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# Influence of environmental stress on microalgae growth and lipid profile: a systematic review

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**Abstract** Microalgae biomass has attracted great interest from researchers as a promising feedstock for biodiesel production. Although enormous research works have been carried out to identify microalgae species with high biomass and lipid productivities, genetic modification of strains as well as optimization of cultivation conditions, however, the progress is yet to be fully satisfied. Based on the present lipid yield and extraction methods, it is still not feasible to commercialize the microalgae biodiesel. One of the promising approaches to elevate lipid accumulation by microalgae is through tuning the cellular mechanisms and metabolic pathway by exposing microalgae cells to various abiotic stress environments such as nutrient starvation, high salinity level and strong light intensity. Nevertheless, a comprehensive analysis of quantitative influences of critical abiotic stress on both microalgae biomass and lipid profile is still limited in the literature. Hence, the present paper aims to deliver insights into selections of single and multiple abiotic stress factors for simultaneous enhancement of microalgae biomass and lipids (polar and non-polar) which could improve the techno-economic viability in the microalgae processing chains and biorefinery industries.

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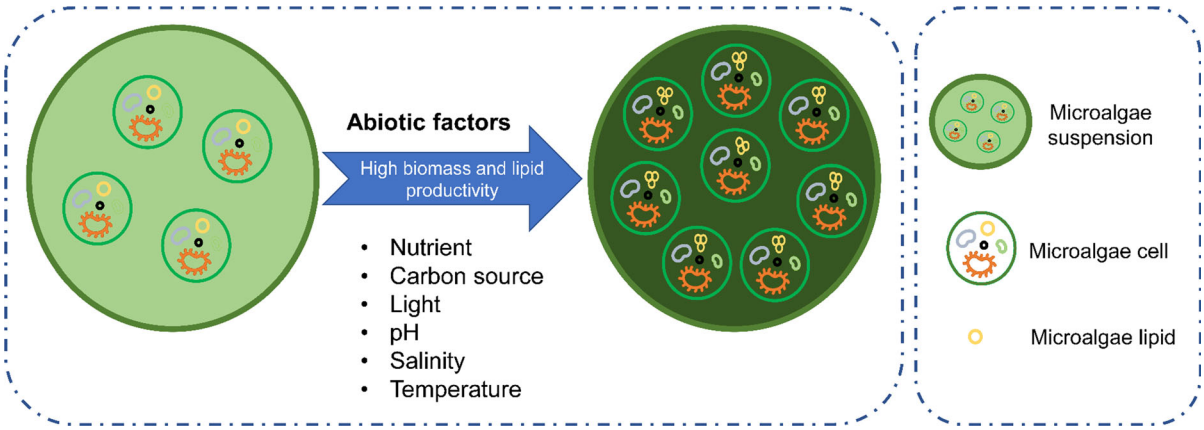
**Graphical abstract**




**Keywords** Microalgae · Biosynthesis · Growth · Lipid productivity · Abiotic stress

## Introduction

Increasing world population and negative environmental impact caused by the excessive burning of fossil fuels, there is an urgent need to explore new and sustainable energy sources (Peter et al. 2021). Currently, 13% of total global energy is contributed by renewable energy, with biofuel contributing 10% of the overall energy usage (Jayakumar et al. 2017). The first-generation biofuels are mainly derived from edible crops such as corn, palm, rapeseed, and sugar cane. Besides, about 50 billion liters (L) of first-generation biodiesel is produced on annual basis (Lee and Lavoie 2013). However, utilization of edible crops for biofuel production is sought to be non-sustainable due to food versus fuel issues, excessive land utilization, and deforestation. Hence, second-generation biofuel has been produced, which includes biodiesel from waste cooking oil and bioethanol from lignocellulosic biomass such as wood chips, sawdust, and grasses (Ramanna et al. 2017). The high contents of carbohydrates in cellulosic biomass make it suitable for biofuel production besides its added advantages of being cheap and abundant in nature without opposing competition to the food supply. Regardless of its wide prospect, second-generation biofuel suffers from a few technical constraints due to high costs related to biomass pre-treatment and processing (Fields et al. 2014). Therefore, microalgae have been recommended by scientists as a feasible feedstock for sustainable biofuel production owing to their lucrative properties.

Microalgae is an autotrophic microorganism, which captures solar energy and utilizes inorganic nutrients to synthesize valuable compounds within their cells, such as lipid, carbohydrate, and protein (Yao et al. 2019). Besides, microalgae cells have several noteworthy physical advantages as well as metabolic versatility over terrestrial crops (Fields et al. 2014), which include high growth rates and high photosynthetic efficiencies (approximately 6–8%) (Raikova et al. 2017) with high tolerance to various stress factors (Dasan et al. 2020). Carbon pollution is one of the critical issues of current millennia and as documented by the UN Environment Programme, the world's atmosphere has reached an alarming level of carbon dioxide ($CO_2$), accounting for 37 billion metric ton annually or 74% of total greenhouse gases emissions (Dasan et al. 2020). Hence, scientists around the globe are continuously improving the carbon capture, utilization, and storage (CCUS) technology in attempt to mitigate climate change and establish a sustainable carbon–neutral future (Jiang et al. 2020). Microalgae, in particular, has emerged as a very promising biological tool to sequester $CO_2$ in present days (Song et al. 2021). Chisti (2007) reported that microalgae cells are able to requester $CO_2$ 10–50 times that of forest in the

same growing area (Chisti 2007). Figure 1 depicts the carbon neutral process of microalgae in which microalgae cells are able to bio-mitigate the tail flue gases from power plants and vehicles (10–20% of $CO_2$ concentration), which serve as carbon source for photosynthesis whilst producing valuable compounds such as lipid for biodiesel production (Song et al. 2021).

Microalgae can be grown in non-agriculture areas by utilizing wastewater, thus reducing the need for land space and chemical fertilizers (Vo Hoang Nhat et al. 2018). Besides able to bio-fix nutrients in the wastewater such as nitrogen and phosphorus into biomass, microalgae can potentially treat various wastewater sources by reducing the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Chai et al. 2021b). For instance, *Chlorella sorokiniana CY-1* was reported to remove 62.07% for total nitrogen (TN), 47.09% for COD, and 30.77% for total phosphorus (TP) (Cheah et al. 2018). Furthermore, *Chlorella pyrenoidosa* accumulated 68% of lipid along with 71% of nutrient removal in palm oil mill effluent (POME) (Low et al. 2021). More interestingly, the capability of microalgae to assimilate wide range of nitrogen sources makes it suitable to treat ammonium-rich wastewater streams that are detrimental to the environment (Chai et al. 2021a). Thus, among a myriad of biofuel products attained from microalgae biomass (i.e., biodiesel, bioethanol, biohydrogen, and biomethane), microalgae biodiesel is the most promising biofuel to replace petroleum-based fuels in the coming years (Fields et al. 2014). This is because most of the microalgae species can accumulate significant amount of lipids than those of oil-bearing crops or animal fats, which can be potentially explored to address the current issue of fossil fuel depletion. Biodiesel produced from microalgae biomass is more environmentally friendly than fossil fuels since it contains less concentration of sulfur (Ramanna et al. 2017). Table 1 presents several microalgae species with high lipid content that have been studied intensively over years for biodiesel generation (Mata et al. 2010).

Although microalgae biomass is highly recognized as a potential feedstock for biofuel production, however, it is still economically infeasible at a commercial scale due to the cost associated with cultivation, harvesting, and biofuel production (Culaba et al. 2020). To overcome this hurdle, researchers are focusing on the manipulations of abiotic stress conditions for yield improvement. Microalgae could alter their biochemical composition under different stress environments, such as elevating lipid concentration within the cells for biodiesel production. In addition, certain microalgae species cultivated under a stress environment could accumulate specific secondary metabolites, which are high-value products with high demand in both

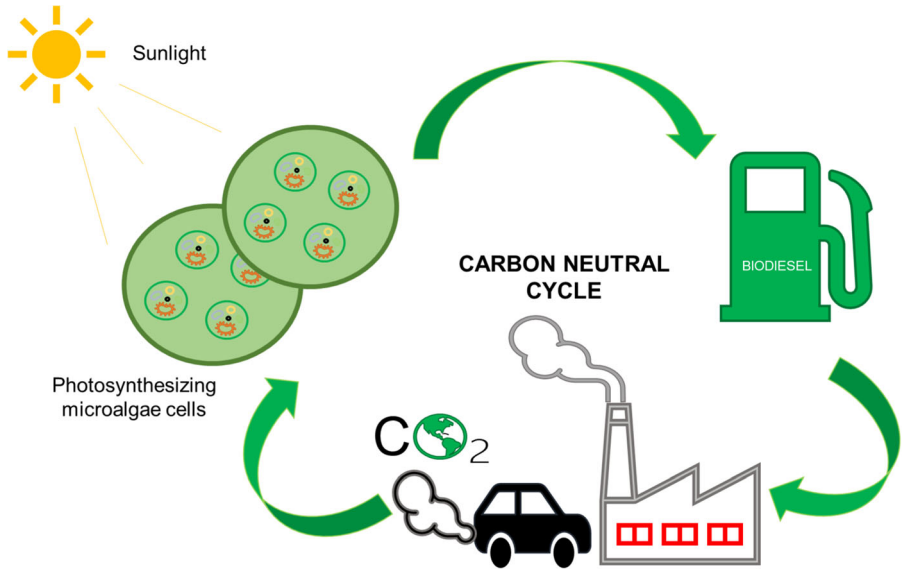



**Fig. 1** Carbon neutral process of microalgae

**Table 1** Lipid contents of microalgae species (Mata et al. 2010)

| Microalgae species | Lipid content (%) |
|---|---|
| *Botryococcus braunii* | 25–75 |
| *Chlamydomonas pitschmannii* | 51 |
| *Chlorella* | 18–57 |
| *Chlorella emersonii* | 25–63 |
| *Chlorella vulgaris* | 5–58 |
| *Monoraphidium* sp. FXY-10 | 56–58 |
| *Nannochloropsis* sp. | 12–53 |
| *Neochloris oleobundans* | 29–65 |

nutraceutical and pharmaceutical sectors. These metabolites include carotenoids (β-carotene, lutein, and astaxanthin), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and phytovitamins (Zhao et al. 2019). Nevertheless, there are some contradictory findings in the metabolite accumulation with microalgae, such as microalgae that have high biomass productivity but low metabolite concentration or vice-versa (Shokravi et al. 2020). Therefore, scientists around the globe are continuously finding approaches to achieve simultaneous high growth rates and metabolite accumulation of microalgae. One of the possible methods is through the manipulation of abiotic stress to tune the cellular metabolism of microalgae and subsequently induce various metabolites from microalgae during their growth phase. Some examples of abiotic stress are such as nutrient deficiency, strong light intensity, high level of salinity, and extreme temperature. (Markou and Nerantzis 2013). Even though genetic modification and metabolic engineering of microalgae appear to be viable options, however, several concerns need to be addressed. For instance, biosafety matters and legislation in certain countries may prevent the use of these modified species. This is because the genetically modified microalgae species have the potential to produce toxins and invade natural ecosystems. Besides, these modified species may produce methane and ammonia which will cause air pollution and global warming (Ramanna et al. 2017). Figure 2 summarizes the advantages and disadvantages of different biofuel generations (Krishna et al. 2021).

To the best of our knowledge, most of the reviews in literature are only focused on the effects of abiotic stress on biofuel products (Paliwal et al. 2017; Zhao et al. 2019), whereas a comprehensive collection of the influences of critical abiotic stress on both microalgae biomass and lipid profile (polar and non-polar) has yet to be annotated. Quantitative information on microalgae growth rates, biomass productivities and polar and/or non-polar lipid yields are crucial to screen out suitable abiotic stress

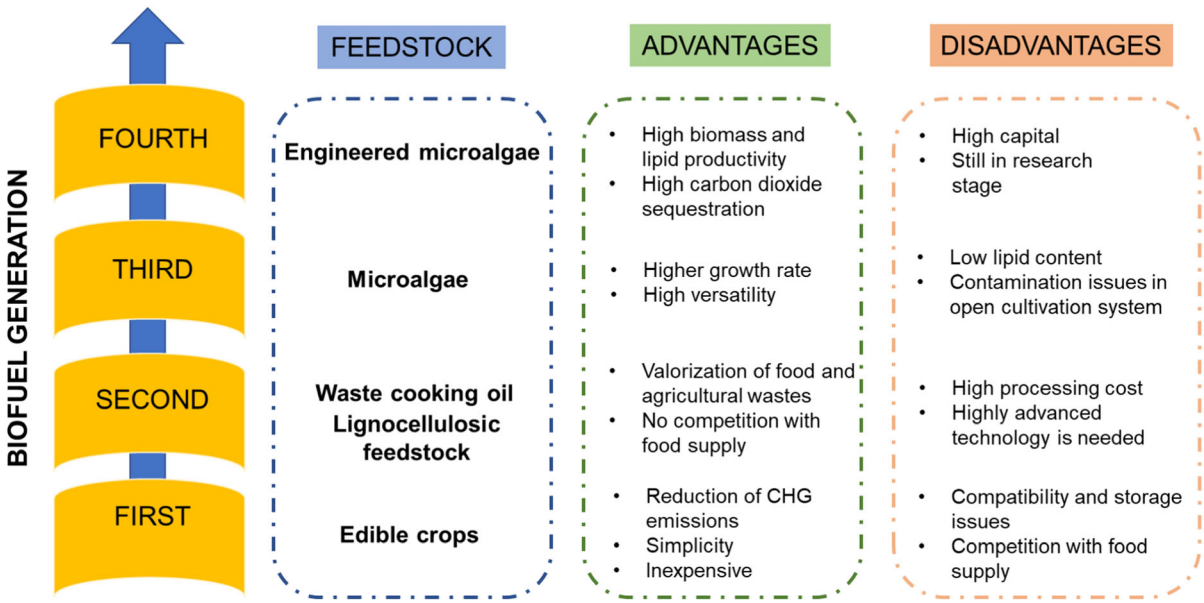



**Fig. 2** Comparison of different types of biofuel generations (Krishna et al. 2021)

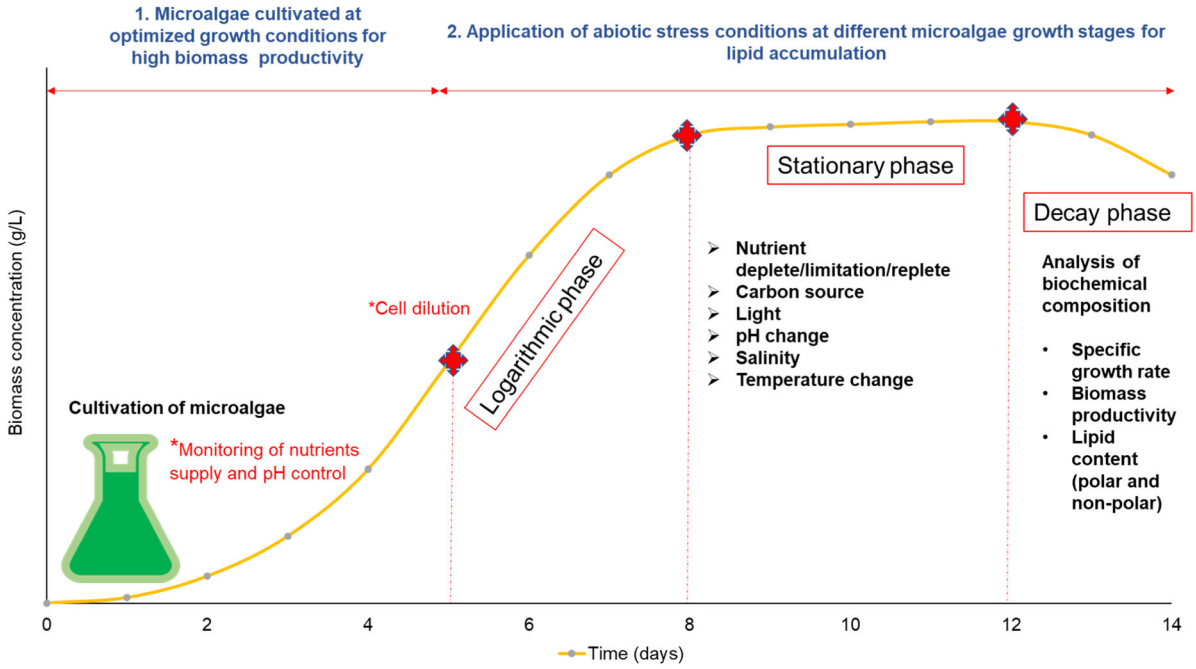



**Fig. 3** Two-stage microalgae cultivation approach

conditions for mass microalgae cultivation to produce biodiesel at commercialization level. We believe this review article will help researchers in microalgae field to elucidate the best single or combination of abiotic stress factors based on the in-depth research works and shed light on achieving the desired biodiesel content of a specific strain without compromising the biomass yield. The compilation of data in the present paper can serve as a blueprint and draw direction for further development of microalgae as a one-pot producer of biodiesel.

## Effects of abiotic stress on lipid profile

### Effect of single abiotic stress

Interest to use microalgae biofuel as a replacement for fossil fuels has sparked efforts to increase lipid productivity from microalgae in recent decades. *Chlorella* sp. is broadly known for providing high fatty acid (FA) profile that is ideal for commercial biodiesel production (Paliwal et al. 2017). Lipid productivity of microalgae is a strain-specific function of physiological responses to various abiotic stresses (Paliwal et al. 2017). A sudden change in the microalgae culture conditions such as temperature, salinity, light, nutrients, and pH, etc. ameliorates microalgae lipid content (Alishah Aratboni et al. 2019). Interestingly, under growth-limiting environmental stress conditions, microalgae cells tend to produce more lipids to survive (Cheng and He 2014). It was reported that, marine microalgae species usually contain 10 to 50 wt% of oil and can accumulate up to 70 wt% of total lipid under stressed conditions (Alishah Aratboni et al. 2019). The nutrient deficiency approach, such as lack of nitrogen and phosphorus source in cultivation medium, has been well documented in increasing the lipids accumulation in microalgae cells (Zhao et al. 2019). In addition to nutrient deficiency, strong light intensity, extreme temperature, and high levels of salinity are also traditionally used to elevate lipid accumulation in microalgae via two-stage cultivation system. In two-stage cultivation approach, microalgae cells are grown under optimized conditions with adequate nutrients to obtain high density of biomass and selective abiotic stress conditions are applied in the later stage for lipid accumulation (Poh et al. 2020) as depicted in Fig. 3.

Microalgae lipids play a crucial role in energy storage which leads to extreme variation in its

composition profile. Microalgae lipids can be generally characterized into two groups, namely storage or non-polar lipids (i.e., saturated fatty acids, SFAs and monounsaturated fatty acids, MUFAs) and structural or polar lipids (i.e., polyunsaturated fatty acids, PUFAs) (Alishah Aratboni et al. 2019). Storage lipids usually appear in the form of triacylglycerols (TAGs) can be converted to biodiesel through transesterification reaction (Paliwal et al. 2017). In addition, TAGs stored within microalgae cells provide basic energy for metabolic processes by playing a fundamental role in photosynthesis under light conditions while involved in the synthesis of polar lipid during dark hours (Alishah Aratboni et al. 2019). Prior research studies have documented that certain microalgae species, for example, the red algae (i.e., *Porphyridium cruentum*) and green microalgae (i.e., *Parietochloris incisa*) could produce a high concentration of PUFAs in TAGs (Alishah Aratboni et al. 2019). More recently, other species, such as *Phaeodactylum tricornutum*, *Thalassiosira pseudonana,* and *Nannochloropsis oculata* could also produce PUFAs in TAGs but at lower concentrations (Paliwal et al. 2017; Alishah Aratboni et al. 2019). PUFAs play an important function in maintaining membrane functions for different metabolic processes, as well as contributing to a variety of intracellular membrane fusion events (Paliwal et al. 2017; Alishah Aratboni et al. 2019). Additionally, these PUFAs (especially omega-3 FAs) have an active role in cell signaling pathways and response delivery to changes in the cellular environment (Alishah Aratboni et al. 2019), which make them highly accumulated during abiotic stress conditions (Carvalho and Caramujo 2018). Examples of PUFAs include eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) which have enormous potential for nutraceutical and pharmaceutical purposes. Under certain abiotic stress environment (i.e., optimal nutrients concentrations, different salinity levels, including light intensity), the potential of high biomass productivity along with a high concentration of EPA and DHA could be attained (da Silva et al. 2019). To achieve acceptable yield of microalgae biomass along with high lipid productivity, numerous abiotic stress methods could be applied which will be discussed in detail in the sub-sections below and the findings have been tabulated in Table 2.

*Nutrients*

Manipulation of nutrients contents in the cultivation medium, including deficiency and/or excess of nitrogen and/or phosphorus, has been widely regarded to affect the FA composition of microalgae (Paliwal et al. 2017). Under nitrogen starvation conditions, upregulation of the key enzymes on the lipid biosynthesis pathways take place, in which malic enzymes become more activated for catalyzing pyruvate synthesis and NADPH (reduced nicotinamide adenine dinucleotide phosphate) formation and both acetyl-CoA and ATP (adenosine triphosphate)-citrate lyase is stimulated for catalyzing acetyl-CoA production (Fu et al. 2019). It is also worth noting that nitrogen starvation was proven to be highly effective to increase lipid concentration within microalgae cells compared to other nutrient stresses such as phosphorus and silicon starvation. For example, *Acutodesmus dimorphus* was able to accumulate 29.92% of lipid under short-term nitrogen starvation conditions (Chokshi et al. 2017). To relate nitrogen starvation to reactive oxygen species (ROS) generation, Chokshi and co-researchers investigated the effects of various stress biomarkers like ROS, cellular enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and non-enzymatic scavenger proline and polyphenols. It was revealed that the presence of ROS at the lowest level in the microalgae culture attained the highest lipid content, which can be related to the extremely high activities of SOD and CAT in this culture (Chokshi et al. 2017).

On the other hand, the correlation between nitrogen concentration in cultivation medium and lipid productivity by microalgae is important to evaluate potential microalgae biodiesel (Chen et al. 2017). Recently, in the diatom culture of *Chaetoceros muelleri*, high lipid content (35.03% of dry cell weight, DCW) and lipid productivity (29.07 mg/L/day) with elevated neutral lipid content were recorded under nitrogen limitation by Lin et al. (2018). That represented an increment of 58% and 10% for lipid content and lipid productivity, respectively as compared to control cultures. However, there was a remarkable biomass loss of about 30% in a nitrogen-limited culture of *C. muelleri*. It was also observed that biomass productivity of *C. muelleri* was greatly inhibited by nitrogen limitation than

**Table 2** Major effects of abiotic stress on polar and neutral lipids content of microalgae

| Abiotic stress | Microalgae species | Lipids | Optimum conditions | Effects | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Biomass | Lipid | Remarks | |
| Polar lipids | | | | | | | |
| Nutrient | *Phaeodactylum tricornutum* | EPA | Nitrate deficiency | Increase in biomass conc. by 13% | Increase in EPA accumulation by 14% | – | Yongmanitchai and Ward (1991) |
| | *Phaeodactylum tricornutum* | EPA | High phosphate (0.5 g/L) | Increase in biomass conc. by 6% | Increase in EPA content by 41% | – | |
| | *Nitzschia laevis* | EPA | Urea as nitrogen source | Increase in biomass conc. by 42% | Increase in EPA productivity by~2-folds | Compared to nitrate as nitrogen source | |
| | *Nitzschia laevis* | EPA | Silicate limitation (16 mg/L) | Increase in specific growth rate by 33% | Increase in EPA accumulation by 75% | – | Wen and Chen (2000) |
| Temperature | *Phaeodactylum tricornutum* | EPA | Low temperature (10 °C) | Significant increase in biomass conc. by 120% | Increase in EPA content by 85% | – | Jiang and Gao (2004) |
| Salinity | *Crypthecodinium cohnii* | DHA | High salinity (9 g/L NaCl) | Increase in growth rate by 61% | Increase in DHA content by 89% | – | Jiang and Chen (1999) |
| | *Nannochloropsis* sp. | EPA | High salinity (40 g/L) | Decrease in biomass productivity by 13% | Decrease in EPA productivity by 18% | | Mitra et al. (2015a) |
| pH | *Pinguiococcus pyrenoidosus* | PUFA | pH 7 | – | Increase in PUFA content including EPA by 24% | | Sang et al. (2012) |
| Carbon | *Aurantiochytrium* sp. KRS101 | DHA | Heterotrophic, sugar refinery washing water (SRWW), total organic carbon of 95 488 mg/L, 35 g/L sea salt | Increase in biomass conc. by 5% | Increase in DHA content by 45% | Compared to culture without salinity stress | Moon et al. (2019) |
| Carbon and silicate level | *Nitzschia laevis* | EPA | Medium glucose level (20 g/L), medium silicate level (32 mg/L) | Increase in biomass conc. by 125% | Increase in EPA content by 78% | Batch | Wen and Chen (2000) |
| Temperature and irradiance | *Nannochloropsis* sp. | EPA | Combined effect of low temperature (10 °C) and low irradiance (30 µmol photons/$m^2$/s) | – | Increase in the EPA content by 3.4-folds | – | Mitra et al. (2015b) |
| Neutral lipids | | | | | | | |
| Temperature | Mixed microalgae | TAG | High temperature (30 °C) | Decrease in biomass conc. by 30% | Increase in neutral lipids by 5-folds | – | Venkata Subhash et al. (2014) |
| | *Acutodesmus dimorphus* | TAG | High temperature (35 °C) | Increase in biomass productivity by 68% | Increase in neutral lipids by 20% | – | Chokshi et al. (2015) |
| | *Scenedesmus quadricauda* | TAG | High temperature (40 °C) | Negligible decrease in biomass conc. by 8.33% | Significant increase in total lipid content by 39.6% | – | Han et al. (2016) |
| | *Navicula phyllepta* MACC8 | TAG | Medium temperature (25 °C) | Increase in total biomass by 53% | Increase in total lipid content by 48% | | Sabu et al. (2019) |

**Table 2** continued

| Abiotic stress | Microalgae species | Lipids | Optimum conditions | Effects | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Biomass | Lipid | Remarks | |
| Salinity | *Dunaliella tertiolecta* | TAG | High salinity up to 0.5 M | Insignificant increase in biomass conc. by 3% | Increase in total lipid content and TAG by 12% | Increase in total lipid by 16% when salt added at mid-log phase or the end of log phase during | Takagi et al. (2006) |
| | *Scenedesmus* sp. | TAG | Salinity stress of 0.4 M | Significant decrease in biomass conc. by 2.4-folds | Increase in neutral lipids by 15% | – | Pancha et al. (2015b) |
| | *Synechocystis* | TAG | 1 M of NaCl | Increase in total biomass by 66% | Increase in total lipid content by 35% | Decrease in biomass productivity by 46% | Paliwal et al. (2015) |
| Light intensity | *Pavlova lutheri* sp. CCNM 2501 | TAG | High light intensity (120 µE/$m^2$/s) | Increase in biomass conc. by 3.4-folds | Increase in TAG by 34% | – | Carvalho and Malcata (2005) |
| | *Pavlova lutheri* | TAG | High light intensity (340 µmol photons/$m^2$/s) | – | Increase in TAG by 13% | – | Guihéneuf et al. (2009) |
| | *Scenedesmus obliquus* | TAG | Low light intensity (500 µmol photons/$m^2$/s) | Insignificant decrease in biomass conc. by 6% | Insignificant increase in TAG by 6% | – | Breuer et al. (2013) |
| Nutrient | *Monodus subterraneus* | TAG | Phosphorus limitation (52.5 µM K2HPO4) | – | Increase in TAG by 6-folds | | Khozin-Goldberg and Cohen (2006) |
| | *Chlorella* sp. | TAG | Nitrogen starvation | Slight decrease in biomass conc. by 2.3% | Increase total lipid content (mainly TAG) by 37% | | Praveenkumar et al. (2012) |
| | *Scenedesmus* sp. | TAG | Nitrogen starvation | Insignificant decrease in biomass conc. by 2% | Increase in total lipid content (mainly TAG) by 47% | Sequential nitrogen starvation conditions unaffected DCW and biomass productivity | Pancha et al. (2014) |
| | *Thalassiosira weissflogii* | TAG | Phosphorus limitation | Insignificant decrease in biomass conc. by 11% | Increase in neutral lipid by 27% | – | Lin et al. (2018) |
| | *Chaetoceros muelleri* | TAG | Nitrogen limitation | Significant decrease in biomass conc. by 43% | Increase in neutral lipid by 18% | – | |
| | *Dunaliella salina* | TAG | Nitrogen limitation (0.5 N), phosphorus deficiency | Decrease in total biomass by 21% | Increase in accumulation of total lipid by 322% | – | Almutairi (2020) |
| | | | Nitrogen and phosphorus limitation | Decrease in total biomass by 48% | Increase in accumulation of total lipid by 289% | | |
| Temperature, salinity, carbon dioxide supply | *Chaetoceros cf.* | | *wighamiibrightwell* | TAG | Low temperature (20 °C), low salinity (25), carbon dioxide supply (+) | Doubling of biomass conc | Increase in total lipid content by 31% |
| Batch, Compared to room temperature | Araújo and Garcia (2005) | | | | | | |

**Table 2** continued

| Abiotic stress | Microalgae species | Lipids | Optimum conditions | Effects | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Biomass | Lipid | Remarks | |
| Nutrient, salt | *Chlamydomonas reinhardtii* | TAG | Nitrogen and phosphate limitation (TAP)+1 g/L sodium acetate | Increase in biomass productivity by 2.1-folds | Increase in TAG by 1.1-folds | – | Ran et al. (2019) |

phosphorus or silicon limitation. Another diatom strain *Thalassiosira weissflogii* was also tested against nitrogen limitation, which was reported to exhibit a similar trend, whereby a dramatic biomass reduction of up to 40% was recorded. At the stationary phase of cultivation, the lipid content and neutral lipid in *T. weissflogii* culture resulted in an increment of 30% and ~ 2-folds, respectively. Lipid productivity is indicated by the increase in total lipid and biomass concentration. Hence, there was a significant drop in the lipid productivity of *T. weissflogii* up to 22%. In some cases, nutrient deficiency can result in an increment of total lipid content but not necessarily lead to higher lipid productivity. Interestingly, for *C. muelleri*, the highest neutral lipid accumulation, lipid content, and lipid productivity were all observed under nitrogen limitation that proved *C. muelleri* as a lucrative feedstock than *T. weissflogii* for the biofuel industry. In fact, the FA composition in *C. muelleri* was mainly comprised of C14-C18 along with 95% of total FA, showcasing high desirability for biodiesel production. The production of SFA and MUFA were improved, followed by a reduction in PUFAs, predominantly in cultures of *T. weissflogii* under nitrogen limitations (Lin et al. 2018). Hence, it is crystal clear that the nutrient deficiency approach is species-specific due to different responses observed towards nitrogen concentration in the cultivation medium.

Most microalgae species undergo biodegradation of proteins, which allows carbohydrate and lipid formation from fixed carbon under nitrogen-deficient conditions, resulting in compromised cell growth and biomass productivity which subsequently affect lipid productivity (Chen et al. 2017). Hence, the selection of appropriate microalgae species coupled with a two-stage cultivation system (first stage: sufficient nitrogen concentration is supplied to promote biomass productivity; second stage: nutrient deficient to allow lipid production) is important to attain high lipid yield. In a study conducted by Praveenkumar et al. (2012), *Chlorella* sp. was cultivated in Chu10 medium that lacked calcium nitrate, $Ca(NO_3)_2$ for 4 days after 16 days of normal growth. It was found that nitrogen-deficient condition reduced slightly on biomass concentration (reduction by 2.3%), whereas 428.26 mg/g of cell lipid content was attained or 37% higher than the control experiment (Praveenkumar et al. 2012). Besides, about 75.6% of SFA and MUFA

were dominated in the FA profile which matched the substantial biodiesel quality. This finding suggested that a two-stage cultivation system under nitrogen-deprived conditions was ideal for culturing *Chlorella* sp. to simultaneously obtain a high yield of biomass and lipid productivity for biodiesel production. A similar finding was also reported by Sabu et al. (2019) that improvement of lipid production by diatom *Navicula phyllepta* MACC8 through two--stage cultivation strategy under nitrogen deficiency condition in the second stage (Sabu et al. 2019).

Biosynthesis of higher concentration of TAGs with decrease content of polar lipids was induced by the nitrogen-starvation environment in *Nannochloropsis* sp. (Axelsson and Gentili 2014). Yongmanitchai and Ward (1991) reported an increment of EPA production in *P. tricornutum* under nitrate-deficient condition whereas *N. laevis* experienced maximum EPA productivity when urea was used as the nitrogen source. However, using ammonia as the only nitrogen source in diatom cultivation was found to be unfavorable for growth and PUFA production (Yongmanitchai and Ward 1991). This could be due to *Nannochloropsis sp.* and *N. laevis* cells were exposed to a nitrogen-deficient environment, the cells intensified the TAG synthesis pathway at the expense of polar lipids, which in turn proving the species-specific response towards nitrogen supply.

Other than nitrogen, phosphorus plays a crucial role in culture media by regulating microalgae growth besides involving several metabolic processes such as energy transfer in cells, nucleic acid, and phospholipid production (Paliwal et al. 2017). Also, the accumulation of neutral/polar lipids regulated by phosphorus stress is species-dependent (Chen et al. 2017). Phosphorus limitation triggered lipid production in *Monodus subterraneus* and *Chlorella* sp. (Lin et al. 2018). For *Monodus subterraneus*, about 6 times increment of TGA was observed along with EPA production when the microalgae are cultivated under phosphorus-deficient conditions (Khozin-Goldberg and Cohen 2006). In contrary to expectations, improvement in EPA content was observed in *P. tricornutum* when cultivated in a medium with a high concentration of phosphorus (Yongmanitchai and Ward 1991). Thus, phosphorus concentration in microalgae cultivation medium played an important role in the overall yield of PUFAs (Harwood 2019). Praveenkumar et al. (2012) studied the effect of phosphorus deficiency on the growth-optimized culture of *Chlorella* sp. by introducing into Chu10 medium that lacked dipotassium hydrogen phosphate ($K_2HPO_4$) for 4 days after harvesting the biomass on day-16. As reported, the biomass concentration decreased significantly to $2.46 \pm 0.06$ g/L (reduction by 4.7%) without significant improvement in lipid content ($319.89 \pm 10.02$ mg/g).

Even though nitrogen starvation could promote intracellular lipid accumulation in microalgae cells, it also reduced biomass yield by as much as 80% which is not economically effective for large-scale microalgae biodiesel production. Adaption of such a strategy could also weaken protein production, and thus resulted in the reduction of ADP (adenosine diphosphate) and NADP (nicotinamide adenine dinucleotide phosphate) (Fields et al. 2014). To overcome this drawback, phosphorus supplementation offered an effective strategy and has been researched to increase microalgae biomass productivity under nitrogen-deficient conditions in several studies. Chu et al. (2013) revealed that sufficient phosphorus (35 mg/L) with nitrogen deficit conditions allowed high lipid productivity (58.4 mg/L/d) from *C. vulgaris*. This is mainly due to phosphorus consumption by microalgae was to maintain the biosynthesis of enzymes involved in lipid synthesis, DNA, ATP, and other metabolites (Chu et al. 2013). On the other hand, a surplus of phosphorus (≤45 mg/L) under a nitrogen-deficient environment was reported to stimulate the growth of *Chlorella regularis* with a 10.2% and 39.3% increase in biomass production and lipid productivity, respectively. However, oversupply of phosphorus (250 mg/L) reduced biomass production by 38.8% which provided insights into the role of phosphorus under various modified conditions (Fu et al. 2019). Under excess phosphorus availability, phosphorus is usually stored by microalgae as intracellular polyphosphate when cultivated in phototrophic and heterotrophic mode. These polyphosphates, with phosphor-anhydride bonds, are rich sources of energy (i.e., ATP) for the biosynthesis of metabolites when cells are exposed to nitrogen starvation. Furthermore, polyphosphates can also be metabolized to form DNA (deoxyribonucleic acid), RNA (ribonucleic acid), or intermediate products within microalgae cells (Fu et al. 2019).

Recently, Sabu et al. (2019) employed a two-stage cultivation technique, in which the first stage involv

high microalgae biomass production followed by multiple abiotic stress environment for lipid production in oleaginous brackish diatom *Navicula phyllepta* MACC8. It was noticed that the lipid content was increased when nutrient silicon and urea were removed, but the biomass yield indicated a significant reduction. In addition, utilization of multiple nutrient stress conditions could result in a positive increment in lipid content. A combination of phosphate and silicate deficiency coupled with temperature reduction resulted to a lipid percentage of 32.13% at the cost of reduced biomass (1.1 g/L). On the other hand, when the microalgae were cultivated under phosphate and urea limited condition with temperature reduction resulted in lipid percentage of 27.58% with a biomass concentration of 1.44 g/L. In this regard, a two-stage cultivation strategy with an application of multiple stress environments would be a plausible approach for simultaneous biomass and lipid production for diatom *Navicula phyllepta* MACC8.

*Temperature*

The effect of temperature plays an important role in lipid production by microalgae. In addition, cultivation temperature also affects nutrients utilization and $CO_2$ fixation by microalgae that subsequently resulted in variation in biomass production and lipid composition. Significant effects of temperature variation on the growth rate of microalgae species have been reported. A study by Renaud et al. (2002) indicated that a higher growth rate of *Chaetoceros* sp. (clone CS256) was observed when the cultivation temperature was raised from 25 to 30 °C (Renaud et al. 2002). One of the possible mechanisms that can be correlated to the low microalgae growth rate as reported by Araújo and Garcia (2005) is the increase in the rate of respiration due to the increment of cultivation temperature above the tolerance level of certain species. Nevertheless, as revealed by the study, a medium temperature of 20–25 °C was suitable to prevent loss of *Chaetoceros cf. wighamii* biomass. As for lipid accumulation, research studies have predicted a general trend between the cultivation temperature and lipid composition of microalgae (Paliwal et al. 2017). It was observed that reducing of cultivation temperature led to increment in polar lipid content in microalgae whereas increasing the temperature resulted in better accumulation of neutral lipids (TAG) (Renaud et al. 2002). For instance, Chokshi et al. (2015) demonstrated that *Acutodesmus dimorphus* could accumulate lipid content up to 22.7% (containing 59% neutral lipid) along with 68% increase in biomass productivity (23.53 mg/L/day) when the cultivation temperature increased from 25 to 35 °C (Chokshi et al. 2015). It was also found that, stress biomarkers such as reactive oxygen species, antioxidant enzymes like catalase and ascorbate peroxidase and lipid peroxidation were the lowest when *A. dimorphus* was grown at 35 °C (Chokshi et al. 2015).

Nevertheless, extreme changes in cultivation temperature may inhibit the growth of microalgae (Renaud et al. 2002). The microalgae growth rate is expected to increase with the increment of cultivation temperature until a certain level before the cells are inhibited. Heat shock reduces protein synthesis due to the production of abscisic acid (ABA) which is known as a stress hormone (Kumar et al. 2012). The released hormones induced additional physical stress to microalgae which can retard biomass productivity (Venkata Subhash et al. 2014). Therefore, Venkata Subhash et al. (2014) explored a dual-stage cultivation strategy, whereby the initial growth phase was intended for biomass production for 8 days under mixotrophic cultivation mode followed by a temperature-stressed starvation phase for lipid production (Venkata Subhash et al. 2014). The initial phase indicated an increment in biomass production while the second phase that operated at 30 °C showed enhancement in lipid productivities (total/neutral lipid, 24.5/10.2%). Interestingly, high utilization of carbohydrates within microalgae cells was observed at 30 °C (57.8%) as compared to 25 °C (50.6%) and 35 °C (26.9%). At the same time, the neutral lipid content was increased 5 times than the control condition with a higher ratio of SFA to unsaturated fatty acids (USFA) and was suitable for biodiesel production.

Nevertheless, a closer look at the result reported by Venkata Subhash et al. (2014) revealed some gaps and shortcomings (Venkata Subhash et al. 2014). Although the microalgae could accumulate higher lipid at 30 °C than that under ambient temperature, however, the efficiency was reduced due to the longtime of cultivation under stress conditions. In addition, the temperature stress also resulted in variation in FA composition within the microalgae

cells that may affect the final biodiesel quality. Apart from that, the microalgae biomass was higher under optimal growth conditions than under high temperature environment. The microalgae biomass yield (1.1 g/L) cultivated under high temperature conditions showed insignificant change after 1 day, then decreased gradually with cultivation time. Therefore, it is obvious that the adverse condition of high temperatures harmed the growth of microalgae cells in the temperature-stressed culture. The same observation was also reported by Kitaya et al. (2008), in which the growth of *Amphidinium* sp. was inhibited between 32 and 35 °C as compared to ambient temperature at 26 °C (Kitaya et al. 2008). Besides, Subhash et al. (2014) also observed a 30% reduction in microalgae biomass yield when cultivation temperature increased to 35 °C (Venkata Subhash et al. 2014).

As a result, Han et al. (2016) attempted to induce the lipid production from *Scenedesmus quadricauda* through cultivation at a high temperature of 40 °C at the end of the cultivation cycle (Han et al. 2016). Interestingly, the lipid content and productivity of microalgae after 1-day stress showed an increment of 39.6% and 33.3%, respectively. However, prolonging the cultivation time resulted in the reduction in biomass production and lipid content in the microalgae. Improvements in results have been observed when Venkata Subhash et al. (2014) adapted a two-stage cultivation method in which simultaneous increment in biomass yield (1.9 g/L) and lipid concentrations (0.4 g/L) by more than 50% in the optimized medium for higher lipid production till the $18^{th}$ day from the start of the second stage at 35 °C. Nevertheless, in the control experiment (single stage), reduction of biomass yield (1.24 g/L) and lipid concentration (0.27 g/L) was observed after the $18^{th}$ day from the start of the first stage due to exhaustion of nutrients (Venkata Subhash et al. 2014). On a contrast note, *Phaeodactylum tricornutum* was cultivated at 10 °C for 12 h, whereby the EPA content was significantly increased (Jiang and Gao 2004). In short, the selection of cold-tolerant species for mass cultivation of PUFA would be a rational choice due to well-adaptation to such environment through up-regulation of FA desaturase and higher lipid content that act as a cryoprotectant to alter the freezing point (Chen et al. 2017). A good example is the *Chlamydomonas* sp. ICE-L that able to accumulate PUFAs up to 78.5% at 0 °C (Chen et al. 2017).

*Light illumination*

When microalgae are irradiated with different light wavelengths, significant influence on their photosynthetic efficiency, cell growth, and biochemical profile are observed due to changes in biosynthetic pathways and metabolic activity (Paliwal et al. 2017). Apart from nitrogen deficiency and an increase in temperature, saturating light conditions often resulted in excess accumulation of neutral lipid (mainly TAGs) in microalgae cells (Srinivas and Ochs 2012). As reported by Klok et al. (2013), TAG production was initiated when microalgae cells were subjected to light stress. Under excessive light supply, TAG could serve as an energy sink to allow the cells to continue to harvest light energy (Klok et al. 2013). Notable numbers of microalgae have capabilities of producing the elevated levels of TAGs upon exposure to high light, namely *Chlorella* sp. (He et al. 2015), *Monoraphidium* sp. (He et al. 2015), *Scenedesmus obliquus* (Breuer et al. 2013), *Pavlova lutheri* (Carvalho and Malcata 2005), and *Nannochloropsis gaditana* (Mitra et al. 2015a). *Chlorella* sp. and *Monoraphidium dybowskii* Y2 cultivated under high intensity of light (400 µmol photons/$m^2$/s) improved carbon conversion to neutral lipids, up to 71.7 and 60.65% of total lipid, respectively, and neutral lipid productivity reached 51.4 and 49.7 mg/L/d, respectively, accompanied by a significant reduction in membrane lipids (He et al. 2015). However, excess light energy can be dissipated leading to a significant reduction in biomass productivity and yield (Klok et al. 2013). Despite the high TAGs contents obtained at high-intensity of light in cultures of *Chlorella* sp. and *Monoraphidium dybowskii* Y2 in the study conducted by He et al. (2015), a significant reduction in the total biomass (g/L DW) were observed in both cultures compared to cultures subjected to medium light intensity (200 µmol photons/$m^2$/s) which accounted for 17% and 20% of biomass loss, respectively (He et al. 2015). Moreover, the highest lipid productivities were also obtained in medium light cultures. Considering the advantage of optimal biomass and lipid productivity, adaptation of medium light intensity for scale-up cultivation could be a win–win strategy.

From the viewpoint of transcriptomes and lipidomes, Gwak et al. (2014) reported that production of TAG and its assembly pathways at the gene level, moderate up-regulation of de novo FA biosynthesis were stimulated and the remodeling of membrane glycerolipids were dramatically reduced when *Haematococcus pluvialis* was exposed to the high level of irradiance (Gwak et al. 2014). However, even though high light irradiance was sought to act as a trigger of TAG, the percentage of TAG accumulation will vary from species to species. Nevertheless, photosynthetic carbon partitioning mechanisms and changes in biochemical compositions in microalgae cells when exposed to different light regimes have still remain unclear. As for indicators of lipid content in microalgae cells, some researchers have adopted Fv/Fm (maximum PSII quantum yield) and the changes of ROS lipid accumulation under light stress were closely related to these two factors (Gwak et al. 2014). Under high light intensity, the value of Fv/Fm may change and over-reduction of the photosynthetic electron transport chain may generate excess ROS. Microalgae cells tended to overcome oxidative stress caused by ROS through several defensive mechanisms, such as increasing ROS scavenging activity or decreasing ROS production. Scavenging of ROS can be facilitated by different enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxide dismutase (POD). Therefore, unraveling the Fv/Fm ratio and ROS scavenging enzymes could further enhance TAG synthesis in microalgae cells (Gwak et al. 2014). On the other side, low light intensity (60 µmol photons/$m^2$/s) yielded the highest TAG content in *Phaeodactylum tricornutum* culture (Remmers et al. 2017). Therefore, it is evident that excessive lighting results in photoinhibition and oxidative damage, which creates impairing of biomass yield with a reduction in lipid content.

Being inversely related to light irradiation, excess structural lipids enriched with PUFAs are produced under low light conditions (Paliwal et al. 2017). PUFAs may involve in the functioning of the thylakoid membrane and are essential for the photosynthesis process of green microalgae cells. When microalgae culture is subjected to low light conditions, the photosynthetic potential of the cells increased, suggesting the requirement for more thylakoid membranes. As a consequence, low light acclimated cells resulted in the accumulation of more PUFA (mainly EPA and/or DHA) (Paliwal et al. 2017). A study by Sukenik and Carmeli (1989) qualitatively proved that microalgae cultivated under low light conditions were characterized by a large relative volume of the chloroplast, a high surface density of thylakoid membranes, and a low relative volume of lipid storage bodies (Sukenik and Carmeli 1989). It is worth noting that, the effect of light illumination towards variation in PUFAs composition is species-specific. In general, high EPA concentration could be attained under low light conditions, whereas DHA levels were the highest under much higher light intensities (Guihéneuf et al. 2009). The successful production of EPA under low light conditions has been widely documented by several scientists in the case of *Nannochloropsis* sp. (Sukenik and Carmeli 1989), *N. salina* (Van Wagenen et al. 2012), and *N. gaditana* (Mitra et al. 2015a).

*Salinity*

Apart from nutrient starvation and few other major abiotic stress factors, salinity also may affect lipid production in microalgae cells. *Dunaliella tertiolecta* culture showed a high concentration of total lipid and TAG when stressed with increasing sodium chloride (NaCl) concentration ranging from 0.5 to 2.0 M (Takagi et al. 2006). Besides, increment in lipid content (23.4%) was found when salinity stress of 1.0 g/L NaCl was introduced to a mixed microalgae culture (Venkata Subhash et al. 2014). Nevertheless, salinity stress is also species-specific and a high concentration of salt in the cultivation medium may inhibit microalgae biomass and lipid productivity (Chen et al. 2017). Investigation of salinity effect on *Synechocystis* sp. CCNM 2501 indicated the microalgae can continue to survive with a supplement of high NaCl concentration at 1.0 M, along with increment in biomass concentration and lipid content. However, the significant results were hampered by low biomass productivity after a certain threshold that accounted for 46% of loss (Paliwal et al. 2015). EPA productivity in *Nannochloropsis gaditana* increased when subjected to a salinity of about 30–40 g/L but was accompanied by a loss in the biomass and productivity (Mitra et al. 2015a).

Osmo-protecting molecules (i.e., glycerol) and ROS may play crucial roles in microalgae cells to cope with a high salinity environment and to

stimulate lipid production as energy storage in the cells (Chen et al. 2017). Transcriptomic analyses of salt-stressed *Nitzschia* sp. revealed that an increased in lipid content is directly linked to the up-regulation of genes governing lipid biosynthesis and the down-regulation of the genes encoding photosynthetic enzymes and glutathione (Cheng et al. 2014). In another work, excess ROS was generated when *C. protothecoides* culture was stressed with 30 g/L NaCl. The ROS generated subsequently mediated the signal transduction to shift the carbohydrate biosynthesis pathway to a lipid with the increment of Acetyl-CoA carboxylase (Wang et al. 2016). Fundamental understanding of lipid production and catabolism under salinity stress should be further explored to attain high lipid productivity from microalgae on a commercial scale.

*Value of pH*

Generally, microalgae grow well at pH 7–8.5 in the cultivation medium (Vadlamani et al. 2017). As microalgae cultivation continues, pH increased due to the accumulation of minerals and oxidation of nutrients. Hence, the pH in the cultivation medium is usually pre-adjusted to 6.5 before being supplied to grow microalgae (Qiu et al. 2017). The changes in the value of pH also cause significant alteration on the microalgae lipid (Paliwal et al. 2017). The solubility of $CO_2$ and other mineral compounds is affected by fluctuations of the medium pH (Ogbonda et al. 2007). The highest level of total PUFAs (38.75%) was recorded at pH 7 in *Pinguiococus pyrenoidous* 2078 culture (Sang et al. 2012). For some microalgae species, i.e., *Spirulina platensis* and *Spirulina fusiformis*, alkaline pH up to 10.0 is a prerequisite for maximal biomass accumulation (Ogbonda et al. 2007). At a pH of 8.5, the highest biomass productivity (96.7 mg/L/d) was attained in the culture of *Ettlia* sp. YC001 (Yoo et al. 2015). On the other hand, high alkaline condition (pH > 9) coupled with nitrogen-deficient and salinity stresses, resulted in increment of TAG production (up to 35% DW) in *Scenedesmus* sp. and *Neochloris oleoabundans* (Santos et al. 2012).

*Silica*

Several studies reported diatoms as one of the promising feedstock candidates for biofuel and pharmaceutical production. Having good oil production capabilities, diatoms able to produce lipid and carbohydrate as their main storage compounds (Jiang et al. 2014). The cell walls of a diatom are comprised of silica in the form of rigid external frustule shells and therefore the effect of silica in the cultivation medium may result in significant changes in the biomass productivity and lipid composition of diatoms. Wen and Chen (2000) documented increased accumulation of EPA (by 75%) along with improved specific growth rate (by 33%) under heterotrophic cultures of *Nitzschia laevis* supplied with 32 mg/L of silicate, highlighting diatoms as an excellent source of PUFAs (EPA and DHA) to potentially replace marine fish oil (Wen and Chen 2000). The study also found that the silicate concentration reduced significantly at the initial stage of cultivation, but the net silicate uptake ceased after 3 days without affecting the cell growth and a residual amount of silicate was present in the medium. A similar occurrence was also noticed in other diatoms species, especially in the cultures of *Thalassiosira pseudonana* (Wen and Chen 2000). Analysis by these early researchers confirmed the silicate consumption pattern of microalgae cells and provided further validation that silicate uptake is mainly limited during cell division and a small concentration of silicate retained in the medium when extracellular silicate uptake ceased.

Jiang et al. (2014) investigated growth characteristics and FA composition of three native diatoms of *Nitzschia* grown at five silicate concentrations in two stages. At the initial stage, cells were grown with different concentrations of silicon concentrations (0.2–10.6 mol/$m^3$) until the exponential phase. Thereafter, they were grown for 4 more days with half of the initial medium replaced by a silicon-free medium after harvesting. The maximum biomass (DW) at the second stage was $0.62 \pm 0.15$ g/L in the treatments with 2.1 mol/$m^3$ silica for *N. perspicua*. Besides, the highest lipid concentration of 255 mg/g was achieved in the second stage with the highest initial silicon present in the first stage for the cultures of LBK-018. For *N. perspicua* and LBK-018, SFA and MUFA showed a declining trend with increased

silicon concentrations whereas PUFAs increased with the increase of silicon concentrations, which was consistent with the argument by Wen and Chen (2000). The FA distribution was majorly C16 in all strains which attributed to the compatibility of this strain for the biofuel industry (Jiang et al. 2014).

In a later study wherein low levels of silicon resulted in at least a 50% increment in lipid concentration in marine diatom *Chaetoceros gracilis* (Adams and Bugbee 2014). Adams and Bugbee (2014) also found that decreased levels of silicon also caused a 5% change from C18 to C16 FAs which is essential for biodiesel production. Similarly, silicon deficiency elevated lipid concentration up to 28.78 wt % in a study conducted by Sabu et al. (2019). Diatoms react to silicon starvation conditions by accumulating lipids as they need silicon to form frustules (cell walls consisted of amorphous silica) (Jiang et al. 2014). However, the biomass concentration was reduced in silicon-deprived media in comparison with nutrient-replete cultures (control), producing 0.84 g/L at the end of the cultivation, which indicated the importance of silicate to support cell reproduction (Sabu et al. 2019). Also, based on the previous findings, increment of TAG concentration was observed under silicon deficient conditions for cell proliferation (Adams and Bugbee 2014). It was hypothesized that this phenomenon could be due to a change in the metabolism of diatom cells during silica deplete conditions, as the energy may divert to form lipid instead of consuming the silicate (Paliwal et al. 2017). Another possible reason is silicon-depleted microalgae cells may direct newly assimilated carbon sources to form lipid instead of carbohydrate or non-lipid cell components (Sabu et al. 2019).

Even though the effect of silicon on lipid production has been vastly studied in some diatom cultures such as *Cyclotella cryptica*, *Hantzschia* sp., and *Nitzschia inconspicua*, an insignificant impact on the biomass yield and lipid production was observed for marine diatoms *Thalassiosira weissfogii* and *Chaetoceros muelleri* when cultivated under silicon starvation conditions (Lin et al. 2018). This could be due to lower overall supplementation of silicon to microalgae cultures in previous studies that were inadequate to achieve higher production. To conclude, broadly translated findings indicate that silicon is an important micronutrient to support cell reproduction and to increase biomass productivity. Moreover, cultivating diatom cultures under silicon stress in two stages is able to alleviate biomass and lipid production as well as to improve the iodine values to meet the standard specification according to the European biodiesel standards (Hu et al. 2015). Apart from that, a wise selection of native strains (i.e., *Nitzschia* sp.) is crucial for high production of biomass and lipid and to ensure economical commercial-scale production of microalgae biodiesel.

*Carbon*

Besides nutrients, temperature, light irradiation, and salinity, the carbon source is another abiotic stress factor that can control microalgae growth and their chemical composition (Tu et al. 2018). Besides nitrogen and phosphorus, inorganic carbon source ($CO_2$) is essential to support efficient growth of microalgae and as a tool for atmospheric $CO_2$ mitigation (Paliwal et al. 2017). In general, photoautotrophic microalgae assimilate $CO_2$ as their direct carbon source to produce storage metabolites such as lipids (Markou and Nerantzis 2013). However, excess supply of $CO_2$ to microalgae culture (i.e., more than □ 10%) may inhibit biomass and lipid production due to induction of low pH when a high amount of $CO_2$ is dissolved in the medium (Pancha et al. 2015a). Different microalgae species require different $CO_2$ concentrations for the robust production of biomass as well as other biochemical components (Chen et al. 2017). For example, *Ettlia* sp. YC001 could tolerate high $CO_2$ concentration up to 10% with biomass yield of 3.1 g/L and lipid productivity of 80.0 mg/L/d (Yoo et al. 2015). *Chlorella* sp. AE10 and AE20 showed high tolerance to elevated $CO_2$ concentration (30%) with optimum biomass production of 3.68 g/L (Cheng et al. 2016). The ability of microalgae to tolerate high $CO_2$ concentration is particularly important to bio-fix $CO_2$ from flue gas along with high lipid production (Chisti 2007).

On the other hand, using organic carbon sources during heterotrophic cultivation of microalgae could significantly increase their growth rate, as well as able to facilitate higher lipid productivity. The introduction of glucose as a carbon source was found to enhance potential biodiesel production of *Scenedesmus* sp. (Pancha et al. 2014). However, the cost of organic substrates (i.e., glucose) in a heterotrophic

medium has been accounted for approximately 80% of the total cost of the growth medium. Therefore, alternative carbon sources have been intensively studied concerning their conversion into FA by microalgae, including hydrolysate of crop straw and sugar-rich crops for economical production of biodiesel (Wang et al. 2016). For instance, *Monoraphidium minutum* produced high SFA content when cultivated using optimum glucose, fructose, and microalgae biodiesel waste residue and sodium acetate (Patidar et al. 2014).

A large and growing body of research works have investigated the production of EPA from marine diatoms but often hindered by light limitation which results in a slow growth rate and subsequently low EPA productivity (Wen and Chen 2003). To overcome this issue, heterotrophic cultivation is widely accepted as an effective method to enhance EPA production in microalgae cultures. For lipid production in heterotrophic cultivation of microalgae, glucose is the most utilized carbon source whereas the role of silicate in the growth and lipid synthesis of diatom cultures has been well reported in the case of *Chaetoceros* sp. and *Nitzschia* sp. (Wen and Chen 2000). Owing to the enormous potential of diatoms for both lipid and PUFAs production, Wen and Chen (2000) investigated the common strain *Nitzschia laevis* for heterotrophic growth study and EPA production under varied glucose (1±40 g/L) and silicate concentrations (2.7±64 mg/L). It was found that the maximum DW of 5.5 g/L and the highest EPA content of 131 mg/L were obtained using a medium glucose level of 20 g/L and a silicate level of 32 mg/L between all concentrations. It was observed that at a lower silicate level below 8 mg/L, glucose was not completely consumed at the end of cultivation and vice versa. Poor glucose assimilation leads to low biomass accumulation within microalgae cells (Wen and Chen 2000). This finding revealed an important understanding of the synergistic effects of silicate and glucose in affecting the biomass and EPA production of heterotrophic cultures. Recently, sugar refinery washing water (SRWW) has been explored for heterotrophic cultivation of *Aurantiochytrium* sp. KRS101 for the productions of value-added docosahexaenoic acid (DHA). Maximum biomass, lipid, and DHA yields of 22.9, 6.33, and 2.03 g/L, respectively were achieved when 35 $gL^{-1}$ sea salt was applied on a stationary phase for osmotic stress (Moon et al. 2019).

Effect of multiple abiotic stresses

In-depth studies on correlations of nutritional stress (i.e., nitrogen and phosphorus) and other environmental stresses (salinity, pH, temperature) might extend the fundamental explanations on the impacts of combined abiotic stresses on microalgae lipid profile. In recent years, the physiological and biochemical changes of microalgae cultivated under different indoor constant light intensities with other abiotic stress conditions such as nutrient deficiency, high level of salinity, and temperature stress have been reported in some research works. *P. tricornutum* exhibited the highest TAG productivity (31.4 mg/L/d) at a high light intensity of 300 mmol photons/$m^2$/s and low temperature of about 20 °C. On the other hand, *I. galbana* showed the highest TAG productivity (21.7 mg/L/d) at a much higher light intensity and temperature of about 400 mmol photons/$m^2$/s and 30 °C, respectively. The combinations of high light intensity and temperature significantly enhanced the lipid content and lipid productivity of *I. galbana* by 70.5% and 84.3%, respectively (Kurpan Nogueira et al. 2015). In another stress combination study conducted by Chen et al. (2016), *Trentepohlia arborum* accumulated simultaneous high concentrations of carotenoids (13.4% of DCW) and lipids (26.3% of DCW) when exposed to high light intensity (150 mmol photons/$m^2$/s) and under nitrogen-limited condition. The lipid content of the nitrate-deficit culture subjected to high light was 55.3% higher than the control culture. Nevertheless, the highest biomass yield was attained at nitrate-sufficient conditions with approximately 1.9-folds differences than that of nitrate-deficit culture (Chen et al. 2016). Hence, considering biomass production, combining high light and nitrogen-limited conditions might not be the best approach for simultaneous production of lipid and carotenoid.

Even though microalgae cultivation under high light intensity and temperature result in higher biomass productivity in many species, this may not be optimal for the productions of PUFAs. As for the combination of different abiotic stresses for PUFAs enhancement purposes, the effects of coupling of low temperature and light were reported in several studies. In a recent study with five polar and cold-temperate microalgae, increments in EPA and DHA synthesis were attained under low temperature and

illumination environment (Boelen et al. 2013). Similarly, Mitra et al. (2015b) observed 3.4 times higher of EPA production from *Nannochloropsis* sp. when the two-stage cultivation process was applied (combination effect of low temperature and illumination) (Mitra et al. 2015b). EPA and DHA content can be high inter- and intra-specifically variable (Boelen et al. 2013). For instance, the highest EPA and DHA production rate was recorded in *Chaetoceros brevis* (174 μg/L/day) and *Emiliania huxleyi* (164 μg/L/day) that cultivated under the same conditions (Boelen et al. 2013). Therefore, through careful selection of microalgae species (i.e., cold-adapted polar), economical mass cultivation for EPA and DHA production is plausible under low temperature and illumination environments. Apart from that, a large and growing body of research has investigated the production of EPA from marine diatoms but is often hindered by light limitation which results in low growth rate and subsequently low EPA productivity. To overcome this issue, heterotrophic cultivation is widely accepted as an effective method to enhance EPA production in microalgae cultures. For lipid production in heterotrophic cultivation of microalgae, glucose is the most utilized carbon source whereas the role of silicate in the growth and lipid synthesis of diatom cultures have been well reported in the case of *Chaetoceros* sp., *Nitzschia* sp. and many more. Owing to the enormous potential of diatoms for both lipid and PUFAs production, Wen and Chen (2000) investigated the common strain *Nitzschia laevis* for heterotrophic growth study and EPA production under varied glucose (1±40 g/L) and silicate concentrations (2.7±64 mg/L). It was found that the maximum cell dry weight of 5.5 g/L and highest EPA content of 131 mg/L were obtained using a medium glucose level of 20 g/L and silicate level of 32 mg/L among all concentrations. It was observed that a at lower silicate level below 8 mg/L, glucose was not completely consumed at the end of cultivation and vice versa. Poor glucose assimilation leads to low biomass accumulation within microalgae cells (Wen and Chen 2000).

Due to lack of studies on combined effects of temperatures and other abiotic stress factors, Araújo and Garcia (2005) studied the effects of different levels of temperatures (20, 25 and 30 °C), salinity (25 and 35 ppt) and $CO_2$ (with and without addition) on the biochemical composition and growth of the marine diatom *C. cf. wighamii* (Araújo and Garcia 2005). It was found that the growth and biomass production of *C. cf. wighamii* was significantly affected by $CO_2$ addition and less impact by temperature and salinity. This finding contracted with previously established results where a significant effect of temperature on the growth rate of other microalgae species has been observed. Renaud et al. (2002) proved higher growth rate of *Chaetoceros* sp. (CloneCS256) when the temperature was raised from 25 to 30 °C (Renaud et al. 2002). As for lipid and carbohydrate content, they were higher at low and medium temperatures (20 and 25 °C) while protein content was unaffected (Renaud et al. 2002). Planned comparisons revealed that other investigations also showed higher lipid content at the low temperature of 25 °C for *Chaetoceros* sp. (clone CS256) than in higher temperatures, while for other species, namely *Rhodomonas* sp., *Cryptomonas* sp. and *Isochrysis* sp., lipid contents were improved when cultivations were operated in medium temperatures between 27 and 30 °C. Similarly, carbohydrates contents were significantly raised at a medium temperature between 25 and 30 °C in *Chaetoceros* sp. and reduced at higher temperatures (Renaud et al. 2002). Therefore, it is apparent that, generally, maximum lipid content coincides with an optimal range in surrounding temperature in most of the species, and the composition negatively altered at temperatures below and above optimum temperature. On the other hand, the carbohydrates were enhanced while lipids and proteins were decreased at the highest salinity of 35 ppt. Even though in some species, an increase in lipid level was observed at higher salinity, this finding was in accordance with the fact that the effect of salinity stress on the biochemical composition of microalgae is highly species-specific. However, few authors documented a similar decreasing trend for protein content with an increase in salinity for other species which matched with finding by Araújo and Garcia (2005). One of the possible mechanisms that can be correlated to the low microalgae growth rate as reported by Araújo and Garcia (2005) is the increase in respiration due to a rise in temperature above the species´ optimum level of cellular function. Nevertheless, this work suggested that low and medium temperatures between 20 and 25 °C at low salinity could be employed to optimize the nutritional value of *C. cf. wighamii* due to higher lipid content without

sacrificing biomass under these conditions. This work also verified the role of $CO_2$ as a backbone in cell growth and protein accumulation. The microalgae cells are prioritizing the excess of assimilated carbon in biomass productivity and protein synthesis instead of lipid and carbohydrate (Chen et al. 2013). However, future research should consider the potential effects of $CO_2$ addition at the different growth stages of microalgae to achieve high biomass productivity and desired lipid profile for biodiesel and nutraceutical purposes.

Recently, Cheng et al. (2017) investigated a two-stage process for a high accumulation of carbohydrates in which the $CO_2$ concentration, light intensity, and initial nitrogen concentration were manipulated in both stages. During the first stage, low $CO_2$ concentration (1%), low light intensity (100 μmol/$m^2$/s), and sufficient nitrogen (1.5 g/L of $NaNO_3$) were provided to *Chlorella* sp. AE10. In the second stage of cultivation, high $CO_2$ concentration (10%), high light intensity (1000 μmol/$m^2$/s), and nutrient starvation (0.375 g/L of $NaNO_3$) were employed. Also, cells were diluted at the beginning of second stage and there was no cell dilution in first stage. Besides cell dilution at the initial phase of the second stage, environmental stress such as the introduction of a high level of $CO_2$, high light intensity, and nitrogen-deficient at the second stage were crucial to elevate carbohydrate production which accounted for 42% of increment. Although the cells were diluted, the microalgae growth was not significantly affected along with higher carbohydrate productivity (Cheng et al. 2017). Since carbohydrate and lipid biosynthesis share the same carbon precursor in microalgae, this approach could be extended for lipid production from lipid-rich microalgae species with an integrated approach from $CO_2$ capture that has not been explored yet. Apart from nitrogen concentrations, microalgae growth and lipid accumulation are also affected by types of nitrogen sources. Recently, Sabu et al. (2019) employed a two-stage cultivation technique involving the initial growth in optimized conditions for biomass production followed by multiple abiotic stress for lipid production in *Navicula phyllepta* MACC8. It was observed that the lipid content was increased once the silicon and urea were completely consumed with a cost of a reduction in biomass yield. A two-stage cultivation strategy coupled with multifactor stress, such as nitrogen-deficient along with limited phosphate and temperature stress, would be a plausible approach to attain high biomass and lipid yield from *Navicula phyllepta* MACC8 for biodiesel production. However, despite several economic benefits of two-stage cultivation, the limitation of this method is apparent as the responses are species-specific (Sabu et al. 2019).

## Challenges and future perspectives

Improvement in lipid yield while maintaining high biomass productivity has been a key challenge to date. Several limiting factors have been identified in the vast applications of abiotic stress conditions to microalgae cultures and possible solutions are discussed in this section. In the present review, photoautotrophic is proposed as the best cultivation mode. Even though high cost is involved in supplying artificial light and proper mixing, the operation cost of photoautotrophic cultivation mode can be offset by the co-production of high-value products along with biodiesel that possesses a high selling price via shifting the metabolic pathways. Moreover, a heterotrophic cultivation system was found only suitable for sugar-preferred microalgae species. Low cell concentration due to light limitation in photoautotrophic mode can be addressed through a two-stage cultivation strategy, in which a high-density culture is achieved in the first stage and metabolites accumulations are triggered in the later stage by abiotic stress applications. Scaling up such systems can be tedious and expensive for commercial production due to the transferring process of microalgae cells. However, it was reported that the harvesting at the first stage followed by abiotic stress induction in the second stage is relatively simple, low cost and fast without the requirement for additional harvesting steps for lipid extraction (Poh et al. 2020).

It is widely documented that among all abiotic stresses, nutrient deficiency is easily achieved by manipulating nutrients composition and is extensively applied to enhance lipid accumulation in microalgae cells. Nevertheless, the main challenge for this approach is to attain simultaneous high biomass and lipid productivity especially when wastewater and organic fertilizers are used as nutrients sources. Being different to modulate chemical nutrients, wastewater streams and compost contain

non-uniform nutrients compositions which make experimental set-ups with deficiency and/or limitation of a specific nutrient to be relatively complex. Hence, a two-stage cultivation strategy is recommended to overcome this problem, in which the microalgae are initially supplied with sufficient nutrients to accelerate biomass productivity and followed by nutrients deficient stage by washing or natural depletion to allow further lipid accumulation within the microalgae cells. In addition, water after each cultivation cycle could be further utilized as low-cost nutrients-deficit media for subsequent microalgae cultivation with appropriate nutrients loading for biomass and lipid accumulation. However, despite several economic benefits of two-stage cultivation, the limitation of this method is strain-specific and the efficiency may differ with species (Sabu et al. 2019). Besides nutrients deficiency or limitation, it is highly suggested to gain the benefits of the phosphorus replete approach to improve the microalgae growth and lipid accumulation synergistically. Agricultural and industrial wastewater could be exploited as promising sources of phosphorus to reduce the cost associated with supplementation of excess phosphorus to microalgae cultivation systems. It was well reported in the existing literature that the phosphorus content in agricultural and food processing wastewater streams were ranged from 3 to 330 mg/L (Jayakumar et al. 2017). More winsome, Lam and Lee (2012) have established the use of organic fertilizer as a potential nutrient source for cost-effective microalgae cultivation (Lam and Lee 2012).

The introduction of high temperature at the end of the cultivation cycle could be a feasible approach at industrial scale for a higher biodiesel production rate. The selection of such species that have high tolerance to elevated temperatures could reduce the cost associated with the installation of cooling accessories in the large cultivation system to maintain low temperature. Nevertheless, the requirement for local microalgae strains that can be easily mass cultivated and able to adapt to the local environment remains a major drawback in the arena of the biofuel industry. Scientific screening methods enable rational decision making on the selection of excellent algae species for the productions of desired products. However, screening of elite microalgae can be heavily tedious and time-consuming. For instance, only tens of new strains were isolated and characterized every ten years and only a few of them (*Chlorella*, *Spirulina*, *Dunaliella*, or *Haematococcus*) were found feasible in the industrial applications (Sánchez et al. 2008). Besides, genetic engineering is required to improve stress tolerance level of microalgae and to elevate biomass and high-value biochemical productivity.

Full utilization of microalgae biomass to produce varieties of high value products (DHA, EPA, and carotenoids) along with biodiesel is vital to ensure sustainable microalgae biorefinery. Nevertheless, the co-production of lipid and PUFAs is not practical due to different light and temperature requirements. Again, a two-stage cultivation strategy could be put into practice, whereby desired biomass and lipid contents are gained at the first stage (medium or high light intensity and high temperature) and then divert the biosynthesis to PUFAs at the second stage. This can be done by lowering the cultivation temperature since the effective light intensity per cell converged to a very low level by default mode as the cells aged and become dense as they grow. In short, light supply not only has significant effect towards microalgae biomass production but also tend to change the biochemical composition within microalgae cell. Thus, effective utilization of light energy has been identified as one of the technological challenges especially at the industrial scale of microalgae cultivation. Therefore, more systematic, and theoretical analyses are required to explore the exact mechanism of light intensity directing microalgae metabolism towards increment in neutral and/or polar lipids.

Apart from that, identifying cheap sources of nutrients with the presence of natural biostimulants is another field to be explored. A considerable number of research works existed on the usage of chemicals (i.e., metals, phytohormones, antioxidants etc.) for augmented production of microalgae compounds, but the exact cost involved for the scale-up is not revealed elsewhere. Hence, finding for alternative chemicals such as biostimulants could be a wiser option. There could be possibilities of origin of crosstalk between signaling molecules and biostimulants in microalgae cell. Besides able to promote microalgae growth, biostimulants may regulate various physiological processes in microalgae. To establish a commercial production system with high biomass productivity and a rapid metabolite turnover

rate, appropriate combination of abiotic stresses is needed to be determined. However, more research works are still required to evaluate the economic feasibility of the simultaneous accumulation of microalgae biomass and lipid for biodiesel production via application of environmental stress conditions.

## Conclusion

The lucrative abilities of microalgae species to modulate their lipid profile in response to the changing environmental condition (abiotic stress factors) should be wisely exploited for the benefit of global sustainability. Knowledge on regulation of suitable single and multiple abiotic stress conditions as well as microalgae genetic response could minimize the contradiction between biomass productivity and lipid content, which determines the final lipid yield. Augmented production of high-value microalgae lipids (polar and non-polar) without sacrificing biomass productivity through careful selection of abiotic stress could improve the economic feasibility of biodiesel production on a holistic scale.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.

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