspective is lipid composition. The length of acyl chains and the degree of saturation are key parameters determining biodiesel oxidative stability, performance properties (Knothe 2011, 2013), and the nutritional value of feeds and supplements. Different stresses applied for inducing lipid accumulation are known to also change lipid profile of algae from free fatty acids to TAGs (Widjaja et al. 2009).

Lipid biosynthesis is usually up-regulated under stress conditions, especially nutrient limitation which prevents cell growth and division, resulting in excess photosynthate shunted towards triglyceride accumulation (Illman et al. 2000; Jakobsen et al. 2008; Lv et al. 2010; Griffiths and Harrison 2009;Rodolfi et al. 2009). An inherent disadvantage of using nutrient depletion to trigger the accumulation of lipids in microalgal cells is reducing cell division (Ratledge 2002). The commonly used growth-limiting nutrients (N, P, Fe, etc.) that promote lipid biosynthesis are essential for protein synthesis, energy generation, and photosynthesis and are needed for rapid growth (Courchesne et al. 2009). Therefore, increasing lipid content is usually accompanied by reduction in lipid productivity. To overcome this problem, a two-stage cultivation strategy is commonly suggested in which algal cells are first incubated under optimal growth conditions to produce large amounts of biomass. Subsequently, the biomass is transferred to a stressful medium in which one or more nutrients are deprived to trigger lipid biosynthesis (Koller et al. 2012; Lyon et al. 2015; Vítová et al. 2015).

Nutrients

The proximate or gross composition, i.e., the percentages of protein, carbohydrate, lipid, and mineral, can vary substantially among microalgae. Under conditions where nutrients are not limited, microalgae typically contain from 10 to 40 % of their dry weight (DW) as protein, 10 to 30 % as lipid, 5 to 30 % as carbohydrate, and 10 and 40 % as ash (Renaud et al. 1999; Volkman and Brown 2005). Because of this species variability, it is difficult to categorize algal classes based on proximate composition alone. Microalgae contain anywhere between 2 and 40 % of lipids (oils) by weight, but relatively few make more than 30 % oil. Lipid accumulation in algae typically occurs during periods of environmental stress, including growth under nutrient-deficient conditions (e.g., Procházková et al. 2014). The lipid and fatty acid contents of microalgae also vary with culture conditions.

Nitrogen

Nitrogen (N) is one of the most abundant elements of algal intracellular components and a key constituent of proteins and nucleic acids (Fan et al. 2014) accounting for 1–10 % of DW of most microalgae (Perez-Garcia et al. 2011). Nitrogen content is an important parameter, since in its absence proteins cannot be synthesized. Hence, this macronutrient plays an important role in governing the growth and metabolism of organisms. N limitation has long been known to be a trigger for lipid synthesis in some algal species (Collyer and Fogg 1955; Spoehr and Milner 1949; Huang et al. 2013; Vítová et al. 2015).

Nitrogen sources Nitrogen goes through biogeochemical cycles producing compounds with different oxidation states that are available to phytoplankton: nitrate, nitrite, ammonium, and organic nitrogen compounds including amino acids, urea, and proteins (Taziki et al. 2015) (Fig. 2). Among the three most commonly used N sources in algal media—nitrate, urea, and ammonium—the latter is more readily assimilated than the other nitrogen sources. Ammonium uptake often leads to the repression of nitrate, urea, and organic nitrogen uptake when these compounds are supplied simultaneously (Fernandes et al. 1993; Giordano and Raven 2014). Ammonium is rapidly incorporated into amino acids, and its assimilation is energetically more favorable than nitrate (Perez-Garcia et al. 2011). However, there are toxicity issues associated with high level of ammonium. It dissipates transmembrane proton gradients needed for both respiratory and photosynthetic electron transport mechanisms (Taziki et al. 2015). This sensitivity/toxicity in response to ammonium is in part due to the pH fluctuations. At high pH, ammonium ions are converted to ammonia (Azov and

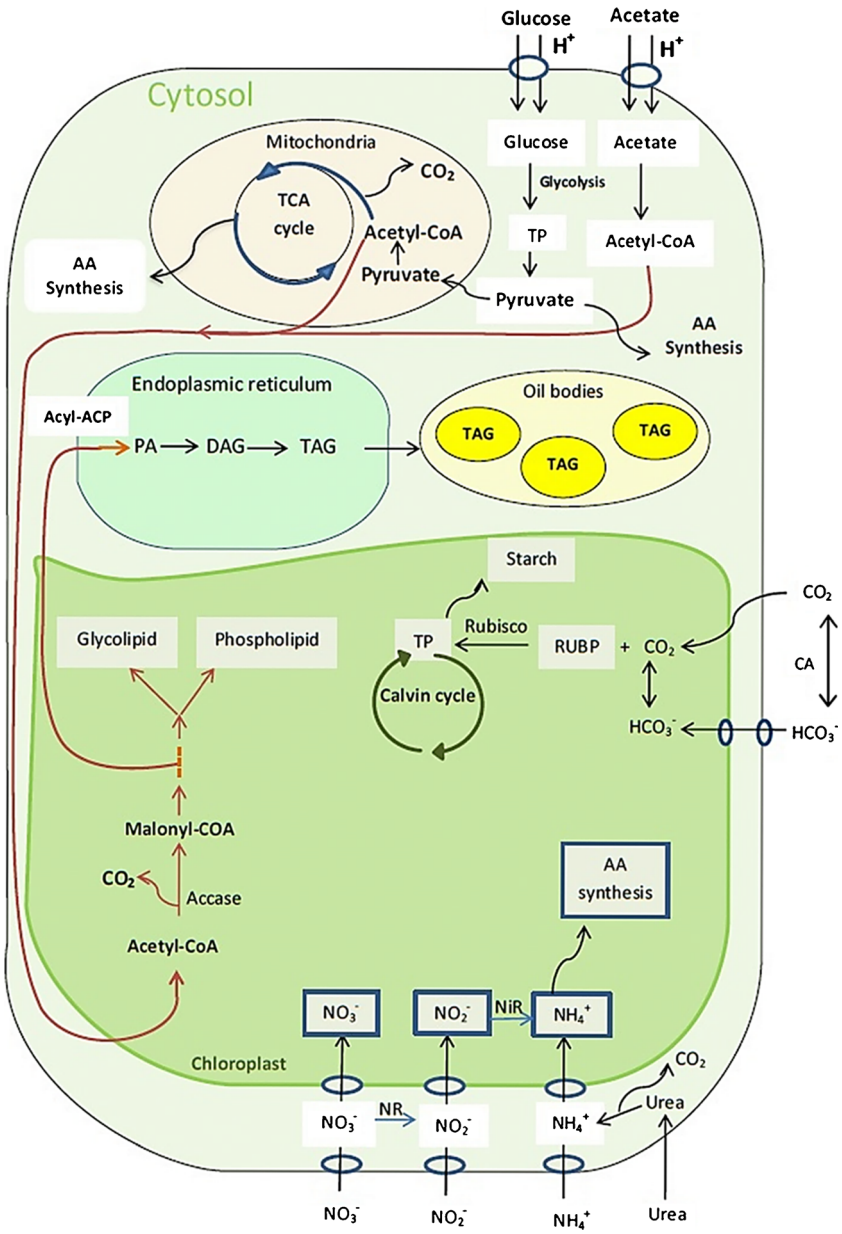

Fig. 2 The assimilation of different nitrogen and carbon sources in algae. *AA* amino acids, *Rubisco* ribulose bisphosphate carboxylase/oxygenase, *RuBP* ribulose 1,5-bisphosphate, *TP* triose phosphate, *CA* carbonic anhydrase, *TCA* tricarboxylic acid, *NR* nitrate reductase, *NiR* nitrite reductase, *ACCase* acetyl-CoA carboxylase, *PA* phosphatidic acid, *DAG* diacylglycerol, *TAG* triacylglycerol, *Acyl-ACP* Acyl-Acyl Carrier Protein. The *ovals* located in membranes indicate the active transport process

Goldman 1982). The inhibitory effects of ammonium may also be related to increasing intracellular pH due to the penetration of undissociated ammonium hydroxide (Giordano and Raven 2014). Ammonium ion also has adverse effects on ribulose bisphosphate (RuBP) concentration and consequently influences photosynthetic carbon fixation (Elrifi et al. 1988). The effect of ammonium ion on RuBP is not a specific reaction between ammonium and RuBP; rather, it is due to the integration of N and C assimilation and a consequence of an overall change in metabolism via the Calvin and Krebs cycles (Huppe and Turpin 1994).

All microalgae are able to assimilate ammonium and, in most cases, nitrate and a variety of other N compounds (Raven and Giordano 2016). While it is generally thought that the presence of ammonium inhibits nitrate, nitrite, urea, and amino acid uptake, there is evidence that in phytoplankton, the uptake and assimilation mechanisms are not as simple or as tightly coupled as previously thought. The inhibitory effects of ammonium on nitrite and nitrate assimilation are due to the products formed during ammonium assimilation, not the effects of ammonium itself (Thacker and Syrett 1972). Syrett and Morris (1963) reported that in *Chlorella vulgaris*, nitrate is not assimilated until ammonium is consumed completely. However, a mixture of oxidized and reduced N forms in some algae species may lead to better growth as a result of lower energy costs of acid–base regulation in N assimilation, due to the simultaneous production of proton and hydroxide ions during the transport of ammonium and nitrate (Giordano and

Raven 2014). Under various environmental conditions, especially light and temperature, and among different algal groups, there is more flexibility in the mechanisms regulating N assimilation (Dortch 1990).

Nitrate assimilation in microalgae is similar to the mechanisms in higher plants, but differences occur due to the evolutionary diversity of microalgae and structural differences between these major taxa (Taziki et al. 2015). However, it must be noted that the research on N metabolism in algae is dispersed and focuses on model organisms. Nitrate reduction to ammonium takes place through sequential reactions involving 2-electron and 6-electron reductions catalyzed by nitrate reductase and nitrite reductase, respectively. Nitrate reduction is highly regulated because of the high energetic cost and because the reactions compete for reducing equivalents with photosynthetic carbon fixation (Buchanan et al. 2000). Urea has long been known to act as an important N source in phytoplankton communities and is suspected to stimulate the formation of harmful algae blooms (Baker et al. 2009). Urease and ATP:amidolyase (UALase) are widespread among the microalgae catalyzing the hydrolysis of urea-producing NH_4^+ and either bicarbonate or CO_2 which are subsequently used by a variety of biochemical pathways including CO_2 fixation (Leftley and Syrett 1973).

The choice of a nitrogen source to promote high biomass production may not support lipid productivity and vice versa. Since the preference of algae for the uptake of different nitrogen sources depends on the expression of specific transporters located on plasma and chloroplast membranes, metabolic responses of algae species to nitrogen source can be species-specific. For example, photosynthesis in *Dunaliella salina* was greater with ammonium than that of nitrate in N replete conditions. In the presence of ammonium, assimilated carbon tended to be allocated to pigments (chlorophyll and carotenoids) and proteins, while nitrate promoted starch accumulation (Giordano et al. 2005). Nitrate has been shown to be the best nitrogen source for both cell growth and lipid production by *Neochloris oleoabundans* and *Isochrysis zhangjiangensis* (Feng et al. 2011; Li et al. 2008). Even though urea and ammonium enhanced the growth of *N. oleoabundans*, they resulted in a 50 % decrease in lipid content compared to the algae grown on nitrate (Li et al. 2008). In contrast, growth and biochemical composition of *Isochrysis galbana* showed no differential response to the N-source including nitrate, nitrite, and urea (Fidalgo et al. 1998). The maximum total lipids in *Scenedesmus* sp. were achieved with peptone as a nitrogen source (Ren et al. 2013).

In addition to influencing lipid content, nitrogen sources also affect lipid composition. The fraction of EPA and PUFAs was strongly increased by the use of urea in *Chaetoceros muelleri* cultures (Liang et al. 2006). In the cultures of *Nitzschia laevis*, ammonium as the sole nitrogen source altered the lipid profile, promoting the synthesis of saturated and monounsaturated fatty acids, whereas nitrate and urea resulted in the synthesis of PUFAs including EPA (Wen and Chen 2001b). EPA content in this diatom was also improved by utilization of tryptone and yeast extract as nitrogen sources (Wen and Chen 2001a). The proportion of PUFA to total fatty acids in *I. galbana* in the presence of nitrate and nitrite was almost the same, while utilization of urea raised this ratio. Urea augmented the percentage of saturated and monounsaturated fatty acids in log phase, whereas in stationary phase, cells showed a decline in these fatty acids and a sharp increase in PUFA (Fidalgo et al. 1998). As summarized in Table 1, the influence of nitrogen substrate on lipid profile can be strain-specific and affect growth rate, lipid content, lipid productivity, and lipid profile in different species.

Nitrogen concentration Nitrogen concentration is regarded as an important factor controlling lipid biosynthesis, and its manipulation leads to remarkable changes in lipid content and the fatty acid profile of microalgae (Vítová et al. 2015). N limitation has long been known as a trigger for lipid synthesis in many algal species (Spoehr and Milner 1949; Collyer and Fogg 1955; Vítová et al. 2015; Negi et al. 2016). In most cases, there is a negative correlation between the nitrogen concentration in the medium and lipid accumulation. For instance, total fatty acid content per cell of *Coccomyxa* showed an 80 % increase under N-deprived conditions (Msanne et al. 2012). In another study, the lipid content of *Chlorococcum* sp. grown in media containing 35.3, 17.7, 8.8, 1.2, and 0.2 mM of nitrate increased significantly from 9, 13, 18, and 32 to 43 % of DW, respectively (Harwati et al. 2012). Nitrogen deficiency limited the protein biosynthesis and increased the lipid/protein ratio in *Nannochloropsis* and *Chlorella* (Converti et al. 2009). In *Scenedesmus* (=*Acutodesmus*) *obliquus*, under nitrogen depletion, the lipid and carbohydrate contents of the algae increased 2- and 1.5-fold, respectively, while the protein content decreased from 41 to 15 % of the biomass (Ho et al. 2012).

Accumulated lipid under nitrogen stress may be due in part to the turnover of non-lipid components (Msanne et al. 2012) by transforming proteins or peptides to lipids or carbohydrates (Siaut et al. 2011). Fatty acid accumulation may also be achieved by the conversion of previously assimilated carbon in the form of starch to nonpolar or neutral lipids (Msanne et al. 2012). Under N limitation, algal strains scavenge nitrogen from photosynthetic pigments and utilize them for metabolic processes. For instance, in *N. oleoabundans* a sharp decrease in chlorophyll content followed by nitrogen limitation was thought to be due to chlorophyll degradation and consumption of freed N for protein synthesis and cell growth (Li et al. 2008). Since chlorophyll and enzymes including ribulose bisphosphate carboxylase/oxygenase (Rubisco), both essential for CO_2 fixation, are greatly diminished when nitrogen is limiting, their degradation may provide carbon skeletons for lipid synthesis (Msanne et al. 2012). For example, in *S. obliquus* and *C. vulgaris* chlorophyll

Table 1 Effect of deletion or changing basic nutrients of culture medium on biomass, lipid content, lipid productivity, and lipid profile of some microalgae in comparison with control condition

No.	Organism	Culture condition	Condition time	Biomass (g/L)	Lipid content (%)	Lipid productivity (mg/L/day)	Lipid profile (%)	Ref.
1	*Dunaliella tertiolecta*	Nitrogen starvation	7 days	NA	NA	NA	SFA 27.0 UFA 73.0	(Chen et al. 2011)
		Control Sodium nitrate (2.3 mM)	7 days	NA	NA	NA	SFA 28.7 UFA 71.3	
2	*Chlorella* sp., BUM11008	Nitrogen starvation	Two phase, 16I–4S	2.52	42.8	53.96	SFA 67.71 MUFA 7.89 PUFA 24.40	(Praveenkumar et al. 2012)
		Control Chu10 medium	20 days	2.58	31.2	40.27	SFA 68.21 MUFA 12.03 PUFA 19.76	
3	*Neochloris oleoabundans*	Nitrogen starvation	Two phase, 7I–6S	1.27	26.65	NA	SFA 25.76 MUFA 49.80 PUFA 22.21	(Popovich et al. 2012)
		Control Sodium nitrate (3.5 mM)	12 days	1.48	14.82	NA	SFA 27.50 MUFA 48.71 PUFA 23.77	
4	*Scenedesmus* sp.	Low nitrate concentration (0.6 g/L)	6 days	3.5	42	NA	NA	(Ren et al. 2013)
		Sodium nitrate (1 g/L)	6 days	3.5	14	NA	NA	
5	*Micractinium pusillum* Y-002	Nitrogen starvation	8 days	0.321	49.9	NA	NA	(Deng et al. 2011)
		Ammonium chloride (0.5 g/L)	8 days	0.341	4.4	NA	NA	
6	*Chlorococcum* sp.	CO_2 (0.04 % *v*/*v*)	6 days	0.53	10.3	5.3	NA	(Harwati et al. 2012)
		CO_2 (6 % *v*/*v*)	6 days	1.32	14.6	19.3	NA	
7	*Chlorococcum* sp.	Acetate (0 mM)	10 days	NA	26.2	10.0	SFA 40 UFA 60	(Harwati et al. 2012)
		Acetate (70 mM)	10 days	0.87	47.2	41.1	SFA 39 UFA 61	
8	Marin *Chlorella* sp.	Glucose (0 g/L)	10 days	<0.5	30	NA	NA	(Cheirsilp and Torpee 2012)
		Glucose (10 g/L)	10 days	3.7	15	NA	NA	
9	*Nannochloropsis* sp.	Glucose (0 g/L)	10 days	<0.5	28	NA	NA	(Cheirsilp and Torpee 2012)
		Glucose (10 g/L)	10 days	3.7	18	NA	NA	
10	*Nannochloropsis oculata* CS 179	Sodium bicarbonate (0 mg/L)	16 days	1.755	(FAME content %) 9.26	FAME productivity (mg/L/day) 9.69	SFA 3.11 MUFA 1.80 PUFA 4.56	(Lin et al. 2012)
		Sodium bicarbonate (400 mg/L)	16 days	1.04	(FAME content %) 11.53	FAME productivity (mg/L/day) 3.21	SFA 2.87′ MUFA 2.14 PUFA 5.85	
11	*Chlorella vulgaris*	Phosphorus starvation	14 days	2	37.7	19.5	SFA 18.87 UFA 81.12	(Chu et al. 2013)
		$K_2HPO_4.3H_2O$ (40 mg/L)	14 days	1.1	37.6	43.17	SFA 19.52 UFA 80.48	
12	*Chlorella protothecoides*	Phosphorus starvation	7 days	2.4	33	190	NA	(Li et al. 2014)
		KH_2PO_4 (1.0 g/L)	7 days	5.9	22	110	NA	
13	*Chlorella* sp., BUM11008	Phosphor starvation	Two phase, 16I–4S	2.46	31.9	39.35	SFA 61.25 MUFA 22.55 PUFA 16.20	(Praveenkumar et al. 2012)
		Control	20 days	2.58	31.2	40.27	SFA 68.21 MUFA 12.03 PUFA 19.76	
14	*Chlorella* sp., BUM11008	Iron starvation	Two phase, 16I–4S	2.54	31.4	39.96	SFA 61.25 MUFA 22.55 PUFA 16.20	(Praveenkumar et al. 2012)
		Control	20 days	2.58	31.2	40.27	SFA 68.21 MUFA 12.03 PUFA 19.76	
15	*Scenedesmus obliquus*	Iron starvation	18 days	0.891	5.75	20.1	NA	(Abd El Baky et al. 2012)
		$FeCl_3$ (20 mg/L)	18 days	1.250	28.12	95.35	SFA 57.17 MUFA 8.12 PUFA 8.12	
16	*Chlorococcum* sp.	NaCl 0 % (*w*/*v*)	10 days	0.60	10.3	6.2	NA	(Harwati et al. 2012)
		2 % (*w*/*v*)	10 days	0.14	29.8	4.0	NA	
17	*Chlorella vulgaris*	NaCl (0 g/L)	7.5 days	5.47	47.71	348	NA	(Duan et al. 2012)
		NaCl (6 g/L)	Two phase, 5I–2.5S	5.47	53.93	393	NA	

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *I-S* incubation days-starvation days, *NA* not available

content decreased, while lipid concentration increased following exhaustion of nitrogen (Piorreck et al. 1984).

Lipids are more energy-rich than carbohydrates, and many algae species under N depletion store assimilated carbon as neutral lipids rather than starch. *Chlorella* species generally accumulate starch under nutrient replete conditions. However, in a low-N medium, *Chlorella emersonii*, *Chlorella minutissima*, *Chlorella vulgaris*, and *Chlorella pyrenoidosa* allocated up to 63, 57, 40, and 23 %, respectively, of their biomass to lipid biosynthesis (Illman et al. 2000). Comparisons of lipid content in exponential and stationary growth phases of many algae reveals a positive correlation between the incubation time and the lipid content of microalgae. As incubation time increases and nitrogen is consumed, cells become N-deficient. Thus, cells in stationary phase are under more nitrogen stress than those in exponential phase. In *C. pyrenoidosa*, at the same concentrations of nitrate, stationary-phase cultures showed higher lipid accumulation than the exponential-phase cells which is consistent with the long-term storage of carbon as lipids (Nigam et al. 2011). The duration of nitrogen deprivation is also an important parameter that can be manipulated to influence the accumulation of lipids and carbohydrates (Ho et al. 2013). Under prolonged environmental stress, starch is often synthesized first as an energy reserve, while lipid is a long-term storage product. During the first 2 days of nitrogen stress, *Chlamydomonas* cells initially accumulated starch up to 14-fold. But, after long incubation of the algae in a nitrogen-deprived medium, total fatty acids and TAG accumulated rapidly, accompanied with starch reduction levels (Msanne et al. 2012). Incubation of *C. vulgaris* for longer periods under nitrogen starvation accumulated more lipids than shorter incubated cells (Widjaja et al. 2009).

Microalgae species demonstrate different physiological behaviors under N-stress. For instance, *Chlamydomonas* and *Tetraselmis* species tend to accumulate starch rather than lipids under N limitation (Yao et al. 2012). *Isochrysis zhangjiangensis* is unusual because nitrogen-replete conditions lead to the overproduction of lipids. Under prolonged nitrogen deficiency, the lipid and protein content of *I. zhangjiangensis* were at the lowest level, whereas the highest carbohydrate content was attained. Thus, a lower protein content due to nitrogen deficiency may result in the activation of carbohydrate synthesis instead of TAG accumulation in this microalgae (Feng et al. 2011). A recent study by Kim et al. (2016) also showed that nitrogen repletion increased lipid content of *Tetraselmis* sp., whereas nitrogen deficiency lowered its lipid percentage. Similarly, Fon-Sing and Borowitzka (2016) showed that several euryhaline strains of *Tetraselmis* produced more lipids in exponential growth than in the stationary growth phase. The cultivation of these strains in high-nutrient wastewaters would be a cost-effective approach to produce biofuels while remediating wastewater.

In most species, nitrogen deficiency not only leads to higher lipid content but can also induce the synthesis of specific lipid classes and lead to fatty acid redistribution in a species-specific fashion (Liu et al. 2012). In general, nitrogen deficiency increases the saturation level of algal fatty acids. Saturated (palmitic acid, stearic acid) and monounsaturated (palmitoleic acid, oleic acid) acids were the major fatty acids up-regulated in *Chlamydomonas reinhardtii* exposed to nitrogen deficiency (Siaut et al. 2011). Comparing nine strains of algae under N-deprived conditions, Breuer et al. (2012) showed that the oleaginous algae had the most prominent changes in fatty acid composition with oleic acid as the most dominant fatty acid. Thus, cultivation of these algae in a medium with nitrogen deficiency can improve the lipid profile for biodiesel production. Palmitic acid was the most abundant fatty acid in *Chlamydomonas* while oleic acid was predominant in *Coccomyxa* under N-limiting conditions (Msanne et al. 2012). Twenty-four-hour interval feeding of nitrogen to *I. zhangjiangensis* resulted in more variation in the fatty acid profile compared to nitrogen-depleted media and enhanced the percentage of unsaturated fatty acids, especially polyunsaturated ones (Feng et al. 2011). As another example, increasing nitrogen level enhanced the fraction of PUFAs in *Tetraselmis* sp. and decreased the percentage of two major fatty acids for biodiesel applications, palmitic acid (16:0) and oleic acid (18:1) (Kim et al. 2016). In contrast, Alonso et al. (2000) reported that monounsaturated and saturated fatty acids accumulated in *Phaeodactylum tricornutum* when nitrogen concentration decreased.

In some microalgae, nitrogen concentration affects the lipid profile more dramatically than lipid quantity. For instance, in *Tetraselmis suecica*, nitrogen starvation did not increase the lipid content but induced important differences in fatty acid composition. It also enhanced neutral lipid proportions about 1.8-fold more than N-sufficient condition (Bondioli et al. 2012). The duration of nitrogen limitation is also an important factor that influences lipid quality. Under longer periods of nitrogen limitation, neutral lipids become the predominant components of cell lipids (Liu et al. 2012). The exposure of *C. vulgaris* to prolonged nitrogen deprivation resulted in increasing the total lipid content and also raised the content of TAGs. As time passed, the lipid composition gradually changed and free fatty acid-rich lipid was replaced by TAG-rich lipid (Widjaja et al. 2009). The ratio of C16 to C18 fatty acids in *Scenedesmus obliquus* reached up to 92.4 % of total fatty acids under nitrogen starvation. After 5 days of nitrogen depletion, oleic acid constituted around one third of total fatty acids in *S. obliquus* (Ho et al. 2012). The optimized nitrogen level that maximizes the concentration of specific fatty acids may reduce the percentage of specific fatty acids. For instance, Breuer et al. (2012) reported that the ratio of valuable PUFAs, EPA, AA, and DHA to the total fatty acids of *P. tricornutum*, *Porphyridium cruentum*, and *I. galbana* decreased in N-deprived conditions, but their concentrations were enhanced due to the overall increase in total lipid content. Thus, in large-scale production operations, certain strain-specific concentrations of nitrogen and specific N compounds need to be determined precisely to optimize the synthesis of desired products.

Carbon

Carbon is a main component of algal biomass that forms the backbone of all structural and fundamental metabolites including proteins, carbohydrates, lipids, and nucleic acids. Therefore, carbon availability is vital to algae growth and metabolism. Due to low CO_2 concentrations in the air (0.04 %) and the limited solubility of CO_2 in water, CO_2 often limits algal growth (Benemann 1997; Vítová et al. 2015; Moreira and Pires 2016). Cyanobacteria and many eukaryotic algae have adapted to the scarcity of CO_2 by developing carbon concentrating mechanisms (Raven et al. 2012) by which inorganic C (C_i) transporters concentrate CO_2, HCO_3^-, and H_2CO_3 inside the cell. Hence, bicarbonate or carbonic acid can affect the algae performance through changing CO_2 availability for Rubisco. In addition to C concentration, C/N ratio has also been reported to act as a regulatory factor in microalgae metabolism. As the C/N ratio increases, nitrogenous compounds are consumed and the accumulation of C storage metabolites occurs (Fidalgo et al. 1995). In some algae, increased lipid content of algal cells under nitrogen deficiency can be attributed to high C/N ratio, rather than the absolute nitrogen concentration (Ogbonna and Moheimani 2015).

Inorganic carbon Microalgae can utilize carbonic acid (H_2CO_3), CO_2, and deprotonated carbonic acid (bicarbonate HCO_3^-) dissolved in water to fuel photosynthetic C assimilation. However, only CO_2 can act as a substrate for Rubisco, the key enzyme involved in CO_2 fixation. This enzyme has both carboxylase and oxygenase activities. With sufficient CO_2, the carboxylase function fixes carbon dioxide. However, in high O_2 levels, oxygen acts as a substrate for Rubisco in a process called photorespiration, and CO_2 fixation rate decreases. Carbonic anhydrase catalyzes the interconversion of CO_2 to bicarbonate and carbonic acid, providing CO_2 to Rubisco (González-Fernández and Ballesteros 2012). Because the affinity of Rubisco for CO_2 is low, CO_2 supplementation is commonly used to stimulate carbon fixation and reduce photorespiration (Raven et al. 2012). In addition, carbon dioxide controls pH during active photosynthesis. It is to be noted that addition of CO_2 to algal cultures must be controlled. The addition of CO_2 results in lowering the culture medium pH. Algae have a range of optimum pH for growth, and any pH lower than optimum pH can negatively affect the growth and productivity of algae. This is the main reason that pH-stat systems (controlling CO_2 addition based on the culture medium pH) must be used in mass algal cultures (Moheimani and Borowitzka 2011).

Diverse microalgae species appear to have different metabolic capacities for adaptation to varying concentrations of CO_2 (Tanadul et al. 2014). It must be noted that increasing CO_2 availability in some algal culture will enhance the production of lipids and fatty acids. A survey of the literature showed that the aeration with CO_2 levels ranging from 1 to 15 % enhanced both biomass production and lipid accumulation to varying degrees depending on the species (Raeesossadati et al. 2014). Moheimani (2012) found that the highest biomass and lipid productivities of *T. suecica* and *Chlorella* sp. were achieved when algae were supplied with CO_2 as inorganic carbon source. Lipid production by *C. vulgaris* increased when the CO_2 level increased from 0.5 to 1 %, but higher concentrations of CO_2 had a negative effect on lipid content (Lv et al. 2010). Enrichment of autotrophic *Nannochloropsis* sp. culture with CO_2 resulted in the highest biomass yield, total lipid content, and PUFAs (Hu and Gao 2003). The fatty acid composition of *C. vulgaris* changed in response to CO_2 concentration where high levels of CO_2 led to enhanced synthesis of unsaturated fatty acids without influencing fatty acid chain length (Tsuzuki et al. 1990). While it is well documented that CO_2 supplementation increases growth rate, there are few studies addressing the influence of inorganic carbon supplementation on the lipid profile of microalgae. The higher solubility of bicarbonate and carbonate in water relative to CO_2 increases the access of algal cells to a carbon source in the medium (Pérez-Pazos and Fernández-Izquierdo 2011). Supplementation of cultivation medium with sodium bicarbonate increased TAG accumulation in *Scenedesmus* sp.WC-1 and *P. tricornutum* strain Pt-1(Gardner et al. 2012). On the contrary, Zhao et al. (2012) reported that the presence of sodium bicarbonate in the culture medium of *Scenedesmus quadricauda* had negative effect on the lipid accumulation and the highest lipid content was obtained under air.

Organic carbon Many algae species are able to utilize organic carbon sources in the presence of light or in the dark (Moheimani et al. 2015). Organic carbon substrates used by algae include glucose, acetate, glycerol, sugars, and sugar alcohols. In some microalgae, lipid biosynthesis is stimulated more by organic carbon in the culture medium than by nitrogen depletion. When the organic carbon is supplied, C availability can exceed cell requirements for growth and the excess carbon is directed towards lipid or carbohydrate synthesis. The addition of organic carbon to the culture medium typically promotes more rapid growth and shifts metabolism from autotrophy to mixotrophy in which the specific growth rate is the sum of autotrophic and heterotrophic metabolism (Perez-Garcia et al. 2011). In mixotrophic cultures, the simultaneous production and assimilation of carbon dioxide during respiration and photosynthesis result in pH stability which may be a reason for the better performance of algae. Furthermore, photorespiration is not seen in mixotrophic cultures due to the simultaneous utilization of dissolved oxygen for the heterotrophic metabolism of organic carbon (Ogbonna and McHenry 2015). With organic carbon supplementation, biomass loss at night declines, presumably because the

respiration of exogenous organic substrates during the dark period preserves photosynthate produced during the day (Chojnacka and Noworyta 2004). There are, however, several drawbacks to supplementing algal cultures with organic carbon, including an increased risk of contamination by heterotroph organisms such as bacteria and fungi. Furthermore, organic carbon supplementation increases the cost of production which is estimated to be about 80 % of the total cost of cultivation medium (Bhatnagar et al. 2011).

A variety of simple sugars including glucose, fructose, galactose, mannose, lactose, and sucrose support the mixotrophic and heterotrophic growth of microalgae (Shi et al. 1999), with species-specific differences in uptake and assimilation (Neilson and Lewin 1974; Sun et al. 2008). Many researchers have succeeded in enhancing the growth and lipid accumulation of algae using inexpensive carbon sources such as sugar cane, cassava, or wastewaters (Cheng et al. 2009; Heredia-Arroyo et al. 2011; Lu 2010). Sugars are catabolized to pyruvate by glycolysis and enter the Krebs cycle via acetyl-CoA, the main building unit for fatty acid synthesis, releasing one molecule of CO_2 from each pyruvate. Thus, the synergistic interaction of sugar catabolism producing CO_2 and acetyl-CoA has been shown to increase algae growth and lipid accumulation (Xiong et al. 2010). However, algae vary in their ability to take up sugars and the physiological responses to specific substrates, due to metabolic differences among microalgae species as well as other culture conditions. For example, lipid accumulation by *Chlorella sorokiniana* in the presence of 5–15 g L^{-1} glucose was augmented, but higher concentrations decreased lipid production (Wan et al. 2011).

Most algae with mixotrophic/heterotrophic capabilities prefer specific carbon substrates for growth and lipid production (Sun et al. 2008). In general, the influence of specific carbon sources on biomass and lipid content in microalgae is more prominent than the effect on lipid composition (Table 1). It should be noted that carbon sources which promote higher biomass do not necessarily induce lipid biosynthesis. There was no significant difference between the lipid content of *Chlorella* (=*Auxenochlorella*) *protothecoides* in mixotrophic cultures with glucose, glycerol, and a mixture of the two (Heredia-Arroyo et al. 2010). Acetate was the preferred substrate for lipid production in *C. vulgaris*, relative to glucose (Heredia-Arroyo et al. 2011). The stimulatory effect of acetate on lipid production is thought to be due to its direct conversion to acetyl-CoA (Perez-Garcia et al. 2011).

The degree of fatty acid saturation is influenced by the presence of exogenous C_o (organic carbon) in the culture medium. Wang et al. (2012) reported that different concentrations of glucose affected the lipid content of *P. tricornutum* without influencing the fatty acid composition. With exogenous acetate, oleic acid (18:1) constituted 41–62 % of total fatty acids in several algal strains while in *Chlamydomonas*, palmitic acid (16:0), and linoleic acid (18:2) were dominant (47–49 %), with only 9–16 % as oleic acid (Park et al. 2012). In contrast, glycerol induced no significant changes in the lipid profile of 14 algal strains (Park et al. 2012). In *Chlorella saccharophila*, *Chlorella vulgaris*, and *Tetraselmis suecica*, several times more lipids were produced under heterotrophic than autotrophic conditions, mainly in the form of triglycerides (Day et al. 1991; Gladue and Maxey 1994; Tan and Johns 1991), while mixotrophic cultures of the diatom *N. laevis* supplemented with glucose increased the production of unsaturated fatty acids (Wen and Chen 2000).

Phosphorus

Phosphorus plays a significant role in most cellular processes especially those involved in generating and transforming metabolic energy via ATP and other high-energy compounds. It also plays a key role in the structure of many biomolecules including nucleic acids, phospholipids, and phosphorus-rich ribosomes (Dyhrman 2016). Phosphorus limitation can induce lipid accumulation of microalgae in a species-specific fashion. In *Nannochloropsis*, lipid content increased to 50 % of DW under P-limitation (Rodolfi et al. 2009). In another experiment, decreasing phosphorus concentration from 150 to 0 % (of P concentration in Guillard medium) enhanced the lipid content of *I. galbana* threefold (Roopnarain et al. 2014). Phosphorus effects are influenced by nitrogen availability because cell growth requires both nutrients. The highest lipid content was achieved in *Chlorella protothecoides* under both N and P deficiency, in comparison with either P or N limitation. However, the maximum lipid productivity was obtained with N deficiency and P availability (Li et al. 2014). The influence of phosphorus elimination on lipid production was similar to nitrogen deficiency in *Parachlorella kessleri* (Li et al. 2013).

Phosphorus concentration also affects the fatty acid profile of some microalgae. According to Reitan et al. (1994), phosphorus deficiency resulted in a reduction in the neutral lipid fraction of *Nannochloris atomus* and *Tetraselmis* sp. In contrast, under growth-limiting phosphate levels, the proportion of phospholipids decreased in *Monodus subterraneus* while TAGs increased six fold (Khozin-Goldberg and Cohen 2006). While N-starvation decreased TAGs containing EPA and ARA, P-starvation had a remarkable stimulatory effect on the proportion of these two fatty acids in *Trachydiscus minutus* (Řezanka et al. 2011). N and P deficiency enhanced total monounsaturated fatty acid content such as C16:1 and C18:1 from 25 to 30 % in *Chlorella*, while the total polyunsaturated fatty acids (PUFAs) decreased significantly (Li et al. 2014). In *S. obliquus*, high concentrations of orthophosphate elevated FAME productivity under nitrogen deficiency (Chu et al. 2014).

Increased fatty acid synthesis has been shown to occur when phosphorus is limited (Siron et al. 1989). When *P. tricornutum* was cultured in a P-deficient medium, the

composition of fatty acids was much like that observed in a senescent batch culture (Siron et al. 1989).

Sulfur

Sulfur is an essential nutrient which is primarily acquired by algae as sulfate (Giordano and Prioretti 2016). Sulfur is a component of sulfolipids and the amino acids cysteine and methionine. Algae may utilize sulfur-containing compounds to control osmosis and influence nitrogen metabolism (Giordano and Raven 2014). Research about the influence of sulfur on lipid content or profile in microalgae is scarce. In comparison to N- or P-limitation, sulfur starvation has less influence on microalgal growth and lipid accumulation. However, the influence of S-starvation on the lipid content of two strains of *C. reinhardtii* was much greater than that of N-deprivation (Cakmak et al. 2012). A comparison of lipid accumulation in eight species of *Chlorella* showed that metabolic responses to a sulfur-deficient medium were species-dependent. Increased lipid content was observed in only two of the eight species: *Chlorella viscosa* and *C. vulgaris* (Takeshita et al. 2014). Sulfolipid concentration in the thylakoid membranes of diatoms was found to be higher than that of other algae taxa (Giordano and Raven 2014). The presence of sulfolipids in thylakoid membranes may be determined by phosphorus availability. Decreased sulfoquinovosyl diacylglycerol levels during sulfur deprivation in *C. reinhardtii* increased phosphatidylglycerol contents two fold, which may represent a compensatory mechanism in which phosphate-containing lipids substitute for lipids that contain sulfur (Sato et al. 2000).

Silicon

Silicon which is considered as an important nutrient for diatoms cultivation is not essential for the growth of other taxa and can be easily omitted from the growth medium of algae without influencing their growth and metabolism. Diatoms require silicon for cell wall (frustule) biosynthesis, and this nutrient is fundamental to the regulation of cell division and lipid accumulation (Perez-Garcia et al. 2011). The presence of silicate in the cell wall of diatoms makes them dense, causing them to sink deep in the water column. Lipid production may be a stress response for the survival of diatoms that increases their buoyancy and allows this non-motile group to rise to the surface to position themselves in optimal light intensities to fuel photosynthesis (Wilhelm et al. 2006). Lipid accumulation can be triggered in diatoms by silicate limitation resulting in a shift in metabolism from cell growth and division to lipid storage (Enright et al. 1986; Taguchi et al. 1987; Wen and Chen 2003). In these organisms, the ratio of carbon to silicon may act as a regulatory factor that determines both the biosynthesis and the composition of lipids. Increased lipid accumulation in *Cyclotella cryptica* under silicon-limited conditions was thought to be due to the enhancement of carbon allocation to lipids or conversion of other products into lipids (Roessler 1988). Variation in silicate concentration changed the lipid profile of *N. laevis*. In this diatom, the percentage of EPA increased when silicate became the limiting growth factor (Wen and Chen 2000).

Iron

Iron may be the most essential micronutrient since it is a constituent of important iron-containing enzymes including peroxidase, nitrate reductase, nitrogenase, and catalase (Marchetti and Maldonado 2016). It plays a role parallel to magnesium in the chlorophyll molecules as iron is in the center of the prosthetic group of cytochromes in respiratory and photosynthetic chains (Rueter et al. 1990). Optimum iron concentration for the growth of microalgae and their responses to iron limitation are species-specific and also depend on the solubility of iron in specific media and the presence of chelating agents. Despite the importance of iron in microalgal growth and metabolism, few studies have focused on the role of iron in the synthesis and/or storage of lipids. As the concentration of iron (Fe_3^+) increased from 0 to 20 mg L^{-1}, the growth rate and lipid content increased in *S. obliquus* (Abd El Baky et al. 2012). No significant increase in the lipid content of *Chlorella* sp.; BUM11008 was observed under iron limitation (Praveenkumar et al. 2012) whereas iron limitation stimulated lipid accumulation in *C. vulgaris* up to 56.6 % of the biomass (Liu et al. 2008). Iron and cobalt deficiency led to lipid accumulation in *Dunaliella tertiolecta* while manganese, molybdenum, and zinc starvation had no influence on its lipid content (Chen et al. 2011).

Salinity

Salinity is a major ecological variable in freshwater, estuaries, and marine ecosystems and seriously affects terrestrial crop production. The harmful effects of salinity on terrestrial plants and algae are associated with ionic, osmotic, nutritional, and oxidative stresses (Mansour 2013; Mansour et al. 2015). Different physiological mechanisms allow algae to tolerate high salt concentration. All marine algae can tolerate up to 0.5 M of NaCl (equal to seawater) while this range of salinity in most freshwater algae has severe impacts on growth (Kirroliaa et al. 2011) presumably because of the energetic cost of salt exclusion or osmoticum synthesis. In addition, salt stress promotes the photoinhibition of photosystem II (PSII) primarily by inhibiting the repair of PSII. Photoinhibition leads to the generation of reactive oxygen species (ROS), which in turn leads to the inhibition of protein synthesis (Nishiyama et al. 2006; Murata et al. 2007; Mansour 2013).

One physiological response of some microalgae to salt stress is the accumulation of intracellular lipid (Takagi et al. 2006; Rao et al. 2007). However, algal responses to salt stress apparently depend on growth phase (Zhila et al. 2011) and the algae

species. For instance, high salinity decreased lipids in *Nitzschia frustulum* (Renaud and Parry 1994), *Cladophora vagabunda* (Elenkov et al. 1996), and *Dunaliella salina* (Al-Hasan et al. 1987). In contrast, Vazquez-Duhalt and Arredondo-Vega (1991) reported that NaCl had no impact on the lipid content of two *B. braunii* strains. In *Isochrysis* sp., *Nannochloropsis oculata* (Renaud and Parry 1994), *Navicula* sp. (Al-Hasan et al. 1990), and *B. braunii* (Ben-Amotz et al. 1985; Rao et al. 2007), high salinity led to the elevation of lipid accumulation.

The stimulating effect of salinity on lipid accumulation can be attributed to osmotic stress (Duan et al. 2012) that may be analogous to nutrient stress in cellular responses. The addition of NaCl to an initial culture medium slowed growth in several studies, but adaptation to high salinity occurred with further incubation (Day et al. 1991; Park et al. 2012; Wang et al. 2012). Increasing initial NaCl concentration from 0.5 to 1.0 M raised intracellular lipid content while an initial NaCl concentration greater than 1.5 M inhibited cell growth (Takagi et al. 2006). By gradually increasing NaCl concentration in *C. vulgaris* cultures at specific growth phases, the negative impact of salt stress on growth and lipid production was minimized (Duan et al. 2012). The addition of low levels of NaCl (2 g L^{-1}) after 80 h of growth, 4 g L^{-1} of NaCl after 100 h during exponential phase, and high level of NaCl (6 g L^{-1} after 120 h) in the stationary phase of *C. vulgaris* maximized lipid yield and minimized the negative effects of salt stress on the growth (Duan et al. 2012).

Fatty acid composition and saturation level in some microalgae also change in response to salinity. The cultivation of *Chlorella* sp. in saline medium slows down the growth and elevates total lipids level, especially the triglycerides (Guckert and Cooksey 1990). The saturation level of membrane fatty acids is significant in the adaptation of algae to harsh environmental conditions, particularly salinity and temperature. In *B. braunii*, alteration in fatty acid composition occurred in response to high salinity, which was thought to be an adaptation to maintain the stability of membrane (Zhila et al. 2011). High NaCl concentration also significantly affected the fatty acid composition of polar lipids in *D. salina* (Peeler et al. 1989). In another study, increasing NaCl concentrations from 0.4 to 4 M in *Dunaliella* cultures enhanced the accumulation of saturated and monounsaturated fatty acids while the fraction of PUFAs declined (Xu and Beardall 1997). Increasing the salt concentration of *B. braunii* LB 572 cultures reduced linoleic acid and augmented oleic and palmitoleic acids proportions (Rao et al. 2007).

pH

pH plays an important role in the physiology of organisms. It determines the ionization degree of chemical compounds and biochemical metabolites, and consequently, it affects their uptake and reactivity. pH influences the availability of inorganic carbon species ($CO_2/HCO_3^-/CO_3^{2-}$) in culture medium and the uptake of essential nutrients such as nitrate and phosphate. Hence, pH fluctuations have severe impacts on microalgal growth and lipid production (Kumar et al. 2010). The optimum pH and the pH range in which microalgae can survive vary among different species. Whereas *Coelastrella* sp. strain PC-3 grew best in neutral pH, *Scenedesmus* sp. (WC-1) preferred alkaline pH values (Gardner et al. 2011). As other examples, *T. suecica* and *Chlorella* sp.can survive when pH range is 6.5–8 and 5.5–8, respectively. The highest lipid productivity was obtained at pH 7.5 and 7 for the strains mentioned above (Moheimani 2013).

The precise mechanism of TAG accumulation due to changing pH levels is not well understood, and it may be related to other parameters of cultivation medium such as nitrogen content besides hydrogen ion concentration. In *Scenedesmus* sp. (WC-1), increasing TAG accumulation in elevated pH (pH > 10) was independent of nitrate depletion (Gardner et al. 2011). pH fluctuation has been reported to modify the lipid composition of microalgae. The cultivation of *Chlorella* CHLOR1 in alkaline pH had a positive effect on TAG accumulation while the fraction of membrane lipids declined. Since alkaline pH inhibited the growth of this strain, it was thought that alkalinity acted to direct carbon to TAG accumulation rather than towards cell growth (Guckert and Cooksey 1990). Increasing pH to 10.0 induced a stressful condition in *N. oleoabundans* that resulted in cell-cycle inhibition and TAG accumulation up to 35 % of the cell DW (Santos et al. 2012).

Conclusion

Algal biomass is considered as an excellent feedstock for the commercial production of biodiesel, as a source of PUFAs for nutritional supplements, and as feed. The selection of algae species and the physiochemical conditions of cultivation are easily manipulated parameters that determine lipid content and profile including C length and saturation level. Understanding the physiological and biochemical responses of a strain to a variety of environmental parameters can be used to shift metabolism towards the production of desired metabolites. A major bottleneck to bioprocess engineering of cultures is that the culture conditions leading to the highest lipid yields often result in the least biomass production and vice versa. High productivity is also advantageous in a two-stage process with the first stage designed to optimize growth followed by a second phase to induce hyper-lipid production (Lyon et al. 2015). Furthermore, in most investigations cited here, the effect of only one component has been investigated: ignoring the point that culture environment is a complicated matrix. The alteration of one nutrient component or concentration may influence the availability of other components, as well. Thus, each strain must be carefully studied in a matrix to assess the best culture conditions for efficient production of specific products.

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