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Microalgal lipids: A review of lipids potential and quantification for 95 phytoplankton species

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ABSTRACT

Phytoplankton have great potential for biodiesel production and offer promises and opportunities in the long term. Phytoplankton species reach higher growth rates, and thus productivity, than conventional forestry or agricultural crops and other aquatic plants. The oil yield in phytoplankton is an order of magnitude larger than terrestrial oleaginous crops. To meet the potential of phytoplankton-based biodiesel there is a need to radically increase lipid yields, which are generally produced under adverse conditions. Nutrients stress and alterations of cultivation conditions are commonly used as lipid enhancement strategies. It is difficult to get a clear picture of the most efficicent factors affecting lipid accumulation and productivity from the abundant literature on this topic, dispatched into a large variety of species and stresses. This article seeks to summarize the widely reported information on TAGs accumulation in phytoplankton and to decipher the regulation mechanisms triggered along the diversity of enhancement strategies. Most of the factors affecting lipid content and composition were analyzed, such as nutrient starvation, temperature, irradiance, salinity, oxidative stress, metals, CO_2 flux, pH and metabolic engineering. In this review, we compiled 213 experiments with lipid analysis, dealing with 95 marine and freshwater phytoplankton (microalgae and cyanobacteria) species. Quantitative indicators (lipid content and productivity), stress level and exposure time, are presented. This review highlights the complexity of comparison between phyla due to differences in culture conditions, analytical methods and/or growth phase. It provides valuable tools for triggering phytoplanktonic lipid biosynthesis and opens the door for enhanced quality and quantity of phytoplankton-based biodiesel.

1. Introduction

The gradual replacement of fossil fuels by renewable energy sources ranks as one of the most challenging problems facing mankind in the short term. Biofuels are expected to offer new opportunities to diversify fuel supply sources and reduce GHG emissions, boosting the decarbonization of transport fuels, increasing the security of food and energy supply and promoting employment in rural areas [1]. One of the most common biofuels is biodiesel, which can replace diesel with little or no modification of vehicle engines. Vegetable oils (edible or non-edible) and animal fats can be used for biodiesel production. There is therefore a conflict with crops, leading to an increase in agricultural food price or pressure for land use change. This can lead eventually to land competition and biodiversity loss. The potential market for biodiesel surpasses by far the availability of plant oils not designed to others markets [2]. In addition, to become a more viable alternative fuel and to survive in the market, biodiesel must compete economically with diesel. This cost depends mainly on the price of feedstocks that accounts for 60–75% of the total cost of biodiesel [2]. To avoid any competition with food crops, biodiesel should be produced from low-cost non-edible oils, such as, frying oils, greases, animal fats and soap-stocks [2]. However, the availability of these wastes is several orders of magnitude below the current demand for biodiesel. Among the possibilities being investigated and implemented at pilot scale, microalgae (eukaryotic) and cyanobacteria (prokaryotic) have the advantage of higher growth rates leading to higher productivity compared to terrestrial plants, with more

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efficient CO_2 fixation [3]. These photosynthetic microorganisms are veritable miniature sunlight-driven cellular factories [4], that capture photons for energy using chlorophyll-a, and various accessory pigments can grow rapidly and live in harsh conditions as efficient CO_2 fixers [3]. They constitute a polyphyletic and highly diverse group of organisms. They are present in all existing ecosystems, mainly aquatic but also terrestrial, such as lakes, springs, ponds, wetlands and rivers [5], representing a large variety of species living in a wide range of environments [2]. They can be soil-less cultivated in non-agricultural lands [6], possibly arid areas with high insolation using seawater, freshwater, brackish or wastewater and also reducing competition for water [3,4]. Several studies demonstrate the ability of microalgae and cyanobacteria to grow effectively in various wastewaters, contributing to water remediation, while the biomass produced can be further exploited [7–10]. Phytoplankton are unicellular, colonial or are constructed of filaments or composed of simple tissues [11]. It is estimated that more than 50,000 microalgae species exist, but only a limited number, of around 30,000 have been studied and analyzed [2]. Cyanobacteria include more than 6000 species in 150 genera and 5 orders [5], more than half of them remaining to be described.

Oil yield is strain dependent, and the productivity of oleaginous species can be much higher than other vegetable oil crops [2], reaching more than 50% of their dry biomass [12], especially when inducing lipid accumulation. Many research efforts have been made to assess the advantages of using microalgae for biodiesel production in comparison to other feedstocks [2]. Each of the three main biochemical fractions (oils, carbohydrates, and proteins) can be converted into biofuels, but lipids represent the highest energy content of the three biochemical fractions.

Microalgae produce a large range of lipids-like compounds, such as glycerolipids, sterols, hydrocarbons and waxes. Glycerolipids are the most abundant and well-described of microalgae lipid classes. These are characterized by a glycerol backbone with one, two or three fatty acids (FAs) groups attached [13]. FAs are one of the major constituents of microalgae biomass and typically make up between 5% and 60% of cell dry weight [14]. Glycerolipids can be divided into two classes based on specific functions: storage and structural lipids. Among structural lipids, membrane lipids are essential for cell and organelle membranes, they usually contain two fatty acid groups and have a polar group bound to glycerol structure. Phospholipids and glycolipids [13,15] are the main representatives of polar lipids while the main form of storage lipids is triacylglycerols (TAGs), with three fatty acid groups attached to the glycerol structure [16].

Fatty acids present in TAGs are targeted for production of transportation biofuels [15]. Lipids from microalgae have a more diversified FAs composition than plant oils [17]. Microalgae mainly make fatty acids with chain lengths of 12, 16 and 18 carbons, but some species can produce fatty acids up to 24 Carbon atoms in length. TAGs mainly contain saturated (SFAs) and monounsaturated fatty acids (MUFAs), such as C14:0 (MA, myristic acid), C16:0 (PA, palmitic acid), C16:1 (POA, palmitoleic acid), C18:0 (SA, stearic acid) and C18:1 (OA, oleic acid), but polyunsaturated fatty acids (PUFAs) can be also present. The long chain PUFAs are most desired in the nutraceutical and food commodities. PUFAs include fatty acids with nutritional benefits such as C20:5 (EPA, eicosapentaenoic acid) and C22:6 (DHA, docosahexaenoic acid) for which no vegetable alternatives exist [15,18]. Some PUFAs present in microalgae lipids are rarely found in plant oils, such as C16:2 (hexadecadienoic acid), C16:3 (hexadecatrienoic acid), C16:4 (hexadecatetraenoic acid) and EPA.

TAG accumulation is motivated by an energy imbalance when phytoplanktonic cells are exposed to an external stress factor, *e.g.,* nutrient starvation perturbing the anabolic processes. Thus, energy demand for anabolism, and in particular for the Calvin cycle, falls lower than the energy supply by the light phase of photosynthesis. This leads to an overreduction of the photosynthetic machinery, causing the formation of damaging reactive oxygen species [19]. TAG is a highly reduced compound, reducing oxidative damage and protecting the photosynthesis process [19]. TAG serve as an alternative energy sink, allowing the cell to continue harvesting light and preventing the formation of oxygen species [19]. TAG prevents an excess accumulation of electrons from the photosynthetic electron transport chain, serving as an electron sink under oxidative conditions [20], as well as maintaining membrane integrity and fluidity [21]. Storage lipids are relative inert and can be packed into vesicles easily [22].

TAGs do not have a clear structural function but are an efficient cellular storage for Carbon and energy during unfavorable conditions. Once favorable conditions are restored, the fatty acyl groups from the TAGs are used to synthesize new membranes and metabolites [23]. In a recent study, concerning to *Dunaliella* sp., enzymes and genes involved in the biosynthesis, catabolism and degradation of fatty acids and TAGs were identified [24].

To be competitive, phytoplankton for biofuels must be cultivated with solar light and grow fast with high lipid content while being tolerant to the wide range of environmental conditions [16], and especially light and temperature fluctuations. They must also be resistant to contamination by other organisms. The choice of phytoplankton species is a key consideration since quantity and quality of lipids highly vary between species. The biochemical composition of phytoplankton is species-specific and is usually regulated by environmental factors. Climatic conditions associated with geographical location, or ecological niche, especially temperature, irradiance play a key role in growth rate and biochemical composition of phytoplankton [25].

Phytoplankton industry is still in its infancy, and the major challenges is to enhance all the steps of the process to make it economically and environmentally sustainable. Reducing the biomass production cost is the main challenge to take advantage of the potential of microalgae and cyanobacteria. On top of identifying new hyper-productive strains, there is a race for enhancing lipid productivity. The seek for strains optimized for high productivity and lipids accumulation motivated metabolic and/or genetic engineering stimulated by environmental factors [26]. Alternative genetic approaches have revealed to be very promising, such as mutation/selection [27,28] or Adaptive Laboratory Evolution (ALE) experiments, based on Darwinian selection to progressively select and improve, through year lasting experiments, the characteristics of the organisms, and especially the lipid content and FA profile [29,30]. However, progress in genetic engineering of phytoplankton was extremely slow until recently [2]. These advances should be viewed with caution because transgenic phytoplankton have tricky legislative drawbacks especially for their potential threat to the ecosystem, especially for outdoor cultivation systems [2]. Moreover, selected metabolic pathways can be also modulated by nutrient deficiency, for instance inorganic Nitrogen or Phosphorus. Nutrients stress and alterations to cultivation conditions are commonly used as lipid enhancement strategies. This review summarizes research on lipid induction in phytoplankton microorganisms and the various abiotic, metabolic, and genetic strategies for improving lipid production. Through the compilation and analysis of data for 95 marine, freshwater and terrestrial microalgae and cyanobacteria species, we provide an extended database on the induction of lipid content/productivity and fatty acids profile for phytoplankton under a wide range of environmental conditions. Most of the factor affecting lipid content and composition were analyzed: i) nutrients starvation (Nitrogen and Phosphorous), ii) temperature, iii) irradiance, iv) salinity, v) oxidative stress, vi) metals, vii) CO_2 flux vii) pH and viii) metabolic and genetic engineering. In addition, conventional and latest trends in lipid enhancement strategies are critically discussed. This review is structured as follows: In the second section, a global overview of the published studies is presented, for the different stress factor and for each phylum. A description of the range of fatty acid profile and saturation that can be found in microalgae are then exposed in the third section together with their influence on the biodiesel quality. The main factors affecting lipid accumulation are reviewed in the fourth section, such as nutrient deprivation, light, and temperature. Other stress factors which attracted

less attention, such as salinity, oxidative stress, CO_2, pH and metals are reviewed in the fifth section. Finally, recent studies exploring how to enhance lipid accumulation by strain selection or metabolic engineering are described in the sixth section.

2. Data acquisition from literature

The data were collected from a wide literature overview for a total of 95 species covering 9 phyla: Cyanobacteria (blue green algae), Rhodophyta (red algae), Chlorophyta, Cryptophyta, Haptophyta, Ochrophyta, Bacillariophyta (diatoms) and Euglenozoa. The classification into classes or phyla is based on various properties such as pigmentation, chemical nature of photosynthetic storage product, organization of photosynthetic membranes and other morphological features [31]. Chlorophyta, Bacillariophyta and Ochrophyta are well represented; however, data on Haptophyta, Cyanobacteria, Rhodophyta, Cryptophyta, Euglenozoa and Dinophyta are scarce. Cyanobacteria generally contain only limited lipid quantities (around 10–12%) [32]. They have however been included, mainly for the studies on the effect of environmental conditions on lipid content. Our dataset included 212 experiments on 95 unique species as shown in Fig. 1. The studies recording lipid content and fatty acid profile under different growth conditions were analyzed. Macromolecular and elemental data for microalgae and cyanobacteria in 117 publications were collected from text, tables and figures. Data were recorded along with the taxonomic information (phylum, genus and species), culture conditions such as temperature, light cycle, irradiance, N-supply, *etc.* and growth phase (exponential or stationary). More than 117 studies with information about N effect (C:N ratio) and 98 studies about other abiotic factors (Phosphorous, oxidative stress, CO_2, pH and metals) affecting lipid content and FAs profile of 37 species were included. Fig. 1 compiles the number of studies by stress factor included in the study.

The data regarding the C:N ratio for 82 species were selected from studies considering different growth phases (exponential and stationary). We focused on marine and freshwater species, with 98 observations under exponentially growth and 92 observations in stationary growth. However, few species have been studied and the data does often not provide the cellular C:N ratio, only reporting lipid content (% DW). For the studies where the C:N ratio was not measured, it was estimated from the percent contribution of macromolecular pools (cell composition: protein, carbohydrate, lipid, and RNA/DNA) using the chemical composition reported by Geider and Roche [33]. These data were thus calculated for each species accounting for the specific experimental condition (temperature, day length and irradiance) which was also included (Table 1). In addition, optimal growth rate, cardinal temperatures (*i.e.*, minimal, optimal and maximal) and optimal irradiance were collected from bibliography for most of the analyzed species (Table 1), to discuss the experimental conditions regarding optimal growth conditions. Fig. 2 shows the number of studies providing information about the N-starvation conditions and the complementary information of optimal growth conditions by specie.

3. Biodiesel quality

Biodiesel consists in fatty acid methyl ester (FAME), produced by the transesterification of biologically derived lipids [2,34]. Thus, lipid composition has considerable influence on the technology of biodiesel production and product quality [35]. The best materials for biodiesel production are TAGs while polar lipids (phospholipids and glycolipids) are deleterious since they cause emulsification and catalyst depletion. Lipids other than TAGs may also reduce the fuel quality by increasing the content of Sulphur and Phosphorus [36].

The unsaturation of FAs profile is crucial for the overall performance of the final biofuel. For instance, biodiesel is mainly constituted of SFAs and MUFAs, since PUFAs decrease the final stability of biodiesel [3]. Carbon chain length and number of double bonds, directly influence the viscosity, ignition quality (cetane number), oxidative stability, and cold-flow property of biodiesel [37,38]. Ignition quality is better for lower fraction of PUFAs. The presence of high fraction of PUFAs, including C18:2 and C18:3, results in a low cetane number, causing a poor ignition quality [39]. PUFAs also cause an increase of viscosity and sediment in biodiesel [40]. Oxidation stability and cold flow performance are known to have inverse relationships to changes in fatty acids composition [41]. For example, the increase in unsaturated fatty acids (MUFAs or PUFAs) would improve the cold flow property, while reducing the oxidative stability. Conversely, increase in SFAs could result in better oxidative stability but poor cold flow property. In addition, SFAs and MUFAs are prone to solidify at lower temperature, thus a certain number of PUFAs can have positive impact on the biodiesel flow properties, especially during winter, in spite of adverse effects on oxidative stability [40]. When the fraction of SFAs is high, it is possible to meet the fuel quality by using some additives, such as a cold flow improver [37]. Short chain FAs containing large proportions of SFAs and MUFAs, are suitable for biodiesel production by increasing energy yield, cetane number and oxidative and thermal stability. Commonly, PA (C16:0), SA (C18:0), OA (C18:1), LA (C18:2) and ALA (C18:3) are strong components candidates for suitable biodiesel production [40].

The types and amounts of fatty acids vary considerably among algae [42]. The relative intensity of individual fatty acids chains is species-specific [43]. The lipid composition by phylum, grown under replete-nutrient conditions, has been characterized [44,45]:

- Bacillariophyta: predominance of POA, PA, EPA and MA.
- Dinophyta: can synthesize high amounts of PA, C18:4, C18:5, EPA and DHA.
- Haptophyta: MA, PA, OA have been reported as their main FAs.

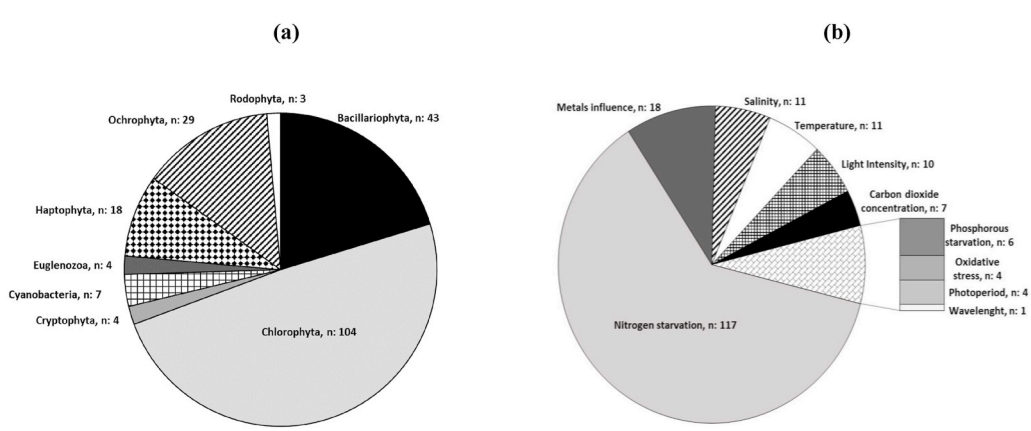

Fig. 1. Number of studies by: (a) phylum and (b) stress factor.

Table 1
Nitrogen starvation effect on lipid accumulation: C:N ratio, experimental conditions (irradiance and temperature), optimal growth rate, cardinal temperatures (*i.e.,* minimal, optimal, and maximal), and optimal irradiance.

Specie/Media[a]		Nitrogen stress effect on lipid accumulation											Optimal conditions for growth							
		N-replete			N-starvation			Experimental conditions		Information about:			Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ_{opt}	Ref.	I_{opt} (μE/m^2s)	Ref.
BACILLARIOPHYTA																				
Amphiprora hyalina	M	**22.1**	-	Exponential NR	**30.2**	-	Stationary ND	80 and 160 [14:10 L:D]	25, 30 and 35	N.D.	X	[172]	20	27.5	35	[173]	1.39	[173]	160	[172]
Amphora sp.	M	**14.0**	6.9	Exponential ND	**24.0**	22.8	Stationary NL	300 [14:10 L:D]	25–28.5	N.D.	N.D	[174]	15	30	40	[175]	0.60	[176]	800	[176]
Amphora capitellata	M	**24.0**	-	Exponential NR	**-**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	-	-	-	-	0.05	[177]	-	-
Biddulpha (odontella) aurita	M	**19.1**	4.5	Exponential ND	**17.2**	10.3	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	−1.5	12	20	[178]	0.81	[117]	-	-
Chaetoceros sp.	F	**11.5**	10.0	Exponential NR	**21.0**	20.0	Stationary NL	70 -90 [24:0 L:D]	23–25	X	X	[25]	5	31	40	[179]	2.50	[179]	230	[180]
Chaetoceros calcitrans	F	**9.9**	5.6	Exponential NR	**10.4**	9.5	Stationary ND	18.5 [24:0 L:D]	20 ± 1	N.D.	N.D.	[181]	10	27–30	30	[182, 183]	2.30	[180]	230	[180]
Chaetoceros calcitrans	F	**16.0**	6.0	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	10	27–30	30	[182, 183]	2.30	[109]	-	-
Chaetoceros muelleri	F	**6.9**	17.8	Stationary NR	**10.9**	19.1	Stationary ND	140-191 [24:0 L:D]	22–30	N.D.	X	[185]	5	27	40	[103]	0.92	[173]	131	[186]
Chaetoceros gracilis	F	**13.7**	6.3	Exponential NR	**28.2**	17.1	Stationary ND	300 [14:10 L:D]	25–29	N.D.	ND	[174]	10	30	40	[109, 187]	1.70	[180]	180	[180]
Chaetoceros gracilis	F	**7.2**	7.0	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	10	30	40	[109, 187]	1.70	[180]	-	-
Cyclotella cryptica	M	**23.0**	5.3	Exponential ND	**36.8**	18.6	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	10	30	35	[173]	1.15	[188]	300	[188]
Cyclotella cryptica	M	**13.2**	-	Exponential NR	**42.1**	-	Stationary ND	80 and 160 [14:10 L:D]	25, 30 and 35	N.D.	X	[172]	10	30	35	[173]	1.50	[188]	-	-
Cyclotella sp.	M	**30.7**	13.9	Exponential NR	**45.5**	27.3	Stationary ND	300 [14:10 L:D]	25–29	N.D.	ND	[174]	−14.8	26.4	28.3	[189]	1.30	[189]	300	[188]
Cyclotella meneghiniana	M	**-**	-	-	**46.0**	-	Stationary NL	146 [16:8 L:D]	N.D	X	N.D	[190]	10	25	30	[191]	-	-	-	-
Cylindrotheca (closterium)	M	**42.0**	-	Exponential NR	**-**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	-	-	-	-	0.97	[192]	-	-
Navicula acceptata	F	**19.2**	-	Exponential NR	**38.2**	-	Stationary ND	80 and 160 [14:10 L:D]	25, 30 and 35	N.D.	X	[172]	20	35	35	[173]	1.31	[173]	160	[172]
Navicula pelliculosa	F	**-**	-	Exponential ND	**44.8**	44.5	-	190 [24:0 L:D]	20–23	N.D.	X	[117]	-	-	-	-	-	-	-	-
Navicula pelliculosa	F	**13.0**	6.6	Exponential NR	**35.0**	16.4	Stationary NL	40 [24:0 L:D]	25	X	N.D.	[48]	-	-	-	-	-	-	-	-
Nitzschia closterium	M	**13.0**	6.4	Exponential NR	**0.0**	-	Stationary NL	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	20	25	35	[193]	-	-	-	-
Ntizschia communis	M	**32.0**	-	Exponential NR	**0.0**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	10	25	35	[173, 194]	0.87	[194]	-	-
Nitzschia dissipata	M	**27.6**	-	Exponential NR	**47.2**	-	Stationary ND	80 and 160 [14:10 L:D]	25, 30 and 35	N.D.	X	[172]	20	25	30	[172, 187]	0.76	[195]	160	[172]
Nitzschia frustulum	M	**26.0**	-	-	**0.0**	-	-	80 [12:12 L:D]	25	X	N.D	[193]	5	28.5	50	[196]	0.18	[197]	-	-
Nitzschia palea	M	**20.0**	-	Exponential NR	**39.5**	25.8	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117, 198]	-	-	-	-	0.14	[199]	25	[199]

(*continued on next page*)

Table 1 (*continued*)

Specie/Media[a]		Nitrogen stress effect on lipid accumulation											Optimal conditions for growth							
		N-replete			N-starvation			Experimental conditions		Information about:			Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ_{opt}	Ref.	I_{opt} (μE/m^2s)	Ref.
Navicula saprophila	F	**16.2**	-	Exponential NR	**34.6**	-	Stationary ND	80 and 160 [14:10 L:D]	25, 30 and 35	N.D.	X	[172]	-	-	-	-	1.32	[172]	160	[172]
Nitzschia thermalis	M	**32.0**	-	Exponential NR	**-**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	-	-	-	-	0.10	[177]	-	-
Phaeodactylum tricornutum	M	**20.0**	-	Exponential NR	**19.0**	-	Stationary NL	60 [24:0 L:D]	17 ± 1	X	N. D.	[200]	−27.7	22.5	25.2	[201]	1.80	[189]	-	-
Phaeodactylum tricornutum	M	**19.9**	6.4	Exponential NR	**23.4**	7.6	Stationary NL	513 - 6780 [solar radiation]	20	X	N. D.	[202]	−27.7	22.5	25.2	[201]	1.80	[189]	-	-
Phaeodactylum tricornutum	M	**0.0**	2.0	Exponential NR	**11.5**	5.3	Stationary NL	60 [24:0 L:D]	15	X	X	[50]	−27.7	23.5	25.2	[201]	1.80	[189]	200	[88]
Phaeodactylum tricornutum	M	**33.0**	-	Exponential NR	**-**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	−27.7	23.5	25.2	[201]	1.80	[189]	200	[88]
Phaeodactylum tricornutum	M	**14.0**	6.0	Exponential NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	−27.7	23.5	25.2	[201]	1.80	[189]	-	-
Phaeodactylum tricornutum	M	**12.0**	9.3	Exponential NR	**20.0**	18.2	Stationary NL	70 -90 [24:0 L:D]	23–25	X	X	[25]	−27.7	23.5	25.2	[201]	1.80	[189]	-	-
Skelotema costatum	M	**23.8**	4.0	Exponential ND	**30.3**	5.2	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	8	24.5	26	[189]	1.72	[83]	309	[83]
Skelotema costatum	M	**10.0**	5.6	Exponential NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	8	24.5	26	[189]	1.72	[83]	-	-
Synedra ulna	M	**23.0**	11.2	Exponential ND	**21.5**	12.6	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	-	-	-	-	0.41	[117]	-	-
Thalassiosira pseudonana	M	**20.4**	5.5	Exponential NR	**42.2**	16.0	Stationary ND	250 [14:10 L:D]	25–27	N.D.	ND	[174]	0	25	32	[203]	1.80	[180]	130	[180]
Thalassiosira pseudonana	M	**19.0**	6.4	Exponential NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	0	25	32	[203]	1.80	[180]	-	-
Thalassiosira weissflogii	M	**22.2**	13.6	Exponential ND	**24.0**	28.6	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	2.9	19.1	26	[204]	1.08	[205]	280	[206]
CHLOROPHYTA																				
Ankistrodesmus sp.	F	**23.4**	7.5	Exponential NR	**38.1**	18.8	Stationary ND	76 [24:0 L:D]	25	X	X	[14]	18	26	31	[187]	2.00	[14]	377.5	[207]
Botryococcus braunii	F	**42.0**	13.3	Exponential NR	**50.0**	16.7	Stationary ND	76 [24:0 L:D]	25	X	X	[14]	5	30	35	[208]	1.25	[14]	850	[208]
Bracteacoccus grandis	F	**12.0**	5.3	Exponential ND	**8.0**	9.7	Stationary NL	60 [24:0 L:D]	17 ± 1	X	N. D.	[200]	2.75	15.3	21	[209]	0.08	[209]	-	-
Chlamydomonas applanata	F	**18.2**	7.1	Exponential ND	**32.8**	23.4	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	-	-	-	-	1.04	[117]	285	[207]
Chlorella ellipsoidea	F	**13.5**	7.2	Exponential ND	**27.2**	24.9	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	20	30	35	[187]	1.87	[187]	250	[210, 211]
Chlorella emersonii	F	**29.0**	10.3	Stationary NR	**63.0**	13.6	Stationary NL	76 [16:8 L:D]	25	N.D.	X	[212]	3	30	38	[213]	2.08	[214]	250	[210, 211]
Chlorella minutissima	F	**19.0**	10.5	Exponential NR	**28.0**	23.2	Stationary NL	130 [14:10 L:D]	20, 25 and 30	X	N. D.	[16]	10	25	45	[215]	0.65	[216]	160	[216]
Chlorella minutissima	F	**31.0**	-	Stationary NR	**57.0**	-	Stationary NL	76 [16:8 L:D]	25	N.D.	X	[212]	10	25	45	[215]	0.65	[216]	-	-
Chlorella protothecoides	F	**11.0**	-	Stationary NR	**23.0**	-	Stationary NL	76 [16:8 L:D]	25	N.D.	X	[212]	-	-	-	-	-	-	-	-
	F	**13.4**	5.3		**29.2**	23.8		190 [24:0 L:D]	20–23	N.D.	X	[117]	5.2	38.7	45.8	[201]	2.00	[201]	275	[189]

(*continued on next page*)

Table 1 (*continued*)

Specie/Media[a]		Nitrogen stress effect on lipid accumulation											Optimal conditions for growth							
		N-replete			N-starvation			Experimental conditions		Information about:			Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ_{opt}	Ref.	I_{opt} (μE/m^2s)	Ref.
Chlorella pyrenoidosa				Exponential ND			Stationary ND													
Chlorella sorokiniana	F	**15.0**	4.5	Exponential NR	**30.0**	16.7	Stationary NL	250 [16:8 L:D]	25	N.D.	N.D.	[217]	13	37	45	[211]	5.90	[211]	450	[211]
Chlorella sorokiniana	F	**20.0**	-	Stationary NR	**22.0**	-	Stationary NL	76 [16:8 L:D]	25	N.D.	X	[212]	13	37	45	[211]	5.90	[211]	-	-
Chlorella vulgaris	F	**12.5**	5.5	Exponential ND	**40.6**	31.3	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	7	32	42	[218]	2.20	[219]	142.1	[220]
Chlorella vulgaris	F	**18.0**	-	Stationary NR	**40.0**	-	Stationary NL	76 [16:8 L:D]	25	N.D.	X	[212]	7	32	42	[218]	2.20	[219]	-	-
Chlorella vulgaris	F	**22.0**	10.9	Exponential ND	**30.0**	21.3	Stationary NL	40 [24:0 L:D]	25	X	N.D.	[48]	7	32	42	[218]	2.20	[219]	-	-
Chlorococcum (oleofaciens & littorale)	F	**12.0**	7.1	Exponential NR	**46.0**	20.0	Stationary NL	300 and 350 [16:8 L:D]	25	N.D.	X	[221]	0	25	30	[222]	2.88	[222]	200	[223]
Desmodesmus sp. F2	M	**9.9**	-	Exponential NR	**53.8**	-	Stationary ND	100–700	25–40	X	X	[224]	5	35	46	[224, 225]	2.53	[226]	700	[226]
Dunaliella primolecta	M	**23.0**	6.6	Exponential NR	**14.0**	11.0	Stationary ND	94660–189000 cal/d [24:0 L:D]	20–23	X	X	[227]	10	30	35	[228]	1.80	[228]	-	-
Dunaliella tertiolecta	M	**21.2**	7.2	Exponential ND	**18.0**	20.8	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	5	32.6	38.9	[189]	1.44	[189]	-	-
Dunaliella tertiolecta	M	**-**	-	-	**64.2**	-	Stationary NL	24 [12:12 L:D]	18	N.D.	N.D.	[229]	5	32.6	38.9	[189]	1.44	[189]	-	-
Dunaliella tertiolecta	M	**15.0**	8.1	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	5	32.6	38.9	[189]	1.44	[189]	-	-
Dunaliella salina	M	**19.0**	-	Exponential NR	**10.0**	-	Stationary ND	76 [24:0L:D]	25	X	X	[14]	-	20	40	[230]	2.50	[14]	-	-
Monoraphidium sp.	F	**11.6**	-	Exponential NR	**17.4**	34.1	Stationary NL	300 [14:10 L:D]	25–29	N.D.	ND	[174]	13	40	70	[231]	1.90	[231]	400	[231]
Nannochloris atomus	M	**11.0**	9.0	Exponential NR	**9.0**	21.3	Stationary NL	70 -90 [24:0 L:D]	23–25	X	X	[25]	5	20.7	32.5	[232]	0.82	[25]	-	-
Nannochloris atomus	M	**21.0**	8.2	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	5	20.7	32.5	[232]	0.82	[25]	-	-
Neochloris (Ettlia) oleoabundans	F	**15.0**	5.6	Exponential NR	**45.0**	16.7	Stationary NL	250 [16:8 L:D]	25	N.D.	N.D.	[217]	5	15	35	[233]	1.20	[234]	180	[235]
Neochloris (Ettlia) oleoabundans	F	**19.0**	-	Exponential NR	**36.0**	-	Stationary NL	60 [24:0 L:D]	17 ± 1	X	N.D.	[200]	5	15	35	[233]	1.20	[234]	180	[235]
Oocystis (polymorpha & submarina)	F	**12.6**	5.4	Exponential ND	**34.7**	44.2	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	5	25	35	[175, 236]	0.60	[207]	109	[207]
Ourococcus sp.	F	**27.0**	6.9	Exponential ND	**49.5**	23.1	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	1.64	27	38	[237]	1.19	[238]	170	[207]
Picochlorum (Nannochloris) sp.	M	**16.0**	6.2	Exponential NR	**28.0**	-	Stationary ND	54 [24:0 L:D]	30	X	N.D.	[34]	15	30	41	[211]	1.80	[211]	250	[210]
Raphidium (Ankistrodesmus) sp.	F	**24.9**	12.4	Exponential NR	**35.3**	33.4	Stationary ND	300 and 150 [14:10 L:D]	25–29	N.D.	ND	[174]	18	26	31	[187]	2.00	[14]	377.5	[207]
Scenedesmus acutus	F	**12.5**	6.8		**10.6**	12.0		50-100 [24:0 L:D]	25	X		[239]	−3.1	26.3	32.7	[189]	0.80	[189]	-	-

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Table 1 (*continued*)

Specie/Media[a]		Nitrogen stress effect on lipid accumulation											Optimal conditions for growth							
		N-replete			N-starvation			Experimental conditions		Information about:			Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ_{opt}	Ref.	I_{opt} (μE/m^2s)	Ref.
				Exponential NR			Stationary NL				N. D.									
Scenedesmus dimorphus	F	**10.0**	7.1	Exponential NR	**34.0**	20.0	Stationary NL	300 and 350 [16:8 L:D]	25	N.D.	X	[221]	−3.1	26.3	32.7	[189]	0.80	[189]	267	[207]
Scenedesmus naegeli	F	**10.0**	5.6	Exponential NR	**39.0**	20.0	Stationary NL	300 and 350 [16:8 L:D]	25	N.D.	X	[221]	−3.1	26.3	32.7	[189]	0.80	[189]	267	[207]
Scenedesmus obliquus	F	**19.0**	5.8	Exponential ND	**41.2**	30.6	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	−3.1	26.3	32.7	[189]	0.80	[189]	267	[207]
Scenedesmus quadricauda	F	**5.4**	5.6	Exponential NR	**10.2**	7.3	Stationary NL	50-100 [24:0 L:D]	25	X	N. D.	[239]	15	30	45	[231]	0.80	[189]	267	[207]
Scotiella sp.	F/ M	**18.0**	12.5	Exponential NR	**34.0**	25.7	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	-	-	-	-	-	-	-	-
Selenastrum sp. *(gracile & minutum)*	F	**20.8**	9.5	Exponential ND	**27.8**	21.0	Stationary ND	190 [24:0 L:D]	20–23	N.D.	X	[117]	0	35	40	[240]	1.73	[240]	400	[240]
Tetraselmis suecica	M	**17.6**	3.0	Exponential NR	**9.2**	6.3	Stationary NL	60 [24:0 L:D]	15	X	X	[50]	10	20	30	[183]	-	-	-	-
Tetraselmis suecica	M	**23.4**	5.3	Exponential NR	**14.6**	10.5	Stationary ND	94660–189000 cal/d [24:0 L:D]	20–23	X	X	[227]	10	20	30	[183]	-	-	-	-
Tetraselmis suecica	M	**10.0**	5.7	Exponential NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	10	20	30	[183]	-	-	-	-
Tetraselmis chui	M	**17.0**	6.7	Stationary NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	-	25	-	[103]	0.7	[103]	-	-
Tetraselmis sp	M	**15.0**	10.7	Exponential NR	**12.0**	16.3	Stationary ND	100 - 120 [24:0 L: D]	20–25	X	N. D.	[241]	2	25	34	[242]	1.66	[25]	-	-
Tetraselmis sp	M	**15.0**	10.3	Exponential NR	**8.0**	18.2	Stationary NL	70 -90 [24:0 L:D]	23–25	X	X	[25]	2	25	34	[242]	1.66	[25]	-	-
CRYPTOPHYTA																				
Chroomonas salina	M	**12.0**	5.9	Exponential NR	**-**	-	-	70 - 80 [12:12 L: D]	20 ± 0.5	N.D.	N. D.	[184]	-	-	-	-	1.10	[219]	-	-
Rhodomonas sp.	M	**14.1**	1.0	Exponential NR	**31.9**	6.9	Stationary NL	60 [24:0 L:D]	15	X	X	[50]	8	16	26	[243]	0.83	[207]	195	[207]
CYANOBACTERIA																				
Anabaena cylindrica	F	**5.0**	17.5	Exponential NR	**3.0**	17.6	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	-	-	-	-	-	-	-	-
Oscillatoria sp.	F	**5.0**	3.8	Exponential NR	**5.0**	7.1	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	-	-	-	-	-	-	-	-
Spirulina maxima	M	**-**	-	-	**6.2**	3.9	Stationary NR	180 [24:0 L:D]	15–45	X	X	[244]	17	33	45	[244]	0.62	[244]	-	-
Spirulina platensis	M	**6.6**	5.4	Exponential NR	**4.7**	4.3	Stationary ND	100 [24:0 L:D]	35 and 42	X	N. D.	[54]	12	34	50	[244]	0.64	[244]	-	-
Synechococcus sp.	M	**-**	-	-	**12.3**	-	Stationary NL	70 [24:0 L:D]	27	X	X	[245]	15	21	26	[246]	2.25	[62]	-	-
EUGLENOZOA																				
Euglena gracilis	F	**13.0**	11.8	Exponential NR	**34.0**	31.7	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	20	29	40	[247]	1.08	[247]	100	[247]
HAPTOPHYTA																				
	M	**20.0**	8.6		**14.3**	30.1		190 [24:0 L:D]	20–23	N.D.	X	[117]	3	25.5	45	[248]	1.20	[249]	-	-

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Table 1 (*continued*)

Specie/Media[a]		Nitrogen stress effect on lipid accumulation											Optimal conditions for growth							
		N-replete			N-starvation			Experimental conditions		Information about:			Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ_{opt}	Ref.	I_{opt} (μE/m^2s)	Ref.
Hymenomonas carterae				Exponential ND			Stationary ND													
Isochrysis galbana	M	**21.9**	9.4	Exponential NR	**38.5**	12.3	Stationary NL	115 [12:12 L:D]	18	X	X	[250]	16	28	34	[103]	1.40	[180]	200	[180]
Isochrysis galbana	M	**14.6**	6.0	Exponential NR	**31.2**	18.7	Stationary NL	60 [24:0 L:D]	15	X	X	[50]	16	28	34	[103]	1.40	[180]	200	[180]
Isochrysis galbana	M	**23.0**	8.7	Exponential NR	**30.0**	17.5	Stationary NL	70 - 90 [24:0 L:D]	23–25	X	X	[25]	16	28	34	[103]	1.40	[109]	200	[109]
Isochrysis galbana	M	**23.0**	7.8	Exponential NR	**0.0**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	16	28	34	[103]	1.40	[109]	-	-
Ochrosphaera (Hymenomonas) sp.	M	**41.0**	-	Exponential NR	**-**	-	-	40 [24:0 L:D]	22 ± 2	X	X	[177]	-	-	-	-	0.08	[177]	-	-
Pavlova (Monochrysis) lutheri	M	**13.0**	6.4	Exponential NR	**32.5**	16.0	Stationary NL	60 [24:0 L:D]	15	X	X	[50]	−5	23	33	[203]	1.30	[203]	100	[180]
Pavlova (Monochrysis) lutheri	M	**22.0**	7.0	Exponential NR	**28.5**	16.7	Stationary NL	70 - 90 [24:0 L:D]	23–25	X	X	[25]	−5	23	33	[203]	1.30	[203]	100	[180]
Pavlova (Monochrysis) lutheri	M	**12.0**	5.9	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[184]	−5	23	33	[203]	1.30	[203]	-	-
Pavlova salina	M	**12.0**	6.1	Exponential NR	**-**	-	-	70 - 80 [12:12 L:D]	25 ± 0.5	N.D.	N.D.	[184]	15	27	30	[183]	-	-	-	-
Prymnesium parvum	M	**16.3**	-	Exponential NR	**9.5**	-	Stationary NL	114.9 [16:8 L:D]	25.7	X	X	[251]	-	-	-	-	0.94	[252]	275	[252]
Tahitian Isochrysis	M	**30.0**	22.5	Exponential NR	**29.5**	32.2	Stationary ND	300 [14:10 L:D]	25–29	N.D.	ND	[174]	10	27.5	35	[193]	1.16	[174]	-	-
Tisochrysis lutea (Isochrysis galbana)	M	**-**	-	-	**35.8**	12.5	Stationary NL	115 [12:12 L:D]	20–23	X	X	[253]	16	28	34	[187]	1.20	[253]	-	-
OCHROPHYTA																				
Heterosigma akashiwo	M	**29.6**	3.1	Exponential NR	**19.8**	1.3	Stationary NL	60 [24:0 L:D]	20	X	X	[50]	4	23	30	[254]	0.57	[254]	-	-
Monallantus (Nannochloropsis) salina	M	**15.0**	5.6	Exponential NR	**48.0**	16.7	Stationary NL	250 [16:8 L:D]	25	N.D.	N.D.	[217]	13	26	36	[211]	1.10	[211]	250	[210]
Monodus subterranea	F	**8.3**	5.3	Exponential ND	**12.5**	29.6	Stationary ND	190 -200 [24:0 L:D]	20–23	X	X	[117, 255]	5	25	35	[256]	1.00	[255]	520	[255]
Monodus subterranea	F	**20.0**	6.7	Exponential NR	**30.0**	17.0	Stationary NL	40 [24:0 L:D]	25	X	N.D.	[48]	5	25	35	[256]	1.00	[255]	-	-
Nannochloropsis oceanica	M	**24.8**	7.8	Exponential NR	**58.7**	27.5	Stationary NL	100 [14:10 L:D]	20	X	X	[4]	−0.2	26.7	33.3	[201]	1.80	[201]	201	[257]
Nannochloropsis oculata	M	**18.0**	6.1	Exponential NR	**45.7**	13.8	Stationary NL	70 - 80 [12:12 L:D]	20 ± 0.5	N.D.	N.D.	[55, 184]	10	25	38	[103, 258]	1.60	[103, 258]	160	[86]
Nannochloropsis oculata	M	**-**	-	-	**40.0**	6.2	Stationary NL	115 [12:12 L:D]	20–23	X	X	[253]	10	25	38	[103, 258]	1.60	[103, 258]	160	[86]
Boekelovia sp.	M	**33.2**	13.6		**23.5**	9.0		300 [14:10 L:D]	25–29	N.D.	ND	[174]	10	23	35	[259]	2.50	[259]	-	-

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Table 1 (*continued*)

Specie/Media[a]		Nitrogen stress effect on lipid accumulation						Experimental conditions		Information about:			Optimal conditions for growth							
		N-replete			N-starvation								Cardinal temperatures (°C)				Growth rate (d^{-1})		Optical Properties	
		% lipid	C:N	Growth condition[a]	% lipid	C:N	Growth condition[a]	Irradiance (μE/m^2/s) Light cycle [L:D]	T (°C)	% Lipid and/or FA	μ	Ref.	T_{min}	T_{opt}	T_{max}	Ref.	μ opt	Ref.	I_{opt} (μE/m^2s)	Ref.
				Exponential ND			Stationary ND													
Tribonema aequale	M	**11.0**	14.9	Exponential NR	**23.0**	36.5	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	0	14	30	[260]	-	-	-	-
RODOPHYTA																				
Porphyridium cruentum	M	**13.0**	7.5	Exponential NR	**6.0**	10.8	Stationary NL	40 [24:0 L:D]	25	X	N. D.	[48]	5.8	19.1	30	[189]	1.30	[189]	-	-
Porphyridium cruentum	M	**9.5**	-	Not determined	**-**	-	-	100 [24:0 L:D]	25	N.D.	N. D.	[261]	5.8	19.1	30	[189]	1.30	[189]	-	-
Porphyridium purpureum	M	**5.3**	-	Not determined	**5.2**	-	Not determined	105.5 [24:0 L:D]	25	N.D.	X	[219]	5	25	35	[262]	1.70	[262]	-	-

[a] M: Marine, F: Freshwater, NR: Nitrogen-replete, ND: Nitrogen-depletion, NL: Nitrogen-limitation. Tmin: minimal temperature for growth, Tmax: maximal temperature for growth, Topt: optimal temperature for growth, Iopt: optimal irradiance for growth. L:D: Light Dark time (in hours). N.D.: Not determined. X: information determined.

- Chlorophyta: PA, C16:4, C18:3 (ALA, alpha linoleic acid) are the main FAs; however, they also include the main species producers of PUFAs, such as EPA and DHA.
- Cryptophyta: have been characterized as producers of PA, SA, C18:2 (LA, linoleic acid), C18:4, EPA and DHA.
- Rhodophyta: high abundance of PA, LA, C20:4 (ARA, arachidonic acid) and EPA.
- Cyanobacteria: high content of POA, LA and ALA.

Hydrotreating or hydrogenation processes can be used instead of transesterification to produce hydrotreated vegetable oils (HVO). The advantages of hydrotreating over transesterification are lower processing cost, and a better yield since most of the microalgal components (lipids, proteins, and carbohydrates), are converted into HVO, therefore with loose constrains on the lipid profile compared to biodiesel. HVO application is however limited for compression-ignition engines by the poor low-temperature properties [46]. Moreover, the high level of Nitrogen in the resulting biofuel is a challenging issue, requiring additional post-treatment to limit the NOx emissions [47].

4. Main factors affecting lipid accumulation and composition

Physical cultivation parameters influence the FAs profile and hence both the quantity and quality of lipids produced, such as light intensity, temperature, nutrient limitation, pH, oxidative stress [15]. Differences in culture conditions, analytical methods and/or growth phase sampled make it complicated to compare the different published results. Even in the same strain, FAs composition variations appear because of different culture media and conditions. After an environmental stress, the physiological state can be affected and the FAs profile eventually modified. The use of FAs profile as a taxonomic tool at species-specific level is possible only when culture conditions are standardized. Comparison of different species can only be indirect and relative to their physiological state [48]. Biochemical composition of phytoplankton is referred to a given state of the culture, describing a particular point in the growth curve. A temporal profile of lipid accumulation, growth rate and C:N ratio at specific time points allow a more accurate calculation of lipid productivity in the growth cycle. Changes in the biochemical composition of a batch culture during the different growth phases must be conditioned in a complex way by a simultaneous exhaustion of the nutrients, and by a progressive accumulation of metabolites in the medium. The study reported by Spoehr and Milner [49] was the first about the cellular composition of microalgae (*Chlorella* sp.) grown under different physiological conditions at different times and indicated a general trend of protein decrease and lipid increase on Nitrogen limitation. In general, microalgae in the exponential growth phase contain more protein, while lipids increase in the later phases of culture; also levels of saturated fatty acids increase during the early stationary phase followed by a slight decrease during the late stationary phase [50]. Generally, stressful conditions are suggested for enriching saturated or mono-unsaturated fatty acids and improving the fuel properties of biodiesel [34]. However, only a few studies have followed the changes in the lipid compositions throughout the different growth phases [50]. Usually, lipid content has been measured at few points, such as before and after Nitrogen deprivation [22]. Thus, Table 1 identified the growth phase (exponential or stationary) to which lipid values were obtained. The knowledge of the biochemical composition of different species at different growth phases allows selecting species with a specific composition and harvest in the appropriate growth stage. Microalgae culture must be harvested (and lipid extracted) before or exactly when the maximal lipid content has been accumulated in the cells, as to avoid harvesting cells when lipid levels start falling during the post-maximal lipid accumulation phase.

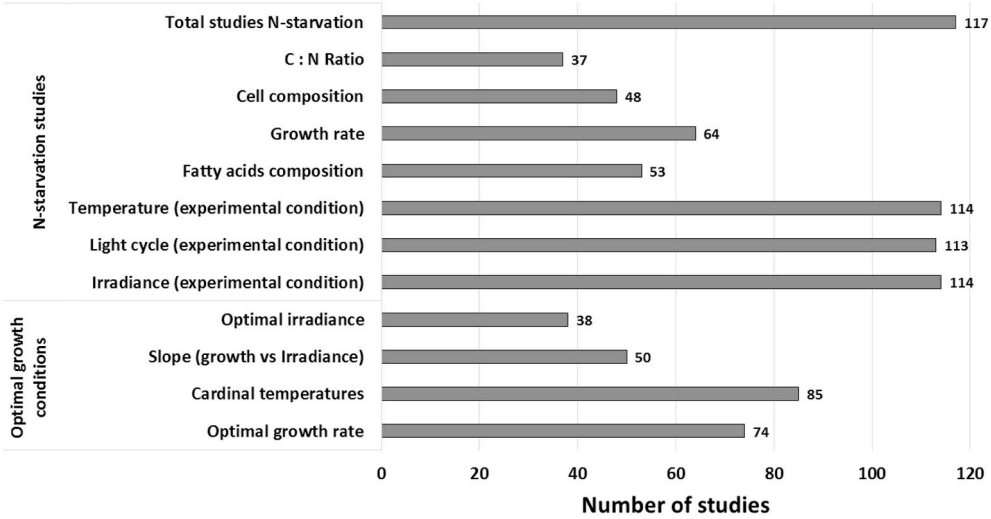

Fig. 2. Number of studies under N-starvation and complementary studies about optimal growth conditions. *Note*: *studies included in Table 1*.

4.1. Nitrogen deprivation

Lipids from algae cultured without stress contain significant amounts of polar lipids (phospholipids and glycolipids) and limited content of TAGs [146,147]. Nutrient deprivation is one of the most widely used and applied TAGs induction techniques for a broad range of phytoplankton species [51]. Nitrogen accounts for 1–10% of the total dry matter in the microalgae [52] and it is the most frequently reported factor in both open and closed systems to enhance lipid accumulation in many species [22,53]. Nitrogen is one of the most cost-effective and easily adjustable factors. *Nitrogen deprivation* causes a decrease in growth, photosynthetic activity, and pigments in many species [54] and induces for some, storage of TAGs [55]. At the same time, it drastically reduces the growth rate, so that the resulting lipid production rate is significantly lower than the product of the maximum lipid content and the growth rate [22].

Nitrogen is required for the biosynthesis of nucleic acids, proteins, and in particular light-harvesting complexes associated with chlorophyll [56]. It is industrially provided as Nitrogen fertilizer (ammonium or nitrate), which not only represents a major cost for microalgae cultivation but also represents a major indirect input of energy. As a consequence, the necessary large amount of Nitrogen necessary to support growth in non-limiting conditions indirectly generates considerable greenhouse gas emissions in the form of CO_2, nitrous oxide, and methane [6]. Reducing the amount of Nitrogen per biomass unit makes therefore also sense both from an economic and environmental viewpoint.

Over 80 years ago, Alfred Redfield discovered an average atomic C:N:P stoichiometry of 106:16:1 in plankton [57]. The Redfield ratio links nutrients availability in the ocean and the elemental composition of plankton. C:N:P ratio reflects their macromolecular composition (protein, lipids, and carbohydrates) of phytoplankton. Protein is the primary reservoir of Nitrogen, while phospholipids and nucleic acids are the major reserves of cellular phosphorous. Carbon is largely determined by the combination of protein, lipid, and carbohydrates. Differences in macromolecular stoichiometry and storage pools, across and within species as a function of changes in environmental conditions, promote changes in the Redfield ratio [58]. There are significant differences in the major macromolecules pools, across the different phylum [58]. These phylogenetic differences in macromolecular stoichiometry predict phylum-level differences in C:N ratio (Table 2).

Major taxonomic groups of phytoplankton differ in their C:N ratio under N-replete and N-deprivation conditions. Under *nutrient replete conditions*, values of C:N range from 3 to 17 mol C:mol N, being mainly distributed about the Redfield ratio, *i.e.,* with C:N of 6.6 [33]. The increase in C:N ratio is the actual indicator to determine if cells are indeed suffering from a Nitrogen limitation [59,60]. Thus, a C:N ratio higher than the values presented in Table 1, for the N-replete condition, can be considered as characteristic of the N-deprivation condition.

Table 2
C:N based on macromolecular composition for different phylum of phytoplankton under nutrient-sufficient exp. growth [58].

Phylum	C:N
Cyanobacteria	6.0
Chlorophyta	6.8
Cryptophyta	7.0
Bacillariophyta	8.3
Haptophyta	7.4
Ochrophyta	8.3
Dinophyta	8.6

The regulating mechanism behind TAGs accumulation in microalgae under Nitrogen deprivation is not elucidated. It results from an imbalance between the energy received and the metabolic energy demand [13]. Under N deprivation, the synthesis of N-rich compounds is no more possible, which deeply impacts cell division. The compounds associated with the light-harvesting processes, including chlorophyll, represent a significant fraction of the intracellular N-pool. They can be remobilized as an alternative N-source to sustain limited growth over a short period [16]. Protein production decreases and carbohydrate and lipid production increase [16]. The larger decrease in the protein content may be due to the degradation and remobilization of proteins to sustain a slower growth. Growth eventually ceases but photosynthetic capacity can be maintained. The accumulation of TAGs during N-stress seems more a consequence of Carbon allocation rather than the induction of genes associated with the lipid biosynthesis pathways. During exponential growth, Carbon allocation is mainly diverted to cell growth and division, while under stressful conditions, there is a switch in Carbon allocation to storage Carbon reserves [16]. When N is depleted, N deficiency promotes the conversion of excess glucose into lipids and leads to a higher lipid transformation rate than cell division rate. The inhibition of cell division without a gradual decrease of lipid transformation results in the accumulation of TAGs in the cells [61]. Another mechanism suggests the repositioning of the chloroplast Nitrogen, leading to mobilization of lipids in chloroplast membranes [62].

Two conditions can be distinguished, depending on the intensity of the Nitrogen deprivation: *Nitrogen limitation* and *Nitrogen starvation*. The

limitation is achieved in continuous cultivation mode, while cells are still growing at a reduced rate given by the rate of Nitrogen uptake. Starvation is generally reached at the end of a batch cultivation mode [13]. Nitrogen limitation is the situation where the production rate of biomass is limited by the rate at which Nitrogen can be consumed. The energy and Carbon imbalance leads to TAGs accumulation while cell division continues [13]. This method of enhancing lipid content is cheap and easy to handle, due to its operational flexibility. Variation in biochemical composition due to growth stage is frequently related to culture age and intensity of Nitrogen limitation [63,64]. Nitrogen starvation is characterized by a phase where Nitrogen is absent in the medium resulting in a minimum Nitrogen quota in the cell [13] associated with a stop of growth [16]. Typically, batch cultures become depleted in nutrients, as they enter stationary stages of growth, with a gradual decrease in Nitrogen quota associated with a protein decline and an increase in total lipid and carbohydrates [65–67]. The lipid accumulation observed in N-starved microalgae may be associated with reaching a critical low concentration of total cell N (Q_{min}, g N/g C) or certain bulk pools of N. It is important to keep in mind that the duration of the N-deprivation is not standardized, and its impact can appear very limited if it does not last long enough [68]. Triggering Nitrogen starvation conditions is the cheapest way to enhance the lipid content of biomass resulting from a first production phase with nonlimiting Nitrogen.

The accumulation of lipids or carbohydrates in living organisms in response to Nitrogen depletion depends on the genetic characteristics of each organism. N-deprivation effects on growth rates and lipid content are species-specific. Different trends can be observed for the lipid content with an increase (Figure A2 in Appendix) but also a decrease for some species, especially for cyanobacteria (Figure A1 in Appendix). This shows that N-deprivation is not a universal strategy for increasing lipid content. For example, under N-deprivation, the lipid content of *Arthrospira* sp. decreases. A proteomic study for this cyanobacterium revealed the upregulation of proteins involved in carbohydrate synthesis and the down-regulation of proteins related to glycogen degradation and inorganic Carbon fixation pathways [69].

Sixty studies were considered stating a lipid content increase due to Nitrogen limitation or starvation (see Table 1). However, it was possible to check the C:N ratio only for half of the studies for which C:N is indeed larger than the minimal one obtained in Nitrogen replete conditions as shown in Table 3. For seventeen studies computations, do not evidence any marked Nitrogen stress (C:N lower than a situation of N-deprivation). Fourteen studies have incomplete information.

The C:N under N-replete condition varied between 5.5 and 7.2. Under N-deprivation the C:N ranges between 15.2 and 28.6 with an average of 15.9 (Table 3). The lipid content average obtained in this study is 17.6% (range between 11.7 and 22.0%) and 34.7% (range between 20.0 and 53.4%), for N-replete and N-deprived condition, respectively.

Table 3
C:N ratio and lipid content (% dry weight) by phylum under N-replete and N-deprivation conditions. The top value is the median, the bottom value in brackets denote the 95% credible interval on the median.

	N-replete condition		N-deprivation		Variation
Phylum (number of studies)	C:N	% Lipid	C:N	% Lipid	% increase lipid
Bacillariophyta (n: 7)	6.3 (5.5, 6.9)	13.7 (12.0, 20.4)	17.1 (16.0, 18.6)	28.2 (20.0, 36.8)	13.8 (8.0, 21.8)
Chlorophyta (n: 14)	5.7 (5.5, 7.1)	13.5 (11.5, 18.4)	21.6 (18.3, 26.3)	36.4 (29.8, 42.2)	22.2 (14.7, 28.3)
Cryptophyta (n: 1)	1.02	14.1	6.9	31.9	17.7
Haptophyta (n: 3)	6.4 (6.0, 7.0)	14.6 (13.0, 22.0)	16.7 (16.0, 18.7)	31.2 (28.5, 32.5)	16.6 (6.5, 19.6)
Ochrophyta (n: 5)	6.1 (5.5, 7.2)	18.8 (11.7, 22.4)	17.0 (15.2, 28.6)	45.7 (21.3, 53.4)	27.7 (7.1, 33.5)

These values match the overview by Finkel et al. [58] who compiled 130 publications under nutrient-sufficient growth conditions. In nutrient unlimited conditions the median macromolecular composition of phytoplankton is 17.3% for lipids, 32.2% for protein, 15% for carbohydrates, 5.6% for RNA, 1.1% for chlorophyll-a, and 0.98% DNA (percent of dry weight). Under the stationary phase of growth (regardless of the factor limiting growth which is often undetermined), the average macromolecular composition is shifted to 22.5% lipids, 27% protein, and 21.8% carbohydrates, without significant difference in ash, chlorophyll-a, or nucleic acid content. These values are similar to the ones reported by Finkel et al. [58].

Most of the studies have described the lipid change through nutrient manipulation, but little research has focused specifically on the changes in FAs profile and distribution between neutral, phospholipids, and glycolipids under different C:N ratios. In general, phospholipids and glycolipids decline, and TAGs and free fatty acids increase under N-deprivation [66,67]. TAGs accumulation is via de novo biosynthesis of fatty acids in the chloroplast and by recycling of membrane lipids, which usually results in the partial collapse of the membrane system [16]. TAGs are deposited in lipid bodies in the cytoplasm [16]. Usually, the FAs profile leads to a decrease in PUFAs [51], *e.g., Chlorella vulgaris* under N -deprivation, shifted from the production of polyunsaturated FAs (C18:2 and C18:3) to saturated or monounsaturated FAs (C18:0 and C18:1) [70].

4.2. Phosphorous deprivation

Phosphorous (P) is indispensable for the structure and function of living organisms [71]. It only accounts for between 0.03 and 0.06% of the total microalgae biomass [72] but is an essential macronutrient for the survival of microalgae [71]. In living systems, P is mainly involved in biology energy transfer mechanisms and cell growth [73]. Phosphorous is also present in cell membranes in the forms of Phosphorous-containing proteins and phospholipids. Phosphate esters constitute the skeleton for the formation of DNA, RNA, and phosphorylated sugars. The high-energy bonds between phosphorous units constitute energy storage in co-factors such as ATP and NADP(H) [74]. The main source of inorganic Phosphorus is phosphate (PO_4^{3-}), but other sources of dissolved organic P are also available in smaller amounts, as phosphate esters, phosphonates and polyphosphates [75].

Microalgae apply physiological and molecular strategies such as phosphorous scavenging or recycling as well as adjusting cell growth to adapt to limiting P concentrations. These strategies also involve adjustments of the Carbon metabolism and lipid biosynthesis [56]. P deficiency affects the normal functioning of phytoplankton cells which require the activation of alternative metabolic pathways [76]. P deficiency decreases the level of photosynthetic phosphorylation, ATP synthesis, and efficiency of the Calvin cycle, affecting chlorophyll synthesis and cell division [77,78]. Cell division drops at low P, leading to a decrease in the requirements for Carbon skeletons for protein and phospholipid biosynthesis. Eventually, more CO_2 is fixed through the Calvin cycle than consumed, and TAGs accommodate the excess of photo-fixed Carbon simultaneously, alleviating the risk of photooxidative damage in the cells [79]. P deprivation induces the accumulation of lipid droplets, indicating the accumulation of TAGs. An excess of Carbon is absorbed continuously by cells, which can enter the Krebs cycle to stimulate TAGs biosynthesis [80]. Under P-limited conditions, the protein involved in metabolic responses such as protein degradation, lipid accumulation, and photorespiration is upregulated while energy metabolism, photosynthesis, amino acid, and nucleic acid metabolism tend to be downregulated [71]. Finally, even if P-deficiency results in a higher lipid yield, limited production of ATP and NADPH (that is required to drive lipid synthesis) over time produces a cessation in lipid synthesis

and its subsequent degradation [73]. Besides, a microalgae response to low P levels is the substitution of phospholipids with non-phosphorous lipids, *i.e.,* the phospholipid content decrease while glycolipid increase [56]. Comparing the performances of different species can only be indirect and relative to their physiological state, Table 4 summarizes the effect of P deprivation on lipid content at different exposure times.

The effect of P on fatty composition varied significantly depending on the algae species. In P- starved cells, El-Sheek and Rady [81] found an enhanced level of unsaturated fatty acids in *Chlorella kessleri*, while Khozin-Goldberg and Cohen [82] found that the content of C20:5, C20:4, C16:0, and C16:1 decrease, while C18:0, C18:1, and C20:3 substantially increase in P- starved cells of *Monodus subterraneus.*

4.3. Irradiance

Irradiance plays a key role for phytoplankton and has been studied a lot, especially in oceanography [83]. The light-driven photo-oxidation process is directly related to Carbon fixation through the Calvin cycle and eventually to growth. The μ-I relationship can be described in terms of three parameters: optimal growth rate (μ_{opt}), initial slope (α), and optimal irradiance (I_{opt}). The maximum growth rate, optimal irradiance, and initial slope are obtained at an optimal temperature of the growth (T_{opt}) [84]. Table 1 shows I_{opt}, $T_{opt,}$ and μ_{opt} estimated for some microalgae species. The optimal irradiance depends on species, varying between 37.5 and 850 μmol photons·m^{-2} s^{-1}, according to Table 1. Higher light intensities generate more energy dissipation by heat (non-photochemical quenching). However, when the optimal irradiance is reached, any further increase in light intensity will damage the microalgae cells due to photoinhibition (which is also affected by temperature). Moreover, at high light an excess of electrons is generated in photosystem II reacting with the photosynthetically produced oxygen, leading to the formation of oxygen radicals [85]. High light generates damages at different levels, but some key proteins, such as the D1 protein in light harvesting complex II are among the first ones to get denatured [55,86].

Minimum light levels still supporting growth for Cyanophyta, Dinophyta and Bacillariophyta have been estimated in the range 5 μmol photons·m^{-2} s^{-1} to 20 μmol photons·m^{-2} s^{-1} for *Chlorophyceae* [87]. However, these values may be misleading, due to the difficulty to measure growth rates at very low irradiance, and μ-I curves often do not include such low-density measurements.

Many studies have focused on the response of photosynthesis (or growth) *vs.* irradiance, fewer works have studied the resulting changes in physiological responses, such as biochemical compositions [88]. Cells grown under saturated light conditions accumulate carbohydrates and TAGs as storage materials, but the optimum level would depend on the microalgae's photosynthetic ability to fully utilize the photo-energy [89]. However, the effect of light intensity on lipid content is contradictory in the literature as shown in Table 5. In Ref. [90], lipid content and lipid accumulation seem to be only affected to a minor extent by light intensity, but other studies [89,91–93] observed that lipid content increases with light intensity with an optimal irradiance for lipid accumulation [93–95]. Light intensity has been shown to have a marginal impact on the FAs composition, increasing the degree of unsaturation with increasing light intensity [90,91]. Alterations of FAs composition to different irradiance might be explained by the fact that these lipids are the main component of chloroplast membranes [96]. An increase in light intensity and light duration (photoperiod) was shown to be related to increased SFAs and decreased MUFAs and PUFAs [96]. SFAs synthesis has been found to require large amounts of photosynthetically produced ATP and NADPH and help in the dissipation of excess light energy, preventing photochemical damage of cells [96]. PUFAs are necessary for the maintenance of photosynthetic membranes function and also play an important role in acclimation to low light conditions [96]. FAs of C16 and C18 series (typical constituents of chloroplasts) shown to be enhanced by illumination for various species such as *Scenedesmus obliquus* [94], *Pavlova lutheri* [97] and *Isochrysis galbana* [98]. Irradiance is not the single factor playing an important role in photosynthesis of microalgae, photoperiod (light-dark periods) and spectral quality (light wavelength) are also crucial [89]. Photosynthesis consist in light reactions when cells are illuminated producing co-factors (such as ATP and NADPH) and electrons to fuel the dark reactions (through the Calvin cycle) occurring independently of light [89]. It has been showed that an increase in light duration at constant light dose has a favorable effect on growth [96], however many studies do not apply a constant daily light dose, so that a longer light period is also a higher supply of energy for the cells. Carbohydrate and lipid are two different ways of storing the energy and Carbon that would later on support cell division during the night [99]. As a consequence of the permanent fluctuation in cell Carbon due to cell division and acquisition during the day, cellular content of protein, carbohydrates and lipids are dependent on the photoperiod [89]. TAGs serve as a sink of excessive energy absorbed by photosynthetic apparatus, dissipating the flux of ATP and NADPH produced in the photosynthesis [91]. Table 5 shows the effect of photoperiod on lipid content. Some studies show that prolongation of dark period increase the lipid content [89,100,101], but an excess dark time also led to decrease total lipid content which is probably re-used in the cell and eventually dissipated through respiration [100,102]. This trend depends on light intensity applied, and therefore probably on the amount of stored lipid.

Table 4
Phosphorous deprivation effect on lipid accumulation.

Stress	Stress Level	Exposure time[a]	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
P deprivation	0%	12 d	*Isochrysis galbana*	47[b]	-	[73]
	25%	12 d		18[b]	-	
	150%	12 d		15[b]	-	
P deprivation	0.1 mg/L	15 d	*Scenedesmus* sp.	53[b]	-	[263]
	1 mg/L	15 d		23,5[b]	-	
P deprivation	16 μM	22 d	*Chlorella* sp.	20,8[b]	12[b]	[264]
	32 μM	22 d		23,6[b]	15.67[b]	
	240 μM	22 d		14[b]	11[b]	
P deprivation	0 g/L	14 d	*Chlorella vulgaris*	37.73	19.5	[265]
	35 g/L	14 d		37.6	43.17	
	0 g/L + N depletion	14 d		54.88	35.02	
P deprivation	0 μM	4 d	*Monodus subterraneus*	15.1 TFA (%DW)	-	[82]
	17.5 μM	4 d		13.4 TFA (%DW)	-	
	52.5 μM	4 d		13.8 TFA (%DW)	-	
	175 μM	4 d		12.9 TFA (%DW)	-	
P deprivation	P-depletion	27 d	*Chlorella zofingiensis*	44.7	44.7	[266]

[a] In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
[b] Approximate values obtained from figures.

Table 5
Irradiance, photoperiod, and wavelength effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
Photoperiod + Light intensity	2.5 Klux L:D = 24:0	21 d	*Chlorella vulgaris*	7*	-	[89]
	2.5 Klux L:D = 16:8	21 d		10*	-	
	2.5 Klux L:D = 12:12	21 d		11*	-	
	2.5 Klux L:D = 8:16	21 d		13*	-	
	5 Klux L:D = 24:0	21 d		8.8*	-	
	5 Klux L:D = 16:8	21 d		10.5*	-	
	5 Klux L:D = 12:12	21 d		12.5*	-	
	5 Klux L:D = 8:16	21 d		13.9*	-	
	2.5 Klux L:D = 24:0	14 d	*Nannochloropsis* sp.	8.1*	-	
	2.5 Klux L:D = 16:8	14 d		10.5*	-	
	2.5 Klux L:D = 12:12	14 d		11.6*	-	
	2.5 Klux L:D = 8:16	14 d		11.1*	-	
	5 Klux L:D = 24:0	14 d		5.8*	-	
	5 Klux L:D = 16:8	14 d		12.36*	-	
	5 Klux L:D = 12:12	14 d		11.6*	-	
	5 Klux L:D = 8:16	14 d			-	
Light intensity	50 μmol photon/m^2/s	12 d	*Scenedesmus* sp.	26.2	-	[91]
	250 μmol photon/m^2/s	12 d		39.2	-	
	400 μmol photon/m^2/s	12 d		41.1	-	
Light intensity	40 μmol photon/m^2/s	12 d	*Chlorella* sp.	22.9	44.05	[92]
	200 μmol photon/m^2/s	12 d		28.7	75.08	
	400 μmol photon/m^2/s	12 d		33	71.85	
	40 μmol photon/m^2/s	12 d	*Monoraphidium dybowskii*	30.7	42.34	
	200 μmol photon/m^2/s	12 d		38.9	85.05	
	400 μmol photon/m^2/s	12 d		43.4	81.81	
Light intensity	200 μmol photon/m^2/s	250 h	*Scenedesmus obliquus*	41*	-	[15]
	500 μmol photon/m^2/s	250 h		42*	-	
	800 μmol photon/m^2/s	250 h		38*	-	
	1500 μmol photon/m^2/s	250 h		42*	-	
Light intensity	60 μmol photon/m^2/s	N.D.	*Scenedesmus obliquus*	11.8*	30*	[94]
	180 μmol photon/m^2/s	N.D.		11*	70*	
	300 μmol photon/m^2/s	N.D.		10*	78*	
	420 μmol photon/m^2/s	N.D.		11.3*	96.5	
	540 μmol photon/m^2/s	N.D.		9.8*	40*	
Photoperiodo + Light intensity	100 μmol photon/m^2/s (Blue LED) L:D = 12:12	13 d	*Chlorella vulgaris*	18	-	[100]
	100 μmol photon/m^2/s (Blue LED) L:D = 16:8	13 d		19.6	-	
	100 μmol photon/m^2/s (Blue LED) L:D = 24:0	13 d		20.8	-	
	200 μmol photon/m^2/s (Blue LED) L:D = 12:12	13 d		23.5	-	
	200 μmol photon/m^2/s (Blue LED) L:D = 16:8	13 d		22.3	-	
	200 μmol photon/m^2/s (Blue LED) L:D = 24:0	13 d		21.7	-	
	300 μmol photon/m^2/s (Blue LED) L:D = 12:12	13 d		16.1	-	
	300 μmol photon/m^2/s (Blue LED) L:D = 16:8	13 d		14.9	-	
	300 μmol photon/m^2/s (Blue LED) L:D = 24:0	13 d		13.3	-	
	200 μmol photon/m^2/s (White fluorescent) L:D = 12:12	13 d		18.5	-	
	200 μmol photon/m^2/s (White fluorescent) L:D = 16:8	13 d		20.9	-	
	200 μmol photon/m^2/s (White fluorescent) L:D = 24:0	13 d		19.3	-	
Wavelength	600 nm (Red light)	5 d	*Chlorella vulgaris*	10.65 TFA (% DW)	-	[267]
	400–700 nm (White light)	5 d		9.69 TFA (% DW)	-	
	450 nm (Blue light)	5 d		11.07 TFA (% DW)	-	
Photoperiod	100 μmol photon/m^2/s L:D = 12:12	9 d	*Nannochloropsis* sp.	25.6	-	[101]
	100 μmol photon/m^2/s L:D = 18:6	9 d		31.3	-	
	100 μmol photon/m^2/s L:D = 24:0	9 d		27.9	-	
Light intensity	135 μmol photon/m^2/s	N.D.	*Isochrysis* sp.	34.2	-	[95]
	140 μmol photon/m^2/s	N.D.		40.3–41.4	-	
	107 μmol photon/m^2/s	N.D.		30.9–35.3	-	
	390 μmol photon/m^2/s	N.D.		34.8–36.6	-	
	620 μmol photon/m^2/s	N.D.		30.7–35.9	-	
	1200 μmol photon/m^2/s	N.D.		34.8–36.9	-	
	107 μmol photon/m^2/s	N.D.	*Nannochloropsis oculata*	33.3–37.9	-	

(*continued on next page*)

Table 5 (*continued*)

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
	100 μmol photon/m^2/s	N.D.		33.5–36.5	-	
	1100 μmol photon/m^2/s	N.D.		30.4	-	
	340 μmol photon/m^2/s	N.D.		22.9–26.3	-	
	243 μmol photon/m^2/s	N.D.		19.7–23.9	-	
Photoperiodo + Light intensity	36 μmol photon/m^2/s L:D = 10:4	12 d	*Chlorella vulgaris*	7 mg/g*	-	[102]
	36 μmol photon/m^2/s L:D = 14:10	12 d		4.6 mg/g*	-	
	36 μmol photon/m^2/s L:D = 24:0	12 d		8 mg/g*	-	
	72 μmol photon/m^2/s L:D = 10:4	12 d		23 mg/g*	-	
	72 μmol photon/m^2/s L:D = 14:10	12 d		19.5 mg/g*	-	
	72 μmol photon/m^2/s L:D = 24:0	12 d		21 mg/g*	-	
	96 μmol photon/m^2/s L:D = 10:4	12 d		22 mg/g*	-	
	96 μmol photon/m^2/s L:D = 14:10	12 d		19 mg/g*	-	
	96 μmol photon/m^2/s L:D = 24:0	12 d		25 mg/g*	-	
	126 μmol photon/m^2/s L:D = 10:4	12 d		27 mg/g*	-	
	126 μmol photon/m^2/s L:D = 14:10	12 d		28.2 mg/g*	-	
	126 μmol photon/m^2/s L:D = 24:0	12 d		25 mg/g*	-	
	36 μmol photon/m^2/s L:D = 10:4	12 d	*Pseudokirchneriella subcapitata*	12 mg/g*	-	
	36 μmol photon/m^2/s L:D = 14:10	12 d		8.7 mg/g*	-	
	36 μmol photon/m^2/s L:D = 24:0	12 d		14 mg/g*	-	
	72 μmol photon/m^2/s L:D = 10:4	12 d		29 mg/g*	-	
	72 μmol photon/m^2/s L:D = 14:10	12 d		27 mg/g*	-	
	72 μmol photon/m^2/s L:D = 24:0	12 d		28 mg/g*	-	
	96 μmol photon/m^2/s L:D = 10:4	12 d		27.5 mg/g*	-	
	96 μmol photon/m^2/s L:D = 14:10	12 d		27 mg/g*	-	
	96 μmol photon/m^2/s L:D = 24:0	12 d		34 mg/g*	-	
	126 μmol photon/m^2/s L:D = 10:4	12 d		33 mg/g*	-	
	126 μmol photon/m^2/s L:D = 14:10	12 d		39.3 mg/g*	-	
	126 μmol photon/m^2/s L:D = 24:0	12 d		35 mg/g*	-	
Light intensity	33 μmol photon/m^2/s	20 d	*Botryococcus braunii*	5.5–26.5	-	[143]
	49.5 μmol photon/m^2/s	20 d		11–29.0	-	
	33 μmol photon/m^2/s + N depletion	20 d		15–31	-	
	49.5 μmol photon/m^2/s + N depletion	20 d		17.5–35	-	
Light intensity	70–150 μmol photon/m^2/s + 15% CO_2	12 h	*Nannochloropsis* sp.	46.1 -> 52.4	-	[93]
	70 to 59.9 μmol photon/m^2/s + 15% CO_2	12 h		46.1 -> 59.9	-	

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.
N.D.: Not determined.

4.4. Temperature

Temperature is an important factor affecting growth rate, Carbon fixation rate, and fatty acid composition [16]. The thermal niche is highly species-specific, but its pattern is common to most of the species [103]. Just above the minimum temperature for growth (named T_{min}) enzymatic activity is reduced and the growth rate low [103]. Temperature enhances growth up to a certain limit (optimal temperature, T_{opt}) where cell mortality appears due to damages in the membrane, and denaturation of proteins involved in the electron transfer chains of photosynthesis. Beyond optimal temperature, growth rapidly drops down to a maximal temperature (T_{max}) where mortality dominates [104]. Temperatures maximal, minimal, and optimal for various species are shown in Table 1. The average temperature by phylum is related to the temperature at the isolation place. Similarities in T_{max} and T_{opt} (see Figure A.3 in Appendix), ranging between 30 °C and 40 °C (except Cryptophyta T_{max} = 26 °C), and between 21 °C and 29 °C (except Cryptophyta T_{opt} = 16 °C), respectively, might result from an over-representation of species issued from temperate areas. The average T_{max} and T_{opt} were 33 °C and 25 °C including all phylum analyzed.

Temperature is also a stress factor that greatly influences lipid productivity and FA profiles in a wide range of microalgae species [105]. The response of microalgae chemical composition to high and low growth temperature varies from species to species. Changes in culture conditions divert the biosynthetic metabolism to lipid synthesis, instead of protein. It has been shown that lipids content is enhanced with increasing temperature [106]. Studies on a large number of species have shown that both low and high temperatures can increase lipid production [107], as shows Table 6. Higher temperatures favor a faster growth rate, with decreased protein content and increased lipid and carbohydrate content [16,108]. Thus, extreme temperatures are preferred for attaining higher lipid profiles.

It is speculated that microalgae modify their fatty acid composition as a strategy to acclimate to change in temperature. Fatty acids are essential in maintaining the integrity and fluidity of the cell membrane phospholipid layer, depending on the degree of fatty acid unsaturation [16]. While many studies have reported on ways to optimize microalgae biomass and lipid productivity, there are fewer reports on the quality of the lipids produced [109], such as the length of the Carbon chain and the number of double bonds. Depending on the species, the levels of unsaturation in FAs increase under low temperatures, whereas those of total saturated FAs increase at high temperatures [110–112], although there are exceptions [16]. Microalgae respond to decreased growth temperature by increasing the ratio of unsaturated to saturated FAs [108]. Temperature is also known to affect carbohydrate production in microalgae, for example in *Spirulina* sp. carbohydrate content increases up to 50% when the temperature was increased from 25 °C to 40 °C [113]. When high-temperature stress was combined with Nitrogen starvation, the freshwater green alga *Scenedesmus obtusus* induced high lipid accumulation up to 47.6% dry weight and alterations of fatty acids by increasing the fraction of saturated fatty acids [114]. Other species such as the microalga *Rhodomonas* sp [115]. and the cyanobacterium *A. platensis* preferred the accumulation of carbohydrates [116].

Table 6
Temperature effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
Temperature	25; 27 °C	N.D.	*Isochrysis* sp.	20.7; 21.7	-	[268]
	25; 33 °C	N.D.	*Prymnesiophyte*	14.7; 13.8	-	
	25; 27 °C	N.D.	*Rhodomonas* sp.	12; 12.7	-	
	25; 30 °C	N.D.	*Cryptomonas* sp.	21.4; 19.6	-	
	25; 35 °C	N.D.	*Chaetoceros* sp.	16.8;12.1	-	
Temperature	25; 20 °C	10 d	*Tetraselmis subcodiformis*	17; 22.25*	-	[112]
	25; 30 °C	10 d	*Nannochloropsis oculata*	20; 24.4*	-	
Temperature	33 °C -> 44 °C	8 d	*Synechocystis* sp.	4.5 -> 3 FAME (%DW)*	12.9 -> 5.9	[269]
	33 °C -> 22 °C	17 d		5 -> 4.2 FAME (%DW)*	9.4 -> 3.4	
Temperature + N-limitation (7 mg N/L)	20; 25; 30 °C	6 d	*Chlorella* sp.	37; 46; 40*	38; 46; 43*	[16]
	20; 25; 30 °C	6 d	*Chlorella minutissima*	30; 34; 35*	25; 35; 45*	
	20; 25; 30 °C	6 d	*Chlorella* sp.	42; 37; 45*	40; 35; 47*	
Temperature	20; 10 °C	10 d	*Thalassiosira pseudonana*	5; 8 pg FAME/cell*	-	[270]
	20; 10 °C	10 d	*Odontella aurita*	800; 600 pg FAME/cell*	-	
	20; 10 °C	12 d	*Nannochloropsis oculata*	1; 2.5 pg FAME/cell*	-	
	20; 10 °C	12 d	*Isochrysis galbana*	4.2; 15 pg FAME/cell*	-	
Temperature	25; 20; 30 °C	N.D.	*Nitzschia closterium*	16; 20; 16*	-	[193]
	25; 10 °C	N.D.	*Nitzschia palea*	14; 21*	-	
	25; 20; 30 °C	N.D.	*Isochrysis* sp.	28; 25; 29*	-	
Temperature	25; 40 °C	N.D.	*Chaetoceros* sp.	20.4; 8.03	20.42; 15.92	[271]
	25; 30 °C	N.D.	*Tetraselmis suecica*	9; 7.5*	25; 27*	
	25; 35 °C	N.D.	*Nannochloropsis* sp.	11.5; 8*	34; 13*	
Temperature	25; 30; 35; 38 °C	14 d	*Chlorella vulgaris*	14.7; 5.9; 6.6; 11.3	20; 8.2; 8.2; 0	[107]
	15; 20; 25 °C	14 d	*Nannochloropsis oculata*	14.9; 7.9; 13.9	9.1; 10; 10.1	
Temperature	25; 20; 30 °C	15 d	*Scenedesmus* sp.	24; 35; 20*	-	[272]
Temperature	13; 25; 37 °C	8 d	*Monoraphidium* sp.	80; 40; 42*	-	[273]
Temperature + P-limitation (0.06 g P/L)	13; 25; 37 °C	8 d		36; 45; 44*	-	
Temperature + N-limitation (0.005 g P/L)	13; 25; 37 °C	8 d		65; 73; 88.2*	-	
Temperature + P-depletion	13; 25; 37 °C	8 d		36; 45; 42*	-	
Temperature + N-depletion	13; 25; 37 °C	8 d		70; 56; 75*	-	
Temperature	13; 25; 37 °C	8 d	*Microcystis aeruginosa*	30; 60; 23*	-	
Temperature + P-limitation (0.06 g P/L)	13; 25; 37 °C	8 d		61; 30; 58*	-	
Temperature + N-limitation (7 mg N/L)	13; 25; 37 °C	8 d		72; 90.65; 80*	-	
Temperature + P-depletion	13; 25; 37 °C	8 d		59; 28; 42*	-	
Temperature + N-depletion	13; 25; 37 °C	8 d		60; 44; 58*	-	
Temperature + N-depletion	17; 25; 32; 35 °C	4 d	*Chlamydomonas reinhardtii*	56*;68*; 76; 74 FAME (% DW)	-	[274]

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.
N.D.: Not determined.

5. Other factors affecting lipid accumulation

Most works have focused on the effects of light, temperature, and N-deprivation on phytoplankton physiology and biochemistry [117]. However, there are other abiotic factors affecting lipid content and productivity, such as salinity, oxidative stress, CO_2, pH, and metals, which are discussed in the sections below.

5.1. Salinity

This factor can affect the growth and biochemical composition of microalgae. Salinity affects microalgae through osmotic stress (restoration of turgor), ion stress, and membrane permeability (regulation of the uptake and export of ions through the cell membrane) [2]. Usually, an increase in the salinity of the medium impacts the FAs metabolism, causing an increase in lipid content as a reserve of energy [118]. NaCl stimulates higher lipid production [111,119–122], with a different range of optimum salinity depending on microalgae species as described in Table 7. However, excess of NaCl in the medium inhibits photosynthesis, reducing biomass and eventually the resulting net lipid productivity [53]. It can also have indirect positive benefits by hindering the growth of competitors and contaminant microorganisms [108]. Salinity stress affects fatty acids profile, enhancing the unsaturated FAs proportion [123], but the changes in FAs profile are species-specific [53]. Salinity is an intricate factor and the correlation between changes in lipid content and salinity has not been well understood and needs to be examined across diverse microalgae strains. The impact of salt on the lipid measurement protocol must also be considered with care. The salinity stress in combination with others factors still needs to be evaluated.

5.2. Oxidative stress

Oxygen (O_2) during its reduction can form partially reduced unstable intermediates termed "reactive oxygen species" (ROS). ROS include the superoxide radicals (O_2^-), hydroxyl radical (^-OH), and hydrogen peroxide (H_2O_2). ROS react with certain molecules and alter or inactive some biochemical activities. Under unfavorable conditions, the generation rate of ROS exceeds their scavenging rate, and resulting intracellular accumulation causes damage by oxidation of cellular components,

Table 7
Salinity effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
Salinity	0.5 M	250 h	*Dunaliella tertiolecta*	60.6	-	[119]
	1 M	250 h		67.8	-	
Salinity	22 PSU	14 d	*Nannochloropsis salina*	18.8	-	[275]
	34 PSU	14 d		37.5	-	
	46 PSU	14 d		21.8	-	
	58 PSU	14 d		26.6	-	
Salinity	0.25 -> 6 g/L	180 h	*Chlorella vulgaris*	47.71 -> 53.93	-	[120]
Salinity	0 mM	20 d	*Chlamydomonas mexicana*	15	-	[118]
	25 mM	20 d		37	-	
	0 mM	20 d	*Scenedesmus obliquus*	18	-	
	25 mM	20 d		34	-	
	0 mM	20 d	*Botryococcus braunii*	17	-	[143]
	86 mM	20 d		13	-	
Salinity	0 mM	18 d	*Botryococcus braunii*	30	-	[121]
	51 mM	18 d		28	-	
Salinity	0 mM	18 d	*Botryococcus braunii*	20	-	[121]
	17 mM	18 d		24	-	
	85 mM	18 d		28	-	
Salinity	0 mM	20 d	*Botryococcus* sp.	6–35.9*	-	[143]
	43 mM	20 d		4–25.8*	-	
	86 mM	20 d		13–19.5*	-	
Salinity + N depletion	0 mM	20 d	*Botryococcus* sp.	12 - 30*	-	
	43 mM	20 d		18*	-	
	86 mM	20 d		5 - 22*	-	
Salinity	2.5% (w/v)	13 d	*Dunaliella salina*	22.4	-	[276]
	22% (w/v)	13 d		13.1	-	
Salinity	13.5 g/L	96 h	*Nannochloropsis* sp.	23*	75*	[277]
	27 g/L	96 h		22*	68*	
	54 g/L	96 h		42*	60*	
	81 g/L	96 h		37*	15*	
Salinity	0.05 M	15 d	*Scenedesmus obliquus*	15*	-	[122]
	0 M	15 d		9.5*	-	
	0.2 M	15 d		21*	-	
	0.3 M	15 d		36*	-	

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.

inhibiting growth and can even resulting in cell death [124]. This biological effect is known as "oxidative stress". ROS production is stimulated by a broad range of stressing factors [125] such as extreme light, high temperatures, heavy metals, UV radiation, ozone, water stress, herbicides, invasion by pathogens, among others. Superoxide radical is unique among the ROS species, being able to act as oxidant and reductant [125]. Hydroxyl radical is among the most reactive ROS highly reactive with organic molecules. Hydrogen peroxide can readily diffuse across biological membranes, whereby causing oxidative stress in many sites even far from its formation.

Microalgae have a ROS-scavenging ability as a protection mechanism, including an array of antioxidants like superoxide dismutase, catalase, ascorbate peroxidase, and various non-enzymatic scavengers such as ascorbate, glutathione, carotenoids, tocopherols. proline and polyphenols [126,127].

Lipid accumulation is also likely to be linked to oxidative stress induced by ROS accumulation [126,128] as shown in Table 8. There is a limited explanation about the correlation between ROS production and

Table 8
Oxidation stress effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
Oxidative stress	N-depletion	2 d	*Acutodesmus dimorphus*	24.3 -> 29.9	-	[126]
Oxidative stress	$NaNO_3$: 5 mM -> 0.05 mM	10 d	*Dunaliella salina*	25 -> 35*	-	[128]
	H_2O_2: 200 μM -> 4 mM	10 d		25 -> 44*	-	
Oxidative stress	TiO_2: 0 g/L -> 0.1 g/L	2 d	*Chlorella vulgaris*	10 -> 11.35 *	17 ->18*	[127]
Oxidative stress	NH_4^+: 7 -> 0.07 mol/m^3	14 d	*Chlorella luteoviridis*	27.7 -> 55*	210 - > N.D.	[131]
	PO_4: 1 -> 0.001 mol/m^3	14 d		27.7 ->60*	210 - > N.D.	
	NH_4^+: 7 -> 0.07 mol/m^3	14 d	*Parachlorella hussii*	35.7 ->70*	300 - > N.D.	
	PO_4: 1 -> 0.001 mol/m^3	14 d		35.7 ->72*	300 - > N.D.	

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.
N.D.: Not determined.

lipid accumulation in microalgae. ROS are involved in the stress-response signal transduction pathway. They are considered as an important factor in the cellular response, *e.g.,* the induction of lipid accumulation by applying exogenously H_2O_2 resulted in increased lipid accumulation, even more effective in lipid production compared to N-starvation [128]. Although a few recent studies have suggested ROS levels and cellular lipid accumulation, underlying mechanism principles are not clear [128]. Some authors state that lipid accumulation is an indirect consequence of the reduction in cell division rate [129]. A direct effect is also hypothesized, where neutral lipid and especially C18 FAs accumulation is a protection mechanism [130]. The modification of the lipid profile has been documented [126] while some fatty acids act as an anti-oxidant defense. Usually, total C18 FAs (and C 16 in a lower proportion) including saturated forms are accumulated, suggesting that they play an important role in the protection mechanism especially for the ROS scavenging mechanism [127]. C18 FAs accumulated under stress conditions make a balance of the over-reduced electrons, they can consume approximately 24 NADPH derived from the electron transport chain [127]. Under oxidative stress, most accumulated lipids contain unsaturated 18 and 16 carbons FAs. Most of FAs produced are C18:3, C18:2 and C18:1 with a smaller proportion of 16 Carbon FAs [131].

5.3. CO_2 and pH

Excess of fixed Carbon from photosynthesis is channeled into storage molecules such as TAGs [22]. However, to obtain maximum biomass and enhanced lipid production, optimum CO_2 levels are required. Table 9 summarizes the effect of CO_2 level on lipid accumulation. Increasing CO_2 concentrations enhances growth by avoiding Carbon limitation [108], but also reduces pH in the medium. Enhancement in lipid production and alterations in the composition of FAs at various CO_2 concentrations have been reported [108]. A global gene expression analysis realized by Ref. [132], revealed that high concentrations of CO_2 induce the up-expression of key genes involved in Carbon fixation and TCA cycle, but down-expression of FAs biosynthesis pathways genes. However, genes involved in TAGs biosynthesis were up expressed at prolonged lipid accumulation phases under high doses of CO_2. CO_2 concentrations also affect lipid profiles, low concentrations of CO_2 have been shown to favor the accumulation of saturated FAs while high CO_2 concentrations stimulate unsaturated FAs [133].

Each species has a specific pH range in which it can grow adequately [134]. The optimal pH range is highly dependent on species and cultivation conditions with a general referendum between 7.0 and 8.0. Cell flocculation and Carbon limitation may take place at a pH higher than 9.0 [134]. Usually, pH in the medium is controlled by manipulating the injection rate of CO_2 compensating for the uptake by the algae. Nevertheless, the effects of pH are difficult to unravel from the CO_2 availability, and thus the enhancement of the Carbon flux in the cell and the enhanced growth rate. This explains the studies which conclude that there is no significant relation between pH and lipid accumulation or fatty acids composition [134–136].

5.4. Metals

Metals ions are involved in various physiological functions affecting metabolism and growth which can affect lipid accumulation [137]. Table 10 describes the effect of metal concentration on lipid accumulation for different phytoplankton species. Metals at small concentrations are indispensable for cellular functions. Usually, the addition of low ion concentrations enhances growth rate and lipid content, but it

Table 9
CO_2 stress effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
CO_2	1%	12 h	*Chlorella vulgaris*	1 g/100 g	-	[278]
	10%	12 h		1.59 g/100 g	-	
	1%	24 h		1.11 g/100 g	-	
	10%	24 h		5.6 g/100 g	-	
CO_2	0.04% -> 2%	11 h	*Chlorella vulgaris*	23->38 μmolFA/gcell·10^{-1}*	-	[279]
CO_2	0.03%	N.D.	*Nannochloropsis limnetica*	30	5.10	[280]
	3%	N.D.		34	24.48	
	10%	N.D.		40	28.0	
	0.03%	N.D.	*Botryococcus braunii*	23	3.58	
	3%	N.D.		28	18.48	
	10%	N.D.		32	19.2	
	0.03%	N.D.	*Stichococcus bacillaris*	24	3.65	
	3%	N.D.		26	13	
	10%	N.D.		30	14.25	
CO_2	0 ml/min	15 d	*Chlorella vulgaris*	20*	4.5*	[281]
	20 ml/min	15 d		26*	9.7*	
	50 ml/min	15 d		24*	13*	
	0 ml/min + N depletion	17 d		41*	4.5*	
	20 ml/min + N depletion	17 d		43*	10*	
	50 ml/min + N depletion	17 d		51*	11*	
CO_2	350 μL CO_2/L	10 d	*Nannochloropsis* sp.	7	-	[133]
	2800 μL CO_2/L	10 d		9	-	
CO_2	2%	8 d	*Nannochloropsis oculata*	29.7	142	[282]
	5%	8 d		26.2	113	
	10%	8 d		24.6	97	
	15%	8 d		22.7	84	
CO_2	0.3 mg/L	18 d	*Scenedesmus obliquus*	4.21	25.1	[139]
	3 mg/L	18 d		8.24	45.32	
	9 mg/L	18 d		20.63	51.96	
	12 mg/L	18 d		33.14	69.23	

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.
N.D.: Not determined.

Table 10
Metal stress effect on lipid accumulation.

Stress	Stress Level	Exposure time**	Species	Lipid Content (%)	Lipid Productivity (mg/L/d)	Ref.
Metal	Fe^{+3}: 0 mM	20 d	*Botryococcus* sp.	6–13.5*	-	[143]
	Fe^{+3}: 0.37 mM	20 d		11 - 30*	-	
	Fe^{+3}: 0 mM + N depletion	20 d		13.5–18*	-	
	Fe^{+3}: 0.74 mM + N depletion	20 d		26 - 36*	-	
Metal	Fe^{+3}: 0 mg/L	18 d	*Scenedesmus obliquus*	5.75	20.1	[139]
	Fe^{+3}: 2.5 mg/L	18 d		9.21	33.24	
	Fe^{+3}: 5 mg/L	18 d		13.52	58.34	
	Fe^{+3}: 10 mg/L	18 d		15.34	75.69	
	Fe^{+3}: 20 mg/L	18 d		28.12	95.35	
Metal	Fe^{+3}: 0 mol/L	22 d	*Chlorella vulgaris*	7.8	-	[140]
	Fe^{+3}: 1.2×10^{-8} mol/L	22 d		11.8	-	
	Fe^{+3}: 1.2×10^{-7} mol/L	22 d		12.2	-	
	Fe^{+3}: 1.2×10^{-6} mol/L	22 d		16.5	-	
	Fe^{+3}: 1.2×10^{-5} mol/L	18 d		56.6	-	
Metal	Fe^{+3}: 1.2×10^{-3} g/L	6 d	*Scenedesmus* sp.	43.2*	33*	[137]
	Fe^{+3}: 1.2×10^{-1} g/L	6 d		10*	249.8	
	Fe^{+3}: 0 g/L	6 d		9,1*	26*	
Metal	Fe^{+3}: 0 mg/L	N.D.	*Nannochloropsis oculata*	34*	51.28	[141]
	Fe^{+3}: 3.16 mg/L	N.D.		43*	62.41	
	Fe^{+3}: 9.48 mg/L	N.D.		46*	66.85	
	Fe^{+3}: 18.96 mg/L	N.D.		48*	76.1	
Metal	Mg^{+2}: 100 μM	4 d	*Monoraphidium* sp.	59.8	17	[138]
Metal	Mg^{+2}: 0 g/L	6 d	*Scenedesmus* sp.	35*	46.8*	[137]
	Mg^{+2}: 7.3×10^{-3} g/L	6 d		43*	246.7*	
	Mg^{+2}: 7.3×10^{-1} g/L	6 d		36.5*	192.5*	
Metal	Ca^{+2}: 0 g/L	6 d	*Scenedesmus* sp.	10.6*	63.5*	[137]
	Ca^{+2}: 9.8×10^{-4} g/L	6 d		47.4*	275*	
	Ca^{+2}: 9.8×10^{-1} g/L	6 d		17*	71*	
Metal	Cu^{+2}: 4 mg/L	15 d	*Chlorella ellipsoidea*	0.087 mg/g*	-	[150]
	Cu^{+2}: 4 mg/L	15 d	*Chlorella emersonii*	0.079 mg/g*	-	
	Cu^{+2}: 4 mg/L	15 d	*Chlorella protothecoides*	0.17 mg/g	-	
	Cu^{+2}: 4 mg/L	15 d	*Chlorella pyrenoidosa*	0.11 mg/g*	-	
	Cu^{+2}: 4 mg/L	15 d	*Chlorella sorokiniana*	0.081 mg/g*	-	
	Cu^{+2}: 4 mg/L	15 d	*Chlorella vulgaris*	0.21 mg/g	-	
Metal	Cu^{+2}: 0 mg/L	N.D.	*Nannochloropsis oculata*	45*	63.48	[141]
	Cu^{+2}: 0.01 mg/L	N.D.		43*	62.41	
Metal	Cu^{+2}: 0.22 mM	72 h	*Euglena gracilis*	1.7 μg/10^5 cell*	-	[151]
Metal	Cu^{+2}: 0 mg/L	96 h	*Chlorella protothecoides*	15*	-	[35]
	Cu^{+2}: 15.7 mg/L	96 h		27*	-	
	Cu^{+2}: 31.4 mg/L	96 h		77*	-	
	Cu^{+2}: 47.1 mg/L	96 h		60*	-	
Metal	Zn^{+2}: 0 mg/L	N.D.	*Nannochloropsis oculata*	20*	28.15	[141]
	Zn^{+2}: 0.023 mg/L	N.D.		43*	62.41	
	Zn^{+2}: 0.069 mg/L	N.D.		35*	42.28	
	Zn^{+2}: 0.138 mg/L	N.D.		40*	61.46	
Metal	Zn^{+2}: 0.88 mM	72 h	*Euglena gracilis*	2.1 μg/10^5 cell*	-	[151]
Metal	Co^{+2}: 0 mg/L	N.D.	*Nannochloropsis oculata*	50*	70.8	[141]
	Co^{+2}: 0.012 mg/L	N.D.		45*	62.41	
Metal	Mn^{+2}: 0 mg/L	N.D.	*Nannochloropsis oculata*	38*	57.6	[141]
	Mn^{+2}: 0.18 mg/L	N.D.		43*	62.41	
	Mn^{+2}: 0.54 mg/L	N.D.		54*	53.11	
	Mn^{+2}: 1.08 mg/L	N.D.		50*	48.77	
Metal	Mo^{+6}: 0 mg/L	N.D.	*Nannochloropsis oculata*	26*	40.69	[141]
	Mo^{+6}: 0.07 mg/L	N.D.		43*	62.41	
	Mo^{+6}: 0.21 mg/L	N.D.		50*	36.56	
	Mo^{+6}: 0.42 mg/L	N.D.		45*	59.44	
Metal	Cr^{+6}: 1.3 μM	96 h	*Euglena gracilis*	4.2 μg/mg*	-	[155]
	Cr^{+6}: 9.8 μM	96 h		4.6 μg/mg*	-	
	Cr^{+6}: 36.2 μM	96 h		2.2 μg/mg*	-	
	Cr^{+6}: 72.3 μM	96 h		1.1 μg/mg*	-	
	Cr^{+6}: 120 μM	96 h		1.6 μg/mg*	-	

**: In most of the cases, the lipid content (and the associated productivity) was assessed at the end of the log phase.
*: Approximate values obtained from figures.
N.D.: Not determined.

becomes inhibiting at high concentrations. They play roles in different key pathways, such as respiration, photosynthesis, and ATP production [138].

- *Iron (Fe^{3+})* is one of the most essential metals required by microalgae [137]. It has been shown to stimulate microalgae growth and to induce considerable lipid accumulation [137,139–141]. The influence depends on the strain [137], but high concentrations inhibit growth and lipid accumulation. Fe^{3+} is one of the most important metals in photosystem (I) and photosystem (II), also playing roles in Nitrogen assimilation, respiration, DNA synthesis [142]. It has a direct impact on lipid accumulation [143,144] but also an indirect effect on lipid productivity by enhancing the growth rate.
- *Magnesium (Mg^{2+})* ions addition has a favorable influence on microalgae lipid accumulation [137,138] and stimulates the growth rate [145]. Mg^{2+}, as other divalent metal ions, enhances the activity of enzymes, such as acetyl-CoA carboxylase and pyruvate dehydrogenase involved in fatty acids synthesis and cell division [137,138,146,147]. A higher concentration is therefore beneficial for fatty acids synthesis. Also, Mg^{2+} is an essential component of the chlorophyll molecule, central in photosynthesis activity [145].
- *Calcium (Ca^{2+})* at moderate concentration can increase the lipid content, whereas at higher concentrations it reduces it [137]. Increasing Ca^{2+} concentration has a negligible effect on growth, but an excess can be lethal [137,148]. Ca^{2+} is an important element used as a biochemical messenger, Ca^{2+} signals regulate neutral lipid synthesis [149] and play a critical role in the signal transduction of environmental changes [137].
- *Copper (Cu^{2+})* ion must be at a very low concentration for efficient growth [35] and it very rapidly becomes strongly inhibiting. Cu^{2+} ions are one of the most toxic metals to microalgae growth [150,151]. Some studies [151] demonstrated that low concentrations of Cu^{2+} already significantly reduced growth. They affect the pyrenoid area, which is essential during the first half of the cell cycle. The reports on Cu^{2+} ions on lipid is not clear in the literature and might be species dependent. In Ref. [141] it has a negative effect on the lipid synthesis, while [151] obtained a marked increase in the total lipid content in presence of Cu^{2+}.
- *Zinc (Zn^{2+})* ions at moderate concentration increase the efficiency of photosynthesis, but when the concentrations are beyond a limit value, it causes harmful effects [141]. Zn^{2+} ions deficiency affects growth and cell morphology in some microalgae species [151], which can be explained by the role of zinc in gene regulation by zinc-dependent enzymes involved in DNA synthesis [152]. Moderate ion concentrations have been shown to promote lipid synthesis [141].
- *Cobalt (Co^{2+})* ions have a strong inhibitory effect like Cu^{2+}, reducing the lipid synthesis and with little effect on growth. Removing Co^{2+} from the medium increased lipid content [141].
- *Manganese (Mn^{2+})* ions are cofactors to some enzymes of photosynthesis and thus can stimulate growth [153]. Mn^{2+} is an activator of nitrate reductase and some enzymes involved in glycolysis and tricarboxylic acid cycle [141]. Appropriate concentrations promote growth and lipid synthesis, but too high Mn^{2+} ions concentrations decrease the lipid content [141].
- *Molybdenum (Mo^{6+})* ions is also toxic. Appropriate Mo^{6+} ion concentrations promote growth and lipid formation, but at higher concentrations, it shows a toxic effect on microalgae [154], whereas the lipid content decreased [141]. Some studies have shown that Mo^{6+} deletion improves growth [141].
- *Chrome (Co^{6+})* ions are highly toxic and affect pigment content, chloroplast disorganization, mitochondrial damage, and cytoskeleton alterations [155]. The study realized by Ref. [155] indicated that Co^{6+} ions impact fatty acids content and distribution. At some moderate value, it can increase lipid content, whereas lipid decreased at higher concentrations. PUFAs related to chloroplast structure were the most affected by Co^{6+}, probably revealing a reparation mechanism for reducing cellular damages by metal stress.

6. Strain selection and metabolic engineering

Even though the presented cultivation strategies can enhance the TAGs content and TAGs productivity, lipid productivity can still be improved by discovering more productive strains. Most of the microalgae species available in nature remain unexplored, thus additional screening for promising strains remains a worthwhile option [13]. Recently, there has been intense interest in isolating new native microalgae species. But enhancing these organisms through *strain selection* and *metabolic engineering* can also lead to increasing the TAGs content.

The first step for optimizing TAGs accumulation is to better understand lipid metabolism. Many aspects involved in TAGs synthesis are not well understood, such as metabolism regulation, genetic regulation, and biological function [13]. The overall lipid biosynthesis pathway and its regulators have not been clearly described, with unknowns in the mechanisms that govern lipid accumulation.

Metabolic engineering focuses on the construction of microalgae capable of synthesizing large amounts of lipid while still growing at a high rate [45]. The strategy targeted to increase the lipid content is overexpression of genes involved in the fatty acids synthesis together with blocking competing metabolic pathways. Some of the most used metabolic engineering strategies are described below.

- *Overexpression of genes*: Genetic manipulation has been used only in few species with contrasting results. The main strategies target the overexpression of genes involved in the early steps of fatty acids, such as the enzyme acetyl-CoA carboxylase, which increases the availability of precursor molecules of lipid synthesis [156]. Recent research [157,158] has detected the conversion of polysaccharides into lipids in microalgae through the overexpression of the ATP-dependent citrate lyases (ACL) genes. Nevertheless, the overexpression of genes related to fatty acids turns out not to significantly affect the lipid content. Hence, research was also focused on enzymes involved in acylglycerols biosynthesis by overexpression of DGAT genes [159,160], finding that each gene affects the lipid accumulation pattern. It is important to emphasize that the genetically modified cells show differences in regulation, the subcellular location of lipid synthesis, or function of TAGs synthesis under adverse conditions. Therefore, considerable research, with insight in transcriptional and post-transcriptional regulation of microalgae lipids is still necessary to develop techniques for transforming others promising microalgae species [13]. Also, more detailed metabolic flux models including gene expression and proteomic analysis would guide the future engineering manipulation for increased lipid production.
- *Blocking competing metabolic pathways*: knocking down competing pathways and directing more Carbon and energy towards lipid synthesis is another successful approach to increase lipid content. In general, a significant amount of energy compounds such as starch are produced instead of lipogenesis. Different studies have been carried out for starch mutants under Nitrogen starvation, giving rise to higher levels of lipids than wild types [161,162]. Also, repression of lipid catabolism by down-regulating or inhibiting TAGs hydrolysis and/or β-oxidation process, limited the re-consumptions of lipids [163].
- *Increasing photosynthetic efficiency:* enhancing photosynthetic efficiency results in an increase in growth rate and eventually to lipid synthesis [164]. Direct mutation towards increasing electron transport rates, even under Nitrogen starvation, contributes to ingincreasing lipid accumulation rates [165].
- *Modification of fatty acids composition:* Elongases and desaturases are responsible for PUFAs biosynthesis. Thus, the regulation and/or

insertion of specific desaturases/elongases modify the fatty acids profile. Different studies have shown how this strategy affects the lipid content and fatty acids distribution [158,166,167].

Alternative approaches by *random mutagenesis* and sorting of the best phenotypes with a flow cytometer is another promising approach [168] that turned out to be effective since the growth rate of the selected mutants is not affected. But several parts of the metabolism can be affected and it is difficult to accurately identify the reasons for the resulting increase in lipid productivity [27,169,170].

Another strategy consists of applying environmental stress in the long term in a continuous reactor. The stress is designed such that the individuals presenting a better fitness with the environment would dominate the population. This Darwinian principle must be tailored to determine the stress that would give an advantage to the individuals accumulating lipids. A 6-month experiment with *Tisochrysis lutea* applying daily fluctuating temperatures contributed to increasing lipid productivity by 34% (+9%) [29]. In Gachelin et al. [171], the amount of DHA in polar lipids was tripled. In all the cases the growth rate was not affected.

7. Conclusions

Phytoplankton cultivation in optimal conditions and species selection are likely to strongly enhance lipid productivity and quality for biofuel feedstock. We have comprehensively overviewed 95 phytoplankton species with improved lipid production through various factors strongly influencing the induction of lipid production. Nitrogen-starvation, temperature, and irradiance have been extensively applied to increase lipid production, but information about other abiotic factors such as Phosphorous-starvation, pH, CO_2, oxidative stress, or metals are still scarce. The diversity of phytoplankton species and the differences in specific responses motivated a compilation of information to target a general overview and define the best strategies for enhanced quality and quantity of microalgae-based biodiesel. The data compiled in this review highlight the complexity of lipid metabolism and its plasticity as a function of growth conditions.

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Credit author statement

Marjorie Morales: Conceptualization, Methodology, Formal analysis, Writing-Original Draft, Visualization. Claude Aflalo: Writing-Review & Editing. Olivier Bernard: Conceptualization, Methodology, Writing-Review & Editing, Supervision.

Appendix A. Supplementary data

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