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Influence of different abiotic factors on lipid production by microalgae – a review☆

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Abstract – Research on lipid production in microalgae, especially to target biofuels, has been widely published in recent decades. This literature is mostly focused on the optimization of culture conditions, metabolic engineering, biorefinery and downstream processes. At the lab scale, lipid production is very promising concerning biofuels production based on microalgae due to their high lipid content. However, the accumulation of knowledge associated with the development of processes and technologies for the production of biomass and associated lipids has so far not achieved the hoped-for commercial success due to still too expensive operating costs compared to fossil fuels. This review aims to present the influence of various abiotic factors on the accumulation of lipids and lipid profile in microalgae, such as the depletion of the culture medium, temperature and light, but also most innovative like the induction by hormones. Other optimization procedures and the use of wastewater to improve the cost-viability are discussed in perspective.

Keywords: microalgae / biodiesel / lipid production / abiotic factors

Résumé – Influence de différents facteurs abiotiques sur la production de lipides par les microalgues – une revue. Les recherches sur la production de lipides chez les microalgues, notamment à visé des biocarburants, ont été largement publiées ces dernières décennies. Cette littérature est principalement axée sur l'optimisation des conditions de culture, l'ingénierie métabolique, la bioraffinerie et les processus en aval. À l'échelle du laboratoire, la production des lipides à partir de microalgues pour la production de biocarburants est très prometteuse en raison de leur forte teneur en lipides. Cependant, l'accumulation de connaissances liées au développement de procédés et de technologies de production de biomasse et de lipides associés n'a jusqu'à présent pas atteint le succès commercial espéré en raison des coûts d'exploitation encore trop élevés par rapport aux énergies fossiles. Cette revue a pour objectif de présenter l'influence de divers facteurs abiotiques sur l'accumulation de lipides et le profil lipidique chez les microalgues, tels que l'épuisement du milieu de culture, la température et la lumière, mais aussi les plus innovants comme l'induction par les hormones. D'autres procédures d'optimisation et l'utilisation des eaux usées pour améliorer la rentabilité sont discutées en perspective.

Mots clés : microalgue / biocarburant / production lipidique / paramètres environnementaux

1 Introduction

Microalgae are photoautotrophic single cell-organisms capable to use solar energy, water, and inorganic nutrients to reduce CO_2 into complex organic compounds. Their large diversity gives them the opportunity to live in diverse environments and is directly correlated to with a large variety of biomolecules such as amino acids, peptides, proteins, carbohydrates (oligo- and polysaccharides), lipid (polyunsaturated

fatty acids (PUFAs) and ω3, −6) and pigments (Rajvanshi and Shankar Sagaram, 2019). The industrial exploitation of these metabolites on various commercial markets such pharmaceuticals, cosmetics, human food or feed is at its early stages, especially when it comes to biofuels. Usually biofuels can be classified as three generations: first generation corresponding to the feedstocks of food, second generation corresponding to lignocellulosic biomass and agricultural wastes and the third generation corresponding to photosynthetic microorganisms. Microalgae have gained more and more attention these last decades due to better characteristics compare to terrestrials plants, like fast growth rate, able to grow in lot of habitats, better CO_2 capture compared to

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Table 1. Examples of the impact of nitrogen and phosphate on lipid production in some microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Nannochloropsis oculata NCTU-3	Nitrogen depletion	50.4%, n.d	n.d	Chiu *et al.* (2009)
Scenedesmus obliquus CNW-N	Nitrogen deprivation	38.9%, $78.7\,\mathrm{mg.L^{-1}.d^{-1}}$	stearic acid (C18:0), oletic acid (C18:1), palmitic acid (C16:0)	Ho *et al.* (2010)
Chlorella vulgaris ESP-31	Nitrogen starvation	63.0%, $51.2\,\mathrm{mg.L^{-1}.d^{-1}}$	palmitic acid (C16:0), oletic acid (C18:1), linoleic acid (C18:2)	Yeh and Chang (2012)
Neochloris oleoabundans HK-129	Nitrogen starvation	27.2%, $30.8\,\mathrm{mg.L^{-1}.d^{-1}}$	oletic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:2)	Sun *et al.* (2014)
Scenedesmus sp. LX1	Nitrogen depletion	33.0%, n.d	n.d	Xin *et al.* (2010a)
Chlorella zofingiensis	Nitrogen starvation	65.1%, $87.1\,\mathrm{mg.L^{-1}.d^{-1}}$	n.d	Feng *et al.* (2012)
Scenedesmus sp. LX1	Phosphate deprivation	53.0%, $75\,\mathrm{mg.L^{-1}.d^{-1}}$	n.d	Xin *et al.* (2010b)
Chlorella zofingiensis	Phosphate starvation	44.7%, $44.7\,\mathrm{mg.L^{-1}.d^{-1}}$	n.d	Feng *et al.* (2012)
Scenedesmus abundans	Phosphate starvation	23.0%, $30.8\,\mathrm{mg.L^{-1}.d^{-1}}$	oletic acid (C18:1), palmitic acid (C16:0), linoleic acid (C18:3)	Mandotra *et al.* (2016)

n.d: not determined

terrestrial plants induce by high-efficiency photosynthesis, high lipid content, smaller and non-arable surfaces. However, the economical barrier, due to high production cost compare to fossil energy, makes the commercialization of biofuel from microalgae unsuccessful. In order to improve cost-effective, various challenges must be took up all along the production process, from the selection of the strain, cultivation including environmental parameters and culture system, harvesting, drying, extraction and conversion of lipid. In nature, microalgae adapt to environmental changes with diverse and varied physiological responses. In view of this phenomenon, the culture conditions in which the microalgae are cultivated are very important to obtain higher lipid productivity. This review present a non-exhaustive list of abiotic factors that can be use to improve the lipid production as well as make change in lipid profile in microalgae.

2 Nitrogen

In microalgae, the most common solution to improve high lipid productivity, especially accumulation of TAG, was nitrogen starvation or N-depleted condition. The strain was first cultivated with nutrient-rich medium to promote cell growth. During the growth phase, the microalgae will consume the nutrients from medium and tend naturally towards nitrogen deprivation to trigger lipid accumulation. Some examples are presented in Table 1.

As an example, in *Nannochloropsis oculata* NCTU-3 the lipid content during growth varying from 30.8% in logarithmic phase to 39.7% in early stationary phase and 50.4% at stationary phase (Chiu *et al.*, 2009). In another study, a two-stage nitrogen starvation strategy, composed of (1) growth under nutrient-rich conditions followed by (2) cultivation under nitrogen starvation was used on *Neochloris oleoabundans* HK-129. The total lipid and specific TAG productivities

were increased by 29.6% and 67.7%, respectively, compare to the single-stage (Sun *et al.*, 2014). The quality of lipids is also impacted. Even if the lipid composition was similar, palmitic acid and oleic acid contents were 1.8 and 2 times higher in nutrient-deficient condition compared to the nutrient-rich condition in *Scenedesmus obliquus* CNW-N (Ho *et al.*, 2010).

The nature of the nitrogen source can also influence the accumulation of intracellular lipids. *Chlorella* sp. has shown the maximum lipid productivity of $0.124\,\mathrm{g.L^{-1}.d^{-1}}$ with $0.100\,\mathrm{g.L^{-1}}$ of urea in batch mode (Hsieh and Wu, 2009). A semi-continuous process combining culture harvest and feeding urea each time the cultivation achieved the early stationary phase has led to the maximum lipid productivity, compared with batch and fed-batch.

Another example was the cultures of *Schizochytrium* sp. ABC101 in three types of fed-batch fermentations that used yeast extract as nitrogen source to regulate lipid accumulation. The limited nitrogen feeding provided the most efficient lipid production, with a maximum dry cell weight and lipids after 60 h (Ju *et al.*, 2020). This production could be upgraded using corn sleep liquor (CSL) as nitrogen source, leading to a maximum dry cell weight and lipids after 84 h. In addition, the CSL being less expensive than the yeast extract, its use made it possible to reduce by half the final cost of lipid production, which is particularly advantageous from the point of view of industrial production.

Stress from nitrogen starvation or N-depleted condition induce both overproduction of starch and lipids. The starch will be partially degraded and the lipid content will increase considerably. The accumulation of starch would then only be a temporary response to the environmental stress, while the accumulation of lipids could serve as long-term storage energy (Zhu *et al.*, 2014). A study from Rai *et al.* (2017) on the longitudinal proteome dynamics of *Chlorella* sp. FC2 have confirmed that lipid accumulation was concomitant with

Table 2. Examples of the impact of metallic trace elements on lipid production in some microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Botryococcus spp. TRG	Fe(III)	25.8%, 46.9 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), stearic acid (C18:0), oletic acid (C18:1)	Yeesang and Cheirsilp (2011)
Chlorella vulgaris	Iron and phosphorus deprivations	56.6%, n.d	n.d	Liu *et al.* (2008)
Chlorella sp. BUM11008	Iron deprivation	31.5%, 49.2 mg.L^{-1}.d^{-1}	stearic acd (C18:0), linolenic acid (C18:2), arachidic (20:0)	Praveenkumar *et al.* (2012)
Chlorella sorokiniana	Iron	33.0%, n.d	n.d	Wan *et al.* (2014)
Chlorella protothecoides	Cu(II)	89.0%, n.d	oletic acid (C18:1), palmitic acid (C16:0), linolenic acid (C18:2)	Li *et al.* (2013)
Chlorella minutissima UTEX 2341	Cd	21.0%, 249.4 mg.L^{-1}.d^{-1}	n.d	Yang *et al.* (2015)
Chlorella minutissima UTEX 2341	Cu(II)	13.1%, 154.2 mg.L^{-1}.d^{-1}	n.d	
Scenedesmus sp. R-16	Mg(II), Ca(II), Fe(III)	47.4%, 275.7 mg.L^{-1}.d^{-1}	n.d	Ren *et al.* (2014)

n.d: not determined

reduction in carbon flux towards carbohydrate, protein and chlorophyll biosynthesis, with a down-regulation of enzymes involved in chlorophyll biosynthesis (porphobilinogen deaminase) and photosynthetic carbon fixation (sedoheptulose-1,7 bisphosphate and phosphoribulokinase). Similar results were obtained in *Chlorella* PY-ZU1 with excess of phosphate and nitrogen deprivation, inducing a down-regulated of the chlorophyll biosynthesis metabolism and an up-regulation of several enzymes involved in carbon fixation via photosynthesis, oxidative phosphorylation, and glycerolipid metabolism which stimulated the biosynthesis (Chu *et al.*, 2019).

Very recently, An *et al.* (2020) employed a new an unusual process based on addition of multiple small-doses of different forms of nitrogen to evaluated growth and lipid production in *Scenedesmus obliquus*. Compared to control, growth and lipid production have been promoted only by daily addition of 2 mg.L^{-1} of $NaNO_3$ or CH_4N_2O. The highest yield of lipid production was obtained with 2 mg.L^{-1} of CH_4N_2O (242.4 mg.L^{-1}), which is 2.75 times higher compare to the M-11 medium condition. Authors suggested that lipid accumulation observed under nitrogen starvation or depletion conditions could be at least partly mediated by oxidative stress. However, the connection between oxidative stress and increased microalgae lipid accumulation is not yet clearly defined, this understanding requires further investigation.

3 Phosphate

Like nitrogen deficiency, phosphorus starvation induces an accumulation of intracellular lipids in many microalgae (Tab. 1). For example, Mandotra *et al.* (2016) have studied impact of K_2HPO_4 concentrations on biomass, lipid concentration and fatty acid profile in *Scenedesmus abundans*. Among different phosphate concentrations, higher biomass productivity, lipid content and lipid productivity were obtained at 60 mg.L^{-1}, follow by 80, 40 and 20 mg.L^{-1}. Furthermore, 60 mg.L^{-1}

phosphate concentration was found to be most suitable for biofuel application. The amount of SFA (saturated fatty acids), MUFA (Monounsaturated fatty acids) and PUFA were 31.70%, 48.35% and 19.95%, respectively.

Study on *Chlorella zofingiensis* has shown that a medium enriched in nitrogen and phosphate provides better biomass production to the detriment of high lipid content. Depletion of the culture medium, in P or N, made it possible to considerably improve the accumulation of intracellular lipids. However, the highest productivity was obtained from the N–P+ condition, indicating that nitrogen deficiency was more effective than phosphate deficiency for lipid accumulation (Feng *et al.*, 2012).

As with nitrogen, phosphate deprivation can affect the quality of fatty acid content. For example, significant changes in the fatty acid and lipid composition of *Monodus subterraneus* were observed in phosphate deprivation condition by Khozin-Goldberg and Cohen (2006). With decreasing phosphate availability from 175 to 52.5, then 17.5 and 0 µM, the proportion of eicosapentaenoic acid (EPA) gradually decreased from 28.2, 20.8, 19.4 and 15.5 (mol% of total fatty acids), respectively. Similar results were obtained for monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and diacylglyceroltrimethylhomoserine (DGTS). At the opposite, the proportion of TAG was increased from 6.5 up to 39.3% of total lipids. The same type of deficiency induces also an accumulation of TAG in *Chlamydomonas reinhardtii* (Sato *et al.*, 2014).

4 Metallic trace elements

Micronutrients and vitamins are elements of culture media present in very small amounts. They very rarely cause a deficiency in microalgae. Like macronutrients, they can also play an important role, acting on algal metabolic functions and increasing lipid content in microalgae as presented in Table 2.

Table 3. Examples of the impact of carbon sources on lipid production in some microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Chlorella vulgaris	CO_2	< 11.9%, 20.7 mg.L^{-1}.d^{-1}	linolenic (C18:2), oletic acid (C18:1), palmitic acid (C16:0)	Yoo *et al.* (2010)
Botryococcus braunii	CO_2	25.8%, 5.51 mg.L^{-1}.d^{-1}	oletic acid (C18:1), palmitic acid (C16:0), linolenic (C18:2)	
Scenedesmus sp.	CO_2	< 11.9%, 20.7 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), linolenic (C18:2), oletic acid (C18:1)	
Chlorella vulgaris ESP-31	Glucose and CO_2	53.0%, 143.9 mg.L^{-1}.d^{-1}	oletic acid (C18:1), palmitic acid (C16:0)	Yeh and Chang (2012)
Chlorella protothecoides	Glucose	32.0%, 200 mg.L^{-1}.d^{-1}	oletic acid (C18:1), linolenic (C18:2), palmitic acid (C16:0)	Chen and Walker (2012)
Phaeodactylum tricornutum	Glucose	40.0%, 38 mg.L^{-1}.d^{-1}	palmitoleic acid (C16:1), hexadecylic acid C16:0, eicosapentaenoic acid (C20:5n3)	Wang *et al.* (2012a)
Chlorella vulgaris	Crude glycerol	15.9%, n.d	oletic acid (C18:1), linolenic (C18:2), palmitic acid (C16:0)	Choi and Yu (2015)
Scenedesmus sp.	Crude glycerol	16.2%, n.d	oletic acid (C18:1), linolenic (C18:2), palmitic acid (C16:0)	
Thraustochytrium sp. BM2	Corn steep liquor	76.0%, 888 mg.L^{-1}.d^{-1}	n.d	Chen *et al.* (2020b)

n.d: not determined

Iron (Fe) is probably the most used metallic element. At high concentration, it is expected to increase lipid accumulation because of its down-regulation which requires fatty acid desaturase enzymes. As an example, replete *Chlorella vulgaris* in a fresh media supplemented with 1.2×10^{-5} mol.L^{-1} Fe^{3+} leading to a lipid content seven times higher than the controlled culture (Liu *et al.*, 2008). A similar effect was observed in four *Botryococcus* strains by Yeesang and Cheirsilp (2011). The lipid content increased in all strains under both nitrogen-deficient and nitrogen-rich conditions with increasing Fe^{3+} up to 0.037 mM, with highest lipid content for *Botryococcus* spp. TRG. This augmentation was more important in nitrogen-deficient condition, probably due to a synergetic effect between iron and nitrogen deprivations.

Copper is an essential micronutrient found in some essential enzymes involved in metabolic pathways. Many studies have pointed out its beneficial effect on highly valuable component accumulation. Study about the impact of Cu(II) stress on *Chlorella protothecoides* was carried out by Li *et al.* (2013) using a two-steps process. The highest lipid content was achieved after 24 h induction by 31.4 mg.L^{-1}Cu(II) which was 4-fold higher compare to the first heterotrophic stage. However, no difference in fatty acid composition was observed.

The effect of heavy metals on lipid production has been also investigated. For example, the use of 0.4 and 0.6 mM Cd significantly increases the lipid content of *Chlorella minutissima* UTEX 2341 by 93 and 42%, respectively (Yang *et al.*, 2015). The lipid productivity was increased for all conditions (except for 2 and 4 mM Zn and 4 mM Mn). The higher lipid productivity was obtained by addition of 0.2 mM Cd followed by 0.6 mM Cd and 0.4 mM Cu.

Different metal ions (Fe^{3+}, Mg^{2+} and Ca^{2+}) were used to test their influence on *Scenedesmus* sp. R-16 growth and lipid accumulation. The maximum lipid productivity for Mg^{2+}, Ca^{2+} and Fe^{3+} were obtained at 7.3×10^{-3}, 9.8×10^{-4} and 1.2×10^{-3} g.L^{-1}, respectively. Moreover, addition of 1.0×10^{-3} g.L^{-1} EDTA could enhance the solubility of iron and calcium ions, leading to increase their availability for microalgae, which promote the lipid accumulation. Compared with the control, the total lipid content and lipid productivity increased by 28.2 and 29.7%, respectively (Ren *et al.*, 2014).

5 Carbon sources

5.1 Inorganic carbon (CO$_2$)

Phototrophic cultivation is the most commonly used cultivation condition for microalgae lipid production probably due to it simple use and its advantage of the consumption of CO_2 as carbon source for both cell growth and oil production. The Table 3 presents some examples of carbon on lipid production in microalgae.

Chlorella vulgaris has produced maximum biomass, lipid yield and lipid productivity at 8% CO_2. Most of its fatty acid were saturated fatty acid, 45% of the total lipid content was composed of unsaturated fatty acids, including 80% of high added-value essential fatty acids belonging to the ω3 and ω6 which have a great importance for human health (Ortiz Montoya *et al.*, 2014). Similar results were obtained on *C. vulgaris* MSU AGM14 cultivated at 8% CO_2, with lipid content and lipid productivity equals to 18.6% and 11.89 mg.L^{-1}.d^{-1}, respectively (Lakshmikandan *et al.*, 2020).

The same microalgal specie cultured under a relatively high CO_2 concentration (10%) has given a similar lipid productivity equal of 6.9 mg.L^{-1}.d^{-1} for 14 days of culture. Similar productivity was obtained from *Botryococcus braunii.* (5.51 mg.L^{-1}.d^{-1}) with oleic acid as main component of the fatty acids content (55%). At the opposite, the lipid

Table 4. Examples of the impact of light on lipid production in some microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Neochloris oleoabundans HK-129	200 μmol photons.m^{-2}.s^{-1}	24.4%, 49.94 mg.L^{-1}.d^{-1}	oletic acid (C18:1), palmitic acid (C16:0), linolenic (C18:2)	Sun *et al.* (2014)
Scenedesmus abundans	6000 lux	32.8%, 39.16 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), oletic acid (C18:1)	Mandotra *et al.* (2016)
Nannochloropsis sp.	700 μmol photons.m^{-2}.s^{-1}	47%, 410 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), palmitoleic acid (C16:1), eicosapentaenoic acid (C20:5n3)	Pal *et al.* (2011)
Nannochloropsis oculata	Blue LED follow by green LED	56.0%, n.d	palmitic acid (C16:0), oleic acid (C18:1)	Ra *et al.* (2016)
Nannochloropsis salina	Blue LED follow by green LED	52.0%, n.d	palmitic acid (C16:0), oleic acid (C18:1)	
Nannochloropsis oceanica	Blue LED follow by green LED	53.0%, n.d	palmitic acid (C16:0), oleic acid (C18:1)	
Scenedesmus obliquus	Blue and red LED with 1:1 ratio	26.0%, 58.3 mg.L^{-1}.d^{-1}	oleic acid (C18:1n9c), palmitic acid (C16:0), α-linoleic acid (C18:3n3)	Abomohra *et al.* (2019)

n.d: not determined

productivity of S*cenedesmus* sp. was 20.65 mg.L^{-1}.d^{-1} (Yoo *et al.*, 2010).

Opposite results were obtained by Lv *et al.* (2010). Indeed, higher lipid contents in *Chlorella vulgaris* were found with different CO_2 concentration ranged 0.5 to 12%. Cultures bubbled with 0.5, 1.0, 6.0 and 12.0% CO_2 have given the following lipid content of 18.1, 20.0, 16.3 and 14.2%, respectively. Furthermore, it seems that lower CO_2 concentration favored the lipid accumulation because of a higher lipid productivity at 1.0% CO_2, which is 2.6 times higher than 12.0% CO_2.

Finally, no significant difference was found in the proportion of lipid classes between 2% CO_2 and air culture conditions in *Chlorella vulgaris*, but alterations in the composition of the fatty acids were observed. The relative content of C18:2 was higher in 2% CO_2, while that of C18:3 was much lower than in air, inducing an increase of C18:2/C18:3 ratio of 3.9 fold (Tsuzuki *et al.*, 1990).

5.2 Organic carbon

Some microalgae and Cyanobacteria are also able, in addition to atmospheric CO_2 to use carbohydrates or simple organic acids such as glucose, glycerol or acetate. The main advantage of heterotrophic culture is that it makes it possible to produce biomass and molecules of interest with a much greater yield compared to autotrophic culture, in some cases reducing production costs. For example, the amount of floridean starch in *Galdieria sulphuraria* increases significantly when it is cultivated in the presence of glucose, whether in heterotrophic or mixotrophic mode (concomitant presence of at least one organic carbonaceous substrate and light). In addition, its lipid content, and more particularly in polyunsaturated fatty acids (PUFA), was greater in heterotrophic conditions (Sakurai *et al.*, 2016). Other examples are presented Table 3.

Lipids obtained from *Chlorella* species in heterotrophic cultivation are considered as a suitable feedstock for biodiesel production. The growth and lipid productivity of an isolated microalga *Chlorella vulgaris* ESP-31 were investigated under different media and cultivation conditions, including phototrophic growth, heterotrophic growth, photoheterotrophic growth and mixotrophic growth by Yeh and Chang (2012). They found that mixotrophic cultivation, with all the culture media tested, has given both the higher lipid content and lipid productivity.

Wang *et al.* (2012a) have conducted a mixotrophic cultivation of *Phaeodactylum tricornutum* with different organic carbon sources (sodium acetate, soluble starch and glucose) at different concentrations (from 0.5 to 5.0 g.L^{-1}). Results showed that the lipid content and lipid productivity of the microalga tending to increase significantly in all tested mixotrophic conditions, especially with glucose. The maximum lipid productivities in mixotrophic cultures with glucose, starch and acetate in medium were respectively 4.6-, 2.0-, and 1.7-fold of those obtained in the control. Total fatty acids were mainly composed of C16:1 (palmitoleic acid), C16:0 (hexadecylic acid) and C20:5n3 (eicosapentaenoic acid, EPA) for all conditions (Wang *et al.*, 2012a). At the opposite, no significant differences in biomass or lipid production were observed in mixotrophic cultivation with acetate in *Scenedesmus abundans* (Gupta and Pawar, 2018).

Crude glycerol is a major by-product accumulated during the production of biodiesel which have usually has little commercial value. Nevertheless, its glut represents option for the value-added conversion. One possibility for its utilization is to use it as a substrate for fermentation, as example in the oleaginous microalga *Schizochytrium limacinum* which can produce significant amounts of total lipids and specially docosahexaenoic acid (DHA, 22:6 n-3) (Chi *et al.*, 2007; Pyle *et al.*, 2008).

For example, mixotrophic conditions with crude glycerol improved both the growth and the accumulation of triacylglycerols (TAG) in *Chlorella vulgaris*, *Botryococcus braunii* and *Scenedesmus* sp. (Choi and Yu, 2015). The three microalgal species had between 2 and 13% higher lipid content compare to the autotrophic condition. The highest TAG content was obtained for all strains with 5 g.L^{-1} glycerol

Table 5. Examples of the impact of temperature on lipid production in some microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Scenedesmus sp. LX1	20 °C	35.0%, 112.0 mg.L^{-1}.d^{-1}	C22:3n8), linoleic acid (C18:2n7), palmitic acid (C16:0)	Xin *et al.* (2011)
Chlorella minutissima UTEX 2341	20 °C	15.0%, n.d	linoleic acid (C18:2n6), oleic acid (C18:1n9), palmitic acid (C16:0)	Cao *et al.* (2014)
Chlamydomonas reinhardtii BAF-J5	32 °C	76.4%, n.d	palmitic acid (C16:0), oleic acid (C18:1), linolenic (C18:2)	James *et al.* (2013)

(15.91, 16.24 and 16.41 % for *Chlorella vulgaris*, *Scenedesmus* sp. and *Botryococcus braunii*, respectively).

6 Light

One of the most essential parameter to the production of algal biomass is the light source. The photosynthesis process is closely linked to the quantity of photons perceived by the cell. A decrease in Photosynthetic Photon Flux Density (PPFD) leads to suppression of CO_2 concentrating mechanism (CCM) and a decrease in CO_2 transport capacity in *Anabaena variabilis* and *Chlamydomonas reinhardtii* (Spalding *et al.*, 1984; Ogawa and Ogren, 1985). To optimize the use of photons, it is necessary to study the influence of light intensity on the process of photosynthesis for each strain. For this it is necessary to determine the growth rates as a function of the PPFD. A study was carried out in *Chlamydomonas reinhardtii* and allows to divide the growth curve into three parts: (1) the first has shown a linear relationship between the incident photon flux and the growth rate, proving that light is a limiting factor for growth; (2) the second corresponds to the light intensities that cause saturation of the photosynthetic process, corresponding to an optimal light intensity meaning all photons were used; (3) Finally, the third corresponds to the higher light intensities which lead to a decrease in photosynthetic activity and therefore have a negative impact on growth. This is photoinhibition (Janssen *et al.*, 2000). From this point of view, use an adequate light intensity favors both overproduction of biomass and microalgal lipids (Khotimchenko and Yakovleva, 2005; Solovchenko *et al.*, 2008; Gim *et al.*, 2016) (Tab. 4).

Sun *et al.* (2014) have observed a correlation between TAG content in *Neochloris oleoabundans* HK-129 and the increase of light intensity. The maximum TAG content and productivity were obtained at 200 µmol photons.m^{-2}.s^{-1}. C16/C18 fatty acids (90.02%), saturated fatty acids (29.82%) and monounsaturated fatty acids (32.67%) were the major constituents of its lipid composition, which is suitable for biodiesel synthesis. In addition, high irradiances stimulated a decrease of chlorophyll a, fucoxanthin and amino acids content and induce the conversion of polar fatty acids to saturated and unsaturated fatty acid in *Isochrysis galbana* (Aguilera-Sáez *et al.*, 2019).

Time exposure to the light could also affect the fatty acid composition. In *Chlorella vulgaris* the maximum of total saturated fatty acid was recorded at 100 µmol photons.m^{-2}.s^{-1} with a 16 h:8 h photoperiod. At the opposite, monounsaturated and polyunsaturated fatty acids decreased with increasing irradiance and light duration. The maximum of total MUFA (15.93%) and PUFA (27.40%) was recorded at 37.5 µmol photons.m^{-2}.s^{-1} with a 8 h:16 h photoperiod (Amini Khoeyi *et al.*, 2012).

Nzayisenga *et al.* (2020) have shown that increased light intensity from 50 to 300 µmol photons.m^{-2}.s^{-1} induced changes in lipid composition in *Desmodesmus* sp., *Scenedesmus obliquus* and *Chlorella vulgaris* with lower amounts of C18:3, and higher contents of C18:1 which became the most abundant fatty acid. This increase in intracellular lipid when microalgae are subjected to high light intensity could be attributed to the oxidative stress from photocatalyst. Under high light stress, microalgae accumulate a large amount of reactive oxygen species (ROS) including singlet oxygen (O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals, resulting in the direct decomposition of the lipids, protein, and nucleic acids. In 2014, Kang *et al.* (2014) have exposed *Chlorella vulgaris* UTEX 265 to an artificial oxidative stress using various concentrations of TiO_2 ranging from 0.1 to 5 g.L^{-1} under UV-A illumination. FAME composition of C18:3 fatty acid (linolenic acid) was highest with 0.1 g.L^{-1} of TiO_2 under 4 days induction time, and slightly decreased, with increased concentration of TiO_2. Furthermore, 0.1 g.L^{-1} of TiO_2 given the best lipid content and lipid productivity in the 2 days induction condition (11.35% and 18.16 m.L^{-1}.d^{-1}, respectively). The same year, Yilancioglu *et al.* (2014) have submitted the microalgae *Dunaliella salina* Tuz_KS_01 to an oxidative stress with different concentrations of H_2O_2. The ROS accumulation and lipid contents increased with increasing H_2O_2 concentrations, leading to lipid accumulation up to 44%.

The wavelength of light is just as important as its intensity. Different wavelength will produce different results in both biomass production and lipid content. For example, *Nannochloropsis salina*, *Nannochloropsis oceanica* and *Nannochloropsis oculata* growth faster under blue light-emitting diode (LED) and have the highest lipid content under green LED. A two-phase culture was then tested, with blue LED in phase 1 to optimize growth follow by green LED to upgrade the amount of lipid. The lipid contents of the three *Nannochloropsis* sp. reached 52, 53 and 56% for *N. salina*, *N. oceanica* and *N. oculata*, respectively (Ra *et al.*, 2016). In *Scenedesmus obliquus*, both blue and red LEDs given the best biomass productivity and lipid content compare to the white light. Furthermore, a combined blue-red illumination with 1:1 ratio has given better results compare to only one wavelength use (Abomohra *et al.*, 2019).

Table 6. Examples of impact of salt on lipid production by microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Dunaliella tertiolecta ATCC 30929	NaCl	67.8%, n.d	n.d	Takagi *et al.* (2006)
green microalga UTEX 2219-4	NaCl	30.1%, n.d	n.d	Wang *et al.* (2011)
green microalga UTEX 2219-4	KCl	27.6%, n.d	n.d	
Monoraphidium dybowskii LB50	NaCl	41.7%, 57.50 mg.L^{-1}.d^{-1}	n.d	Yang *et al.* (2014)
Scenedesmus obliquus	NaCl	36.0%, n.d	palmitic acid (C16:0), oleic acid (C18:1n9c)	Kaewkannetra *et al.* (2012)
Chlorella minutissima UTEX 2341	NaCl	31.8%, n.d	linoleic acid (C18:2n6), oleic acid (C18:1n9), palmitic acid (C16:0)	Cao *et al.* (2014)
Chlorella vulgaris	Multiple NaCl induction	58.6%, n.d	n.d	Duan *et al.* (2012)

n.d: not determined

7 Temperature

Among the various culture conditions, enhancement of lipid production in microalgae by temperature is studied long ago (Norman and Thompson, 1985). The effect of temperature on microalgal growth and lipid production is similar to that of light intensity. Actually, microalgal growth as well as lipid production exponentially increases to a certain extent as the temperature increases and reaches an optimal level, dependent on each microalga strain, as presented in Table 5.

As an example, Jiang and Gao (2004) have showed that the production of EPA and PUFA by *Phaeodactylum tricornutum* increased by 50 and 20%, respectively, when the temperature switch from 25 to 20 °C after 6 days of culture, respectively. In *Nannochloropsis salina*, both TFA and EPA contents increased under a low culture temperature (17 °C) compared to moderate temperatures of 21 and 26 °C (Hoffmann *et al.*, 2010).

James *et al.* (2013) have compared lipid production at different temperatures, in both quantity and quality, between two strains of *Chlamydomonas reinhardtii*: a wild type and its starchless mutant BAF-J5. There was significant increased in total fatty acid content for all temperature in the mutant compare to wild type. Furthermore, shifting temperature from 25 to 32 °C resulted of maximum quantity of fatty acid in BAF-J5, which is 6.15 fold higher than wild type. Shifting temperature to 17 °C, reduced both the total amount of fatty acid from 68.29 to 55.63% DW and proportions of unsaturated fatty acid, especially C18:1.

Finally, Wei *et al.* (2015) studied temperature effects on lipid properties of microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata*. They found that increased temperature led to a decrease in neutral lipid and polyunsaturated fatty acids (PUFA) while saturated fatty acids increased (SFA). In *Tetraselmis subcordiformis* these changes were accompanied by an increased in monounsaturated fatty acids (MUFA), whereas it was decreased for *Nannochloropsis oculata*. These studies demonstrate how a critical environmental factor, such as temperature, can modulate both the amount and composition of fatty acids and it is important for costly refining of biofuels.

8 Salinity

Dunaliella species provide the best examples of microalgae that can tolerate high salt concentrations. The ability of *Dunaliella* species to proliferate over practically the saturation range of salinities makes them good candidates to study salinity effects on lipid production in microalgae. As an example, increase NaCl concentration from 0.5 to 1.0 M resulted in 11% of more intracellular lipid content and 39% of the proportion of triacylglyceride (TG) in *Dunaliella tertiolecta* ATCC 30929. Also, addition of 0.5 or 1.0 M NaCl at mid or the end of log phase during cultivation, with initial NaCl concentration of 1.0 M gave a significant increase in lipid content (70%). However no significant change in TG fraction was observed (Takagi *et al.*, 2006). Increased NaCl concentrations from 13 to 40 g.L^{-1} enhanced TFA content in *Nannochloropsis* sp. EPA production was slightly higher at the lowest level of salinity (Pal *et al.*, 2011).

The effect of salinity on lipid storage was also considered in *Scenedesmus obliquus*, using NaCl solutions of varying concentrations (0.05, 0.2, and 0.3 M). Algal oil storage of up to 36% DW was observed in the culture supplemented with 0.3 M NaCl at day 15 after salt stress, which was 3.8 fold higher than before the salt stress induction (Kaewkannetra *et al.*, 2012).

In the diatom *Nitzschia laevis* Hust, the maximum specific growth rate and biomass concentration were obtained at 10 g.L^{-1} NaCl. Increase salinity induces a diminution of neutral lipids (NL) from 78.6 to 43.4% of total lipids, especially TAG. On the contrary, the content of phospholipids (PL) increased from 11.6 to 39.9% of total lipids. EPA represents the major PUFA and its highest content was obtained at 20 g.L^{-1} NaCl (Chen *et al.*, 2008).

In order to avoid inhibited cells growth promote by NaCl stress, Duan *et al.* (2012) decided to applied multiple osmotic stress with different NaCl additions to *Chlorella vulgaris* to improved lipid production. After 180 h, three induction of NaCl stress (2 g.L^{-1} at 80 h, 4 g.L^{-1} at 100 h and 6 g.L^{-1} at 120 h) achieved a lipid yield of 3.16 g.L^{-1} and corresponding to 58.6% of intracellular lipid content, which were 21 and

Table 7. Examples of the impact of hormones on lipid production by microalgae.

Microalgae	Stress conditions	Lipid content, lipid productivity	Major lipids	References
Scenedesmus quadricauda SDEC-13	Diethyl aminoethyl hexanoate	28.4%, 32.69 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), oleic acid (C18:1), linolenic (C18:2)	Jiang *et al.* (2015)
Chlorella ellipsoidea SDEC-11	Diethyl aminoethyl hexanoate	32.5%, 39.13 mg.L^{-1}.d^{-1}	palmitic acid (C16:0), linolenic (C18:2), oleic acid (C18:1)	
Scenedesmus obliquus GU732418	Indole-3-acetic acid and diethyl aminoethyl hexanoate	n.d	g-linolenic acid (C18:3n6), palmitic acid (C16:0)	Salama *et al.* (2017)
Ourococcus multisporus GU732424	Indole-3-acetic acid and diethyl aminoethyl hexanoate	n.d	γ-linolenic acid (C18:3n6), palmitic acid (C16:0), linoleic acid (C18:2n6c)	
Chlorella vulgaris FR751187	Indole-3-acetic acid and diethyl aminoethyl hexanoate	n.d	γ-linolenic acid (C18:3n6), palmitic acid (C16:0), oleic acid (C18:1n9c)	
Desmodesmus sp. JS07	Indole-3-butyric acid and indole-3-propionic acid	34.9%, n.d	n.d	Singh *et al.* (2020)
Desmodesmus sp. JS07	Indole-3-butyric acid and benzylaminopurine	42.4%, n.d	oleic acid (C18:1), palmitic acid (C16:0), palmitoleic acid (C16:1)	
Monoraphidium sp. QLY-1	Strigolactone	48.8%, 63.7 mg.L^{-1}.d^{-1}	n.d	Song *et al.* (2019)
Nannochloropsis oceanica CASA CC201	Methyl jasmonate	n.d	palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1)	Udayan *et al.* (2020)
Tetradesmus obliquus BPL16	Salicylic acid	30.1%, 326 mg.L^{-1}.d^{-1}	n.d	Esakkimuthu *et al.* (2020)
Tetradesmus obliquus BPL16	Jasmonic acid	27.5%, 357 mg.L^{-1}.d^{-1}	oleic acid (C18:1), palmitic acid (C16:0)	
Tetradesmus obliquus BPL16	*p*-coumaric acid	30.7%, 341 mg.L^{-1}.d^{-1}	oleic acid (C18:1), palmitic acid (C16:0), C16:2	

n.d: not determined

22.8% higher than control. Another addition of $6\,\text{g.L}^{-1}$ at 140 h did not upgrade both lipid content and lipid yield. These result suggested that application of multiple osmotic stress could be a promising method to lipid overproduction while decreasing the loss of biomass. Other examples are presented in Table 6.

9 Hormones

The approaches presented in the previous parts have been known and studied for many years, on various microalgae and *Cyanobacteria*. Recently, the literature has seen a growing number of publications about the use of hormones to improve lipid production in microalgae (Tab. 7).

Many types of hormones have been used more or less successfully. Among the most promising ones, Jiang *et al.* (2015) have shown that very low concentration of DA-6 (10^{-7}M) could enlarge cell size and enhance cell growth of *Scenedesmus quadricauda* SDEC-13 and *Chlorella ellipsoidea* SDEC-11. Biomass productivity was 14 and 336% higher than without DA-6, respectively. The higher lipid productivity occurred in 10^{-7}M medium, representing 30% and 15% of increase compare of controls (Jiang *et al.*, 2015).

In 2017, Salama *et al.* (2017) found that indole-3-acetic acid (IAA, auxin) and diethyl aminoethyl hexanoate (DA-6)

positively affect the growth and lipid productivity of *Scenedesmus obliquus* GU732418, *Ourococcus multisporus* GU732424 and *Chlorella vulgaris* FR751187. The amounts of PUFA were significantly increased.

In *Desmodesmus* sp. JS07, supplementation of culture medium with $10\,\text{mg.L}^{-1}$ of indole-3-butyric acid (IBA) and indole-3-propionic acid (IPA) significantly increase the lipid content and lipid yield. Higher lipid content was observed with $10\,\text{mg.L}^{-1}$ benzylaminopurine (BAP) treatments. More, a combination of IBA ($10\,\text{mg.L}^{-1}$) and BAP ($5\,\text{mg.L}^{-1}$) resulted to better biomass concentration and lipid content, sign of synergistic effect of auxin and cytokinins (Singh *et al.*, 2020).

Another hormone, the strigolactone was used on *Monoraphidium* sp. QLY-1 cultures and led to enhance lipid content and lipid productivity under $1\,\mu$M induction compared (Song *et al.*, 2019). Nevertheless, higher lipid content and lipid productivity were obtained in nitrogen deficiently without strigolactone induction (Song *et al.*, 2020). Similar study performed on *Nannochloropsis oceanica* CASA CC201 have shown that treatment with 10 ppm of methyl jasmonate (MeJA) also promoted biomass and lipid yields, with an increase of 22% and 49% more than control (Udayan *et al.*, 2020). Increase MeJA concentration beyond 10 ppm induce a decrease of essential ω3 fatty acid but promoted monounsaturated fatty acid production, especially oleic acid.

Table 8. Examples of the impact of co-cultivation with microorganisms on lipid production by microalgae.

Microalgae	Co-cultivated microorganisms	Lipid content, lipid productivity	Major lipids	References
Tetraselmis striata KCTC1432BP	*Pelagibaca bermudensis* KCTC 13073BP	n.d	palmitic acid (C16:0), linolenic acid (C18:2), stearic acid (C18:0)	Park *et al.* (2017)
Tetraselmis striata KCTC1432BP	*Stappia* sp. KCTC 13072BP	n.d	palmitic acid (C16:0), linolenic acid (C18:2), stearic acid (C18:0)	
Chorella minutissima UTEX 2341	*Escherichia coli* ATCC 25922	24.5%, 83.9 mg.L^{-1}.d^{-1}	n.d	Higgins and VanderGheynst (2014)
Chlorella vulgaris	*Mesorhizobium sangaii* 13218	51.2%, 96.8 mg.L^{-1}.d^{-1}	oleic acid (C18:1), palmitic acid (C16:0), linolenic acid (C18:2)	Wei *et al.* (2020)
Chlorella sacchrarophila FACHB 4	*Cellvibrio pealriver* PR1	53.9%, 61.9 mg.L^{-1}.d^{-1}	n.d	Xie *et al.* (2021)
Chlorella vulgaris MSU-AGM 14	*Streptomyces rosealbus* MTTC 12.951	17.5%, 21.1 mg.L^{-1}.d^{-1}	oleic acid (C18:1), palmitic acid (C16:0), linolenic acid (C18:2)	Lakshmikandan *et al.* (2021)
Chlorella protothecoides	*Aspergillus fumigatus*	n.d	linolenic acid (C18:2), palmitic acid (C16:0)	Chu *et al.* (2021)

n.d: not determined

Another interesting approach is to use the *p*-coumaric acid as a suggested novel growth regulator typically found in wastewaters of agricultural origin. 1 mM increased the lipid accumulation up to 30.7%, leading to a higher lipid production in *Tetradesmus obliquus* BPL16. Furthermore, neutral lipids were enhanced under 10 mM of jasmonic acid and 1 mM of *p*-coumaric acid to the detriment of both phospholipids and glycolipids. The SFA content decreased of 37–40% and the MUFA content increased of 36–56%. Oleic acid was the major constituent, but its proportion was drastic increase up to 81.7 and 73.6% of TFA, respectively (Esakkimuthu *et al.*, 2020).

10 Co-cultivation with microorganisms

Diverse strategies can be used to get a biologically plausible optimization of the microalgae culture such as using indigenous microalgae added with other microorganisms such as fungi or bacteria. In nature or industrial processes as wastewater treatment plant, microalgae co-exist with many other microorganisms in complex microbial communities which may have influence on microalga growth and lipid production (Cho *et al.*, 2015). These positive effects can be explained by interactions between the microorganisms and microalgae called symbiosis. The oxygen produced by the microalgae along the photosynthetic process can be used by the microorganisms, which produced in response carbon dioxide for microalgae. Additionally, there is no competition for the carbon source since microalgae and microorganisms consume inorganic and organic carbon, respectively. This fact results in an enhanced yield of biomass and higher concentrations of secondary metabolites (carbohydrates, proteins, and lipids) (Tab. 8).

The impact of two isolated bacteria (*P. bermudensis* and *Stappia* sp.) have been studied on *T. striata* growth and lipid content. The results showed that *P. bermudensis* and *Stappia* sp. have stimulated the increased of cell density of *T. striata* on the 25th day compared to the axenic *T. striata* culture. The analyses of lipid content in the treated and axenic cultures suggested that the axenic cultures had better capacity to accumulate lipids. However, total lipid productivity was still substantially promoted by the bacterial treatment compare to control because of the increase of total biomass production (Park *et al.*, 2017).

Mixotrophic cultivation also significantly affected total fatty acid composition in *C. minutissima*. Compared to axenic *C. minutissima*, provision of organic substrate had a positive effect on both fatty acid and neutral lipid content in co-cultures of *C. minutissima* and *E. coli*. This was particularly true at 10 g. L^{-1} glucose where total fatty acid and total lipid contents increased to 40% and 32%, respectively. Compared to axenic culture, co-culture with 10 g.L^{-1} glycerol substrate leading to a large increase in oleic acid and linolenic acid contents. This resulted of a decrease of C18:3 and C16:0 content (Higgins and VanderGheynst, 2014). A later study revealed that a positive effect may be traced to *E. coli*'s provision of thiamine derivatives and degradation products to *A. protothecoides* (Higgins *et al.*, 2016).

In 2020, Wei *et al.* (2020) have co-cultivated *Chlorella vulgaris* with *Mesorhizobium sangaii* under nitrogen deficiency condition. The maximum biomass, lipid content and productivity of algae in the co-cultures were 1.5, 2.2, and 3.3 times higher than those of the pure algae culture. Furthermore, the neutral lipid productivity was 3.5-fold higher compared to control, resulting in an increase of 50% of neutral lipid proportion. FAME profile was also affected. The proportion of PUFA and MUFA were increased whereas the proportion of SFA was decreased. In addition the content of C18:1 and C18:2 were also significantly increased thanks to the consortium system (Wei *et al.*, 2020).

More recently, a continuous two-step (CTS) cultivation strategy based on separated co-culturing was developed. At the first-step co-cultivation, *Chlorella sacchrarophila* was grown in bioreactor while *Cellvibrio pealriver* was grown in a dialysis bag as inner bag inside the bioreactor. Then, the inner bag was removed which then start the second-step cultivation of *C. sacchrarophila*. Results shown that after 3 days of co-cultivation, the production of microalgal biomass was similar with that obtained in single reactor. The microalgal growth almost ceased after that the inner bag being removed. The maximum biomass production was determined as 1725.41 mg. L^{-1} which was lower compared to microalga grown in one-step co-cultivation. At the opposite, the microalgal lipid content immediately increased after removing the inner bag and reached 53.89% after 15 days cultivation, which was almost 2.5-fold higher than the one-step co-cultivation. The microalgal lipid production in CTS and one-step co-cultivation were determined as 929.79 and 607.90 mg.L^{-1}, respectively (Xie *et al.*, 2021).

Chlorella vulgaris and *Streptomyces rosealbus* were co-cultivated to evaluate the impact of co-culture system in microalgae growth and lipid production. The co-cultured microalgae produced 1.42 g.L^{-1} of biomass and, which represented 33% higher biomass productivity compare to the control. The maximum lipid production and productivity were increased by 80%. This result can be explained by the symbiosis between *C. vulgaris* and *S. rosealbus* that triggered Trp biosynthesis to increased volume. Trp is the main precursor for IAA secretion pathway. An augmentation of around 82–140% of IAA production was recorded in co-culture system compared to monoculture, which lead to significant elevation of microalgal biomass and lipid productivity. The co-culture has shown no significant effect on the total FAMEs. However, the SFAs, MUFAs and PUFAs ratios showed significant variations compared to control, with an increase of 70% of PUFA and a decrease of 12% of MUFA (Lakshmikandan *et al.*, 2021).

As microalgae/bacteria co-culture, co-culturing oleaginous yeast and microalgae have been studied extensively in the recent years for enhancing lipid productivity. In most of the cases, this strategy resulted in increasing the lipid productivity in both microalgae and yeast cultures.

Arora *et al.* (2019) have recently highlighted both the metabolic links between the lipid biosynthesis pathway of yeast and microalgae, and factors affecting the lipid productivity in the co-culturing system. According to them, the strain selection is the most important parameters. The best yeast and microalgal species to promote lipid production in co-culture system belong the genera of *Rhodotorula* sp. and *Chlorella* sp. The second more important abiotic factor was the microalgae/yeast ratio. They determined that the optimal value was 1:1.

More recently, Chu *et al.* (2021) have compiled results of the biomass and lipid concentrations obtained from separately cultured fungus and algae or co-cultured pellets. Compared with mono-cultivation, the significant increase in biomass and lipid yields could be observed in co-cultured microalgal/fungus co-culture, probably due to the symbiosis system. The maximum biomass and lipid yield were obtained using *Aspergillus fumigatus* co-cultivated with *Chlorella protothecoides*. It was 2 and 2.15 higher compared to the respective

monocultures. However, the microalgal/fungus combination has no significant effect on the FAMEs profile, since it was mainly composed of the same lipids that the microalgal mono-culture (C16:0 and C18:2) (Chu *et al.*, 2021).

11 Multi factor optimization

11.1 Experimental approach

Each environmental parameter could be optimized independently each other in order to improve the lipid production in microalgae. However, the culture process is a complex system in which many parameters can interact with each other. It is therefore particularly advantageous to co-optimize these parameters by taking account of their interactions in order to improve the lipid productivity. Recent reports have presented the potential of combining abiotic stress factors to improve the lipid productivity in microalgae.

In 2016, Kwak *et al.* (2016) studied the combined effect of various stress conditions on lipid productivity in *Chlamydomonas*, *Chorella*, *Scenedesmus* and *Neochloris* strains. Compared to the only nitrogen starvation stress, its combined effect with high salt stress condition has improved the lipid productivities of *Chlamydomonas* species up to 50% and 30% for *Chlorella protothecoides*. The lipid productivities of *C. vulgaris* and *N. oleoabundans* were improved up to 39% and 37% respectively under the combined condition of N-starvation and 30 °C temperature compared to the maximum with single stress condition. In *C. zofingiensis*, the increase of lipid production up to 44% with the combined effects of 30 °C culture temperature and high NaCl concentration. The optimal combined stress conditions in *Scenedesmus* sp. were high temperature and 10-pH. It induced 1.54-fold higher lipid productivity compared to the lipid productivity under only the 10-pH condition.

The lipid content of *H. pluvialis* could also be increased by melatonin induction coupled with nitrogen deficiency and photoinduction at 150 μmol photons.m^{-2}.s^{-1}. The highest lipid content was 42.84% which was 1.22-fold greater than that of the control samples (Ding *et al.*, 2018). In *Chlorella* sp. BUM11008, the combined iron and potassium-phosphate deprivations leading to a substantial increase in lipid content of the cell with significant increase in linolenic acid and arachidic acid, which were the major PUFA and SFA of the fatty acid composition (Praveenkumar *et al.*, 2012).

More recently, Song *et al.* (2020) have demonstrated that combined effect of nitrogen deficiency with strigolactone induction could increase the lipid content and lipid productivity in *Monoraphidium* sp. QLY-1. Authors observed that the jasmonic acid (JA) content was also increased rapidly under nitrogen deficiency with or without SL conditions. JA is generally regarded as an oxylipin signaling molecule that plays diverse functions in plants and microalgae especially in defense and stress responses. Authors suggested that the increased in lipid production may be related to the stimulation of JA synthesis under combined nitrogen deficiency and SL treatment in microalgae. An exogenous JA supplementation was tested and led to the highest lipid content (57.12%) and productivity (322.65 mg.L^{-1}.d^{-1}) under nitrogen stress combined with SL treatment, validating the role of JA in regulating lipid synthesis and oxidative stress response in microalgae

(Song *et al.*, 2020). Also, Zhao *et al.* (2021) have demonstrated the effect of melatonin on lipid accumulation in *Monoraphidium* sp. QLY-1 under salinity stress. The melatonin induction significantly promoted the lipid content, which was 1.16- and 1.44-fold higher than those induced by salinity stress alone and control, respectively.

Also, combined effect on growth and lipid accumulation has been recently demonstrated between nitrogen starvation and IAA on *N. oceanica*. A drastic decease of SFA associated to drastic increase of MUFA and PUFA were obtained with combined factors. These changes are more and more important as the concentration of IAA increases (Touliabah and Almutairi, 2021).

Very recently, Chen *et al.* (2020a) have chosen to use glycerol as a low-cost carbon source as well as an inexpensive nitrate source (corn steep liquor) to investigate the potential of *Thraustochytrium* sp. BM2 for cost-effective lipid production. Lipid content and productivity reached 76 % and 37 mg.L^{-1}.h^{-1} when corn steep liquor and glycerol were used at 12.5 g.L^{-1} and 10 g.L^{-1}, respectively. Lipid content could be further enhanced to 79% by addition of sea salt (2% w/v). Crude glycerol with acid and $CaCl_2$ pretreatment enhanced the overall lipid productivity to 131 mg.L^{-1}.h^{-1}. A semi-continuous culture with harvesting of 50% of the microalgal culture every cycle allowed to enhanced overall lipid productivity to 158 mg.L^{-1}.h^{-1} (Chen *et al.*, 2020b).

11.2 Statistical approach

Optimizing the microalgae biomass productivity through the optimization of different abiotic factors is one of the crucial improvements to increase lipid production. As it has been demonstrated, these optimal conditions are strain dependent.

Several studies have shown the benefit of using statistical models to optimize growth parameters. For example, Miranda *et al.* (2016) have used a simple 2^3 factorial design combined with the Response surface methodology (RSM) in order to optimize nitrate, phosphate and salt concentrations to improve the lipid yield of *Ankistrodesmus* sp. and *Chlamydomonas* sp. Their results indicated significant interactions between the three factors for both biomass and lipid productivities, with a confidence level equal to 95% for the two species. The maximal biomass productivities for *Ankistrodesmus* sp. and *Chlamydomonas* sp. (34 mg.L^{-1}.d^{-1} and 90 mg.L^{-1}.d^{-1}, respectively) were achieved with concentrations of nitrate at 0.04 and 0.08 g.L^{-1}, phosphate at 0.02 g.L^{-1} for both microalgae, with absence of sodium chloride for *Ankistrodesmus* sp. and independent level of sodium chloride for *Chlamydomonas* sp.

More recently, a Central Composite Face-centered (CCF) design with RSM were used to determine the optimum light intensity and CO_2 concentration to obtain the best biomass and lipid productivity of *Ettlia* sp. YC001. Based on the model, the maximum biomass and lipid productivity were 28 ± 1.5 g.m^{-2}.day^{-1} at 730 µE.m^{-2}.s^{-1} with 8% CO_2 concentration, and 4.2 ± 0.3 g.m^{-2}.day^{-1} at 500 µE.m^{-2}.s^{-1} with 7 % of CO_2 concentration (Kim *et al.*, 2018).

In 2020, a wastewater isolated co-culture microalgae-yeast (CCMY) was evaluated as potential biomass and lipids-efficient producer. It was composed of *Scenedesmus obliquus*, *Scenedesmus* sp. and a number of yeast species including *Candida pimensis*. A Box-Behnken Experimental Design (BBED) was applied to determine the best combination of nutrients and their effect on the biomass and lipids. They determined that the maximum lipid accumulation in CCMY was achieved when the sodium nitrate:potassium phosphate ratio is the smallest one and the potassium phosphate concentration is lower than ferrous sulfate concentration (Suastes-Rivas *et al.*, 2020).

Usually, the number of variables in these statistical studies was restricted to a maximum of 3. However, lipid productivity of microalgae is significantly affected by all aforementioned abiotic factor. A similar study using a three level Box-Behnken design was used to investigate the effect of six variables on lipid productivity and CO_2 fixation of *Chlorella protothecoides* (Binnal and Babu, 2017). Fifty-four experiments and a statistical optimization was performed using RSM. The optimum were determined as follow: pH-6.51, Temperature-28.63 °C, light intensity-5.31 klux, Photoperiod-15.36 h:8.64 h, CO_2 concentration-6.26% (v/v) and aeration rate−2.92 lpm. The highest lipid productivity and CO_2 fixation under these conditions were 274.15 mg.L^{-1}.d^{-1} and 273.66 mg.L^{-1}.d^{-1}, respectively.

Finally, Chiranjeevi and Mohan (2016) have done a design of experimental methodology using Taguchi orthogonal array to evaluate the specific influence of eight important factors (light, pH, temperature, carbon concentration, nitrates, phosphates, magnesium ion concentration and carbon source) on the biomass production. The relative influence of the abiotic factors has been classified as follows: Carbon > Nitrates > Light > Temperature > pH > Carbon sources > Magnesium > Phosphates. In order to understand the influence of the interaction between the individual parameters, the authors assigned them a severity index (SI). The results showed significant interactions among the factors. Carbon concentration with nitrates showed highest SI (85.13%), followed by temperature and light (60.4%) then pH with magnesium (SI 59.3%). The lowest SI was attributed to carbon concentration and light interaction (32.6%). An analysis of variance (ANOVA) with the percentage of contribution of each factor in interaction have confirmed that carbon concentration as the most influential factor, accounting for 16.8% of the overall variance followed by nitrates (12.8%) and light (9.3%). At least, light, carbon concentration and nitrates contributed a majority of 38.9% at their individual levels on the microalgae biomass production indicating their critical role in the optimization.

12 Conclusion and perspectives

The production costs of biomass and lipids will depend on the considered systems (strain, environmental parameters and culture system). Some microalgae, called oleaginous microalgae, are able of producing larger quantities of fatty acids and must be favored for the production of biofuels. It is clear that different species react differently to different stresses by producing different fatty acids or by changing their biochemical composition. This review focused on how environmental factors can play an important role in improving lipid production in microalgae. Of all possible approaches,

nitrogen deprivation appears to be applied most often. Changes in temperature, salt stress, appropriate concentration of metallic trace elements and supplementation of the medium with hormones can also induce lipid accumulation but seem more difficult to control in a large-scale culture system. The identification of other approaches such as the influence of CO_2, the use of organic substrate, light intensity have also been studied in many species. Studies have shown that the optimization of these parameters by statistical or experimental methods makes it possible to significantly improve the lipid production, in particular through the increase in cell growth. However, the exact combination allowing optimal lipid productivity in a given culture system will be specific to each strain of microalgae. Further work is therefore required and recent advances have been made with the use of other approaches, such as the use of the neurotransmitter (Zhao *et al.*, 2020), a pretreatment of atmospheric-pressure plasma (Almarashi *et al.*, 2020), co-cultures with bacteria and filamentous fungi (Huo *et al.*, 2020; Chu *et al.*, 2021) and transgenic organisms (James *et al.*, 2013; Han *et al.*, 2020). 2-step hybrid systems including optimization of growth in a rich medium and then transfer of part of the culture under conditions favoring lipid production have also been developed (Aziz *et al.*, 2020).

Li *et al.* (2019) has reported that the cost of microalgae production processes was strongly affected by the cost of the raw materials used. An alternative eco-friendly solution to reduce these costs could come from the use of wastewater as nutrient sources. According to Acién *et al.* (2012), the utilization of wastewater in the production of algal biodiesel could reduce the total production costs to \$ 1.5 kg^{-1} and using flue gases from industrial sources can decrease the cost of microalgae production to \$ 2.08 kg^{-1}. To date, some types of wastewater rich in organic matter, such as piggery wastewater, municipal wastewater and monosodium glutamate wastewater have been successfully utilized for the mixotrophic cultivation of microalgae (Wang *et al.*, 2010, 2012b; Ebrahimian *et al.*, 2014; Aketo *et al.*, 2020). Recent work has highlighted methods that can improve lipid production in microalgae cultivated with wastewater (Gao *et al.*, 2019; Tan *et al.*, 2020). Finally, Ren *et al.* (2019) have shown that ultrasonic irradiation could be a promising process to significantly enhance the lipid production by microalgae cultivated in a non-sterile domestic wastewater. From this point of view, the use of wastewater to produce biofuels from microalgae appears to be a cost-friendly and eco-responsible alternative solution since it contributes to the pollutants removal and recycling of water.

Although there is extensive literature on the impact of different abiotic factors taken independently of each other, studies have shown that the co-optimization of these parameters by statistical or experimental methods are future avenues with a view to improve lipid production. However, the optimization of growth associate to culture factors alone remains insufficient to reach profitability thresholds, particularly for applications in the field of biofuels. For this, it is necessary to take into account in its entirety the entire production process and improve all stages. This includes the optimization of the culture parameters, but also the choice in the culture technology (photobioreactor), the method of harvesting and separating the biomass with the culture medium, the extraction of the target compounds, as well as the purification step and that of any biochemical modifications to convert the molecules into biodiesel.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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