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Review

A Review on Synchronous Microalgal Lipid Enhancement and Wastewater Treatment

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Abstract: Microalgae are unicellular photosynthetic eukaryotes that can treat wastewater and provide us with biofuel. Microalgae cultivation utilizing wastewater is a promising approach for synchronous wastewater treatment and biofuel production. However, previous studies suggest that high microalgae biomass production reduces lipid production and vice versa. For cost-effective biofuel production from microalgae, synchronous lipid and biomass enhancement utilizing wastewater is necessary. Therefore, this study brings forth a comprehensive review of synchronous microalgal lipid and biomass enhancement strategies for biofuel production and wastewater treatment. The review emphasizes the appropriate synergy of the microalgae species, culture media, and synchronous lipid and biomass enhancement conditions as a sustainable, efficient solution.

Keywords: microalgae; lipid enhancement; biofuel; wastewater treatment

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1. Introduction

Day by day, humankind creates many aspects of environmental pollution, mostly due to urbanization and industrialization. Fossil fuel depletion and the release of untreated wastewater are among the two most severe problems in recent times contributing to air and water pollution. Fossil fuel depletion increases the level of greenhouse gases in the environment. At the same time, untreated wastewater contains heavy metals, excess nutrients, and pathogens. Thus, both fossil fuel depletion and the release of untreated wastewater threatens human and ecological well-being. The rapidly growing pollution level has led to a call for desperate sustainable alternatives to be considered. Otherwise, an unpredictable climate and environmental changes may be activated, causing brutal health and ecological disruptions and, consequently, triggering the requirement of clean, sustainable energy resources and wastewater treatment at the same time.

Biological treatment seems to be a clean, sustainable alternative for environmental pollution mitigation. Microalgae are attributed with the distinct ability to offer ecological services and react to the sustainability challenges concurrently. In recent times, microalgae have gained attention worldwide for their significant potential in biofuel production and wastewater treatment besides mitigating atmospheric CO_2. Microalgae can grow in nutrient-rich wastewater and reduce pollutants in the water bodies [1,2]. Microalgal bioremediation (phycoremediation) of wastewater also provides biofuels or high-value biomass yield for biofuel production simultaneously [3]. Pittman et al. [4] concluded that microalgae cultivation for biofuel devoid of wastewater is expensive with no positive energy return. Lundquist et al. [5] studied various microalgae-based wastewater treatments combined with biofuel production. They observed that the studies that utilized wastewater for microalgae cultivation could produce cost-effective biofuel, especially on a large scale.

Many previous studies have been reported on the phycoremediation of wastewater [6] and microalgae-based fuel production [7,8] (Table 1). Additionally, various studies have wastewater treatment combined with microalgae biofuel production. Min et al. [9] studied algal biomass production for biofuel while treating swine manure-based wastewater. They

observed that the nutrient removal rate from the wastewater significantly correlated to the algal biomass productivity. The NH_3-N, TN, PO_4-P, and COD reduction rates were observed to be 2.65, 3.19, 0.067, and 7.21 g/m^2 × day, respectively, and the algal biomass productivity ranged from 8.08–14.59 and 19.15–23.19 g/m^2 × day. However, a very low lipid content of 1.77–3.55% was observed for the harvested algal biomass. Mar et al. [10] studied the growth of *Desmodesmus* sp. S1 in oil refinery wastewater. Efficient wastewater treatment was achieved with 82% COD removal, 53% total phosphorous removal, metal ions (Fe^{3+}, Al^{3+}, Mn^{2+}, Zn^{2+}) removal, and pungent smell removal, whereas in *Desmodesmus* sp. S1, biomass and lipid contents were 2.98 g/L and 21.95%, respectively. Daneshvar et al. [11] reported the biomass productivity of 187 mg/L/d and lipid content of 9.07% for *Chlorella vulgaris* in pulp and aquaculture wastewater. They witnessed efficient wastewater treatment showcasing 75.5% COD removal, 76.5% total nitrogen removal, and 92.7% total phosphorous removal. Thus, from most previous studies, it was observed that microalgae are efficient for wastewater treatment, but biomass production and lipid content are inversely related. This inverse relation of microalgae biomass production in wastewater and lipid content might be a bottleneck. High biomass production and high lipid content using wastewater as a culture media is a cost-effective, sustainable option. It is also feasible to stock up lipids in microalgae to a considerable amount by changing physio-chemical factors.

Table 1. Previous studies on microalgae cultivated in wastewater.

Microalgae	Wastewater	Results	Reference
Chlorella sp. *UMN271*	Swine manure-based	Biomass productivity ranged from 8.08–14.59 g/m^2 × day and lipid content of 1.77–3.55%	[9]
Desmodesmus sp. *S1*	Oil refinery	Biomass and lipid content were found to be 2.98 g/L and 21.95%, respectively	[10]
Chlorella vulgaris	Pulp and aquaculture	Biomass productivity of 187 mg/L/d and lipid content of 9.07%	[11]
Mix consortium	Carpet mill treated	Biomass productivity of 41 mg/L/d and lipid content of 12.2%	[12]
Chlorella pyrenoidosa	Soybean processing	Biomass productivity of 0.64 g/L/d	[13]
Chlorella vulgaris	Tertiary-treated domestic	Biomass productivity of 197 gL^{-1} and lipid productivity of 0.164 gL^{-1}	[14]
Scenedesmus bijuga	6% effluent from poultry litter anaerobic digestion	Biomass productivity of 31–76 mg/L/d	[15]
Chlamydomonas sp. *TAI-2*	Untreated industrial	Biomass yield of 1.5 g/L	[16]
Desmodesmus sp.	Municipal	Biomass productivity of 500 mg/L/d and lipid content of 3.3%	[17]
C. sorokiniana	Domestic wastewater with urea supplementation	Biomass productivity of 200 mg/L/d and lipid content of 61.52%	[18]
Scenedesmus obliquus *Chlorella sorokiniana* *Ankistrodesmus falcatus*	Aquaculture	*A. falcatus* showed the highest biomass productivity (160.79 mg L^{-1} d^{-1}) and lipid productivity (57.72 mg L^{-1} d^{-1})	[19]
Desmodesmus spp. *S. obliquus*	Municipal wastewater with different leachate	33% increase in lipid content	[20]
Chlorella sp.	Municipal wastewater	Lipid content of 34.83%	[21]
Scenedesmus obliquus	Municipal wastewater	0.33–0.38 g L^{-1} of total lipid	[22]

There are many review papers available that focus on using microalgae for wastewater treatment, biofuel production, or even integration of both processes. However, this review paper emphasizes the microalgae lipid enhancement strategies by not compromising the biomass productivity and utilizing wastewater as the growth media. Challenges such as high prices can be reduced by coupling wastewater treatment with high biomass and lipid productivity for biofuels. As the review was on synchronous microalgae lipid enhancement and wastewater treatment, the major keywords utilized for identifying literatures were

microalgae, wastewater, lipid enhancement, and biofuel. The time frame for sorting the literature search was from 2002 to 2021.

2. Functioning of Microalgae

Microalgae are photosynthetic organisms functioning in presence of nutrients, light, and CO_2 to grow (Figure 1). Besides nutrients, light, and CO_2, many other environmental factors (temperature, pH) determine their growth rate. As microalgae uptake nutrients for growth, many studies have utilized microalgae for nutrient removal from wastewater. This process of excess nutrient removal from water bodies by microalgae is known as phycoremediation. Microalgae have also found their usage in biofuel production as they are rich in lipids.

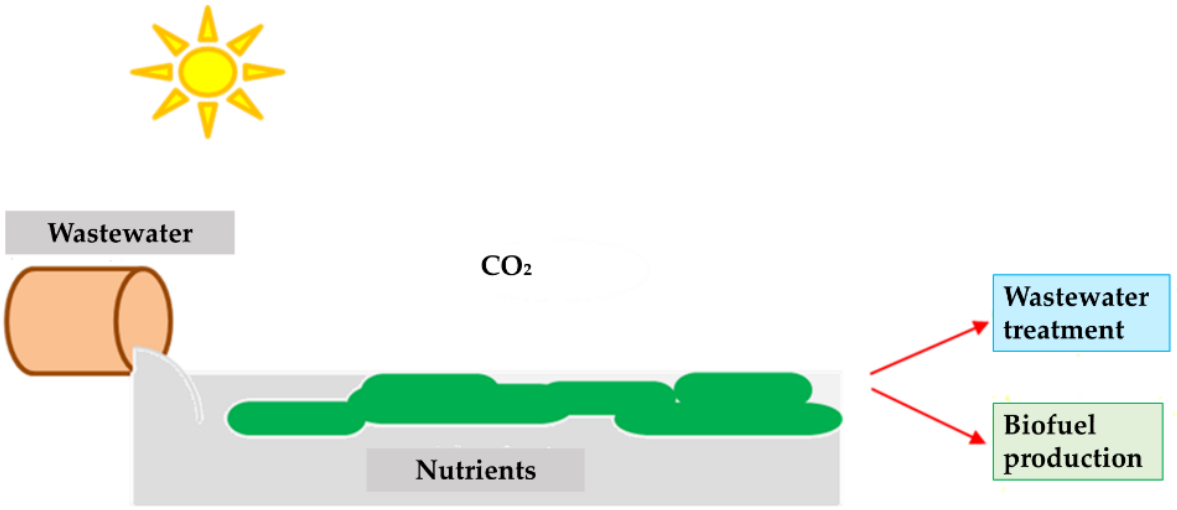

Figure 1. Pictorial representation of the functioning of microalgae for wastewater treatment and biofuel production.

2.1. Wastewater Treatment

Microalgae cultivation for wastewater treatment is an old practice. Microalgae are capable of producing oxygen (O_2) through photosynthesis during cultivation. The produced oxygen can be used to degrade organic contaminants and phycoremediate inorganic compounds available in the wastewater. Thus, photosynthetic oxygen eradicates the necessity to supply air through the conventional aeration process, decreasing the cost drastically. To reduce the probability of eutrophication, microalgal ponds were designed to treat the secondary effluent before release in aquatic bodies. Microalgae can also eliminate the nutrients (N, K) more proficiently in wastewater than conventional treatment routes.

2.2. Biofuel Production

Microalgae can convert atmospheric CO_2 into carbohydrates and lipids [7]. Lipids produced by microalgae can be divided into polar lipids (glycerophospholipids), which have an important role in cell structure, and non-polar lipids (triacylglycerols, TAGs) mainly responsible for energy storage. Lipids are stocked up in microalgae in diverse ways based on the species, growth phase, and environmental conditions. When photosynthesis occurs in microalgae, non-polar lipids such as TAGs accumulate in the cells [23]. TAGs are modified into fatty acid methyl esters by trans-esterification for biofuel production [8].

Microalgal lipid production is around 15–300 times more than the oil-bearing crops (palm, corn, soybean, and sunflower) [24]. Microalgae are competent and preferable to produce biofuels as they do not require land and freshwater for cultivation. The best way to produce high amounts of these lipids is to sustain global energy demands through high microalgal biomass productivity. Previous studies have reported that accomplishing both factors synchronously is very difficult [25]. Although there are many challenges for microalgal biofuel production, researchers believe that microalgal biofuel is still a

promising alternative for fossil fuel. A new focus in microalgal biofuel research is to optimize an ideal condition to achieve a high growth rate of microalgae while producing high lipid content under a stressful environment. Therefore, various approaches to enhance the lipid production rate in microalgae have been investigated.

3. Strategies for Lipid Enhancement

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Selection of Proper Microalgae Species or Strain

In various kinds of literature, altering the culture condition has been mentioned as a strategy for lipid enhancement in microalgae. However, biofuel production's primary drawback cannot be conquered if the microalgae species/strain is not suitable for the purpose. Microalgae have been under the limelight for their lipid-producing capability [26]. Lipid content and growth rate in microalgae vary from species to species. Lipid production by microalgae for commercial purposes undergoes tough challenges as microalgae display two different traits, either low lipid accumulation with high biomass production or vice versa. The microalgae *Botryococcus braunii* has a lipid content of 25–75% but a low growth rate, *Chlorella vulgaris* has a lipid content of 28–58% and a very high growth rate, *Dunaliella salina* has a low lipid content of 6–25% and a moderate growth rate, and *Nannochloropsis oculata* has a lipid content of 23–30% and a high growth rate [27]. Although lipid production in microalgae is species-specific, sometimes it is also strain-specific. The same microalgal species of a different strain can vary in lipid composition as well. Yamaguchi et al. [28] studied two different *B. braunii* strains under the same culture conditions and media. They reported that the lipid composition is different for both the strains, although biomass and lipid yields are similar. Growth characteristics and lipid production can vary for the same species/strain based on the growth media composition and other physio-chemical factors (light, carbon dioxide, temperature, salinity) [29]. Ruangsomboon [30] chronologically changed growth conditions (nitrogen, phosphorus, iron, light, salinity, and cultivation time) for *B. braunii* (KMITL2) in chlorella medium. At 25 °C, 222 mg/L phosphorus concentration and continuous illumination of 200 μE m^{-2} s^{-1}, the maximum lipid yield of 0.48 mg/L and biomass yield of 0.8 g/L was obtained. When phosphorus concentration was increased to 444 mg/L, lipid yield (0.45 g/L) and biomass concentration (1.91 ± 0.03 g/L) were also enhanced.

Hence, the first step for the microalgae cultivations is to determine or engineer the proper species/strain for the specific function and intention. As enhancing lipid productivity is hindered by swapping between lipid accumulation and cell growth, numerous endeavors are being made to decipher these bottlenecks, from choosing oleaginous species, strain development, and strain acclimation, to diverse cultivation media and conditions [31].

3.2. Selection of Proper Growth Media

The growth of microalgae necessitates the need for light, carbon dioxide, water, and nutrients, nitrogen and phosphorus being the chief nutrients required for their growth. For phototrophic culture, BG-11 and Chu 13 are the most used growth media with CO_2 supplementation [32–35], as BG-11 and Chu 13 contain all the significant nutrients necessary for the growth of microalgae, whereas for mixotrophic culture, synthetic media with organic carbon sources are mostly utilized [36]. Yeh and Chang [37] investigated the growth and lipid productivity of *Chlorella vulgaris* ESP-31 in different media (Basal medium and Modified Bristol's medium) and cultivation conditions. At phototrophic, photoheterotrophic, and mixotrophic conditions, *C. vulgaris* ESP-31 obtained a biomass concentration of 2–5 g/L in nitrogen-rich media. *C. vulgaris* ESP-31 growth on nitrogen limiting media exhibited enhanced lipid build-up (20–53%) with a slower growth rate.

When all the culture media were utilized at mixotrophic conditions, enhanced lipid content (40–53%) and enhanced lipid productivity (67–144 mg/L/d) were achieved. Hence, growth media and cultivation conditions influence microalgal growth and lipid accumulation to a great extent.

A huge quantity of water is essential for the microalgae cultivation process. Yang et al. [38] assessed that to generate 1 kg of microalgal biofuel, 3726 kg of water is necessary, and this prerequisite can be reduced by 90% via wastewater utilization. Saranya and Shanthakumar [39] investigated the performance of *Chlorella vulgaris* (NRMCF0128) and *Pseudochlorella pringsheimii* (VIT_SDSS) using combined sewage and tannery effluent at varying dilution. At a higher dilution (up to 30%) of tannery effluent both the species exhibited a drop in pollutant concentration: >65% for NH_3-N, 100% for PO_4-P, >63% for COD, and >80% for total chromium. They observed the maximum biomass yield of 3.51 g/L for *Chlorella vulgaris* at 30% tannery effluent, whereas it was for *Pseudochlorella pringsheimii* 2.84 g/L at for 20% tannery effluent. Additionally, *P. pringsheimii* exhibited an enhanced lipid accumulation of 25.4%, which was greater than that of *Chlorella vulgaris* (9.3%). They also reported that at 20% dilution, the lipid accumulation of *P. pringsheimii* was 5 times and 1.5 times more than undiluted sewage- and BG11-grown microalgae. Hence, it is evident that *Pseudochlorella pringsheimii* when grown in 20% tannery effluent assists in biomass growth, lipid enhancement, and treatment of wastewater.

Utilization of post-fermentation leachate from biogas plants, including the wastewater after the initial stage of anaerobic digestion, is a favorable route for microalgae biomass production and wastewater treatment. The post-fermentation wastewater comprises a high content of nitrogen and phosphorus, significant amounts of dissolved carbon dioxide, and low organics. *A. prothecoides* when cultivated in post-fermentation wastewater reported 79.45% nitrogen removal, 78.4% phosphorus removal, and the maximum lipid content of 44.65% [40].

Wastewater can serve as an excellent medium for microalgae cultivation as it contains the nutrients necessary for its growth. The utilization of wastewater as a growth medium for microalgae is an economical step as wastewater is easily available in plenty and there is no need to add nutrients as a supplement. The addition of nutrients for microalgae growth can be expensive if required in huge quantities. Wastewater utilization reduces the surplus cost of nutrient addition, especially in large-scale cultivation [41]. Furthermore, using wastewater as a growth medium for microalgae induces wastewater treatment. Therefore, wastewater utilization for microalgae growth is an economical and sustainable alternative.

3.3. Selection of Proper Stress Condition

Microalgae tend to stock up a substantial amount of lipids under stress. However, the stressful culture condition where lipid (TAGs) synthesis takes place simultaneously stimulates protein biodegradation, compromising on cell growth and biomass productivity. There are quite a few physio-chemical stress factors that perk up the stimulation of lipid accumulation such as light, temperature, CO_2, nutrients, and salinity (Table 2). Diverse microalgae species/strains respond differently to different stress conditions, thereby producing different quantities and composition of lipids in different microalgae species/strains. This alteration in the microalgae lipid metabolism can enhance the lipid basal level.

Table 2. Different microalgae responses to various stress conditions.

Microalgae	Media	Stress Condition	Result	Reference
Desmodesmus spp.	Municipal wastewater with leachate	Intensity of 53 µmol/m^2/s and light cycles of 12:12. High ammonia concentration (≥167 mg/L)	Biomass productivity of 1.95 g/L and lipid content of 20%	[20]
P.kessleri NKG021201	Municipal wastewater	Illumination at 40 mmol/m^2/s at 25 °C	Biomass productivity of 125 ± 8 mg/L/d and lipid content of 38 ± 1%	[29]
Nannochloropsis oculate *Chlorella vulgaris*	Bold's Basal Medium for *C. vulgari* f2 for *N. oculata*	20 °C for *N. oculata* and 30 °C for *C. vulgaris* Nitrogen deficiency CO_2 contained in air (about 300 ppm)	Lipid yield enhanced to 15.31% for *N. oculata* and 16.41% for *C. vulgaris*	[42]
Scenedesmus sp.	BG11	Light intensity of 55–60 µmol photon·m^{-2} s^{-1}, light/dark ratio of 14:10 at 25 °C Phosphate deficiency	Lipid accumulation of 53%	[43]
C. mexicana GU73240 *S. obliquus* HM103382	Bold basal medium	Illumination at 40 µmol (photon)/m^2s at 27 °C for 20 days Increased NaCl dose	Highest dry weight (0.8 and 0.65 g/L) and lipid content (37 and 34%) of *C. mexicana* and *S. obliquus* repectively	[44]
N. oceanica DUT01	f/2 seawater BG 11	14/10 h light/dark cycle under an intensity of 60 µmol m^{-2} s^{-1} at 25 °C 2% CO_2	Lipid productivity of 31 mg L^{-1} d^{-1}	[45]
Ankistrodesmus falcatus	Blue-Green (BG11) medium	Nitrogen rich Photon flux of 120 µmol m^{-2} s^{-1}, under a 16 h:8 h light dark cycle at 25 °C Iron sufficient and deficient	At 3 mg L^{-1} the lipid content and productivity decreased whereas lipid content and productivity enhanced at 6 mg L^{-1} iron concentration	[46]
Chlorella vulgaris *Chlorella kessleri* *Scenedesmus obliquus*	Secondary treated urban wastewater	Illumination of 143 µmol·m^{-2}·s^{-1} and 14/10 light/dark cycle at 20 ± 1 °C 4% CO_2	*Scenedesmus obliquus* yielded the highest biomass concentration (1.4 g/L) and lipid content (36.75%) *C. vulgaris* reached the highest biomass productivity (0.107 g/L·d) followed by *S.obliquus* (4.4 mg Total N/L·d)	[47]
Mixed consortium mainly consisted of Chlorella sp.	Mixed waste streams (liquid digestate obtained after the filtration of the compost, liquid coming from the septic system sludge treatment plant and an effluent coming from the wastewater treatment plant)	Photoperiod of 12:12 h at 20 °C CO_2 supplemented at a rate of 0.2 volume of air per volume of medium per minute (vvm).	Biomass productivity (105.2 mg·L^{-1}·d^{-1}) was negatively affected by CO_2 addition and positively affected by light intensity. Higher lipid contents (17.2%) were found at low light intensity	[48]
C. protothecoides UTEX-256	Pretreated dairy wastewater	5% CO_2 was supplied light intensity was 150 µmol m^{-2} s^{-1} for 9 days	40% pretreated whey was most productive for biomass and lipid fractions, respectively, 4.54 and 1.80 gL^{-1} with daily productivities 0.50 and 0.20 g$L^{-1}d^{-1}$	[49]
Chlorella vulgaris	Chicken waste compost mixed with tap water	1 day of nutrient starvation with 6 g/L of salinity stress at dark	Lipid content was recorded at 40.28%	[50]
Chlorella vulgaris	BG11 medium	Illumination at 135 µmol photon m^{-2} s^{-1} at 25 °C low concentration of Cr(VI)	Biomass productivity of 28.3–35.9 mg L^{-1} d^{-1}	[51]

3.3.1. Effect of Light

Light is one of the most indispensable factors for the production of microalgal biomass. Light provides energy for the process of photosynthesis and microbial growth. The intensity of light and its wavelength can drastically modify microalgal growth and lipid accumulation (Table 3). Wahidin et al. [52] in *Nannochloropsis* sp (marine microalga) noticed an enhanced lipid accumulation of up to 31.3% after 8-day cultivation under 100 µmol m^{-2} s^{-1}

light intensity and a photoperiod of 18 h light: 6 h dark cycle. They also observed a gradual decline in cell density and growth rate during the 24 h photoperiod cycle. Zhu et al. [8] reported that extreme light intensities affected the lipid content by photo-inhibition and photo-oxidation. Generally, microalgae encompass a light saturation limit of 600 ft. candles approximately. Exposure of microalgae to light intensity more than the light saturation limit causes oxidative stress [52]. Excessive photo-assimilation causes lipid stock up as a self-defense mechanism to prevent photo-oxidative damage, thus transforming the surplus light energy into chemical energy [53,54]. For *Chlorella* sp. L1, the high light intensity of 400 µmol photon m^{-2} s^{-1} exhibited neutral lipid productivity of 51.4 mg L^{-1} d^{-1} by He et al. [55]. Ruangsomboon [30] reported that *Botryococcus braunii* KMITL 2 exposed to high light intensities of 200 and 538 µE $^{-2}$ s^{-1} stocked up more lipid than those grown at lower light intensity (87.5 µE m^{-2} s^{-1}). On the other hand, *Phaeodactylum tricornutum* at a low light intensity of 60 µmol photons m^{-2} s^{-1} exhibited the highest TAG yield of 112 mg $mol_{ph}{}^{-1}$ [31] This indicates that the light intensity, which may be low for few microalgae species, can be high for some other microalgae species. The microalgae *Nannochloropsis* sp. produces the enhanced lipid content (47% of dry weight) at a light intensity of 700 µmol photons/m^2/s [56]. Liu et al. [57] reported that lipid accumulation in *Scenedesmus* sp. enhanced 11-fold at a light intensity of 400 µmol photons/m^2/s, whereas at a light intensity of 600 µmol photons/m^2/s, microalgae species *C. sorokiniana*, *C. viscosa*, *C. emersonii*, *C. vulgaris*, *P. beijerinckii*, and *P. kessleri* CCALA255, NIES-2152, and NIES-2159 produced enhanced lipids [58]. Liao et al. [59] confirmed that *Chlorella vulgaris* at a high light intensity of 560 µE m^{-2} s^{-1} exhibited a 92.89% enhanced lipid yield, greater than the lower intensity of 160 µE m^{-2} s^{-1}. In some microalgae species such as *Scenedesmus obliquus*, lipid content remained the same when light intensity changed from 200 to 1500 µmol photons/m^2/s [60].

Table 3. Different microalgae responses to light conditions.

Microalgae	Stress Condition	Result	Reference
Botryococcus braunii KMITL 2	Light intensities of 200 and 538 µE $^{-2}$ s^{-1}	More lipid accumulation than lower light intensity of 87.5 µE m^{-2} s^{-1}	[30]
Phaeodactylum tricornutum	Light intensity of 60 µmol photons m^{-2} s^{-1}	TAG yield of 112 mg $mol_{ph}{}^{-1}$	[31]
Nannochloropsis sp.	100 µmol m^{-2} s^{-1} light intensity photoperiod of 18 h light: 6 h dark cycle	Enhanced lipid accumulation of up to 31.3%	[52]
Chlorella sp. *L1*	400 µmol photon m^{-2} s^{-1} light intensity	51.4 mg L^{-1} d^{-1} lipid productivity	[55]
Nannochloropsis sp.	Light intensity of 700 µmol photons/m^2/s	Enhanced lipid content (47% of dry weight)	[56]
Scenedesmus sp.	143 µmol·m^{-2}·s^{-1} and 14/10 light/dark cycle at 20 ± 1 °C	Lipid accumulation enhanced	[57]
Chlorella vulgaris	Light intensity of 560 µE m^{-2} s^{-1}	92.89% enhanced lipid yield	[59]
Scenedesmus obliquus	200–1500 µmol photons/m^2/s light intensity	Lipid content remained the same	[60]

Besides light intensity, previous studies are reporting the influence of varying light wavelengths on microalgae growth and metabolism. The microalgae chlorophyll has an absorption band (630–675 nm) in red and blue (450–475 nm) spectral region [61]. The phenomenon of microalgae using light energy obtainable within the wavelength of 400–700 nm is known as photosynthetically active radiation (PAR). Das et al. [62] exposed *Nannochloropsis* sp. to different light wavelengths (red, green, blue, and white). They reported the highest fatty acid methyl esters (FAME) content under green LED (550 nm). Nevertheless, the maximum volumetric FAME yield was achieved under blue LED (470 nm) owing to maximum biomass productivity at this wavelength. Hultberg et al. [63] observed that *Chlorella vulgaris* exposed to light at green wavelengths illustrated enhanced polyunsaturated fatty acids (C16:3 and C18:3). According to Rai et al. [64], the best growth and the highest lipid accumulation for *Chlorella* sp. were obtained in red light, whereas green

light demonstrated weak biomass growth and less lipid production. Wong et al. [65] demonstrated that *Chlorella vulgaris* grown in wastewater using blue light achieved the highest lipid content (34.06%) after 10 days of 18:6 light/dark periods, with an initial cell number of 106 cells/mL. Severes et al. [66] reported that a combination of red (600–700 nm) and blue (400–500 nm) light wavelength for a culture of *Chlorella* sp. increases biomass production. They also observed that red light wavelength doubled the dry weight of lipids in *Chlorella* cells during the growth period.

Microalgae seem to produce more polar lipids during low light intensity due to enhanced chloroplast membrane synthesis. A gradual increase in light intensity tends to stock up more neutral lipids without hampering the biomass yield. Both low and extremely high light intensity leads to adverse growth and responses in cells. Therefore, determining the ideal intensity of light helps acquire optimal microalgal growth and enhanced lipid production [53,67]. However, the intensity of light required to attain the maximum growth, and lipid content varies from species to species. Hence, complementing irradiation of the light bands for the constitutive pigments (chlorophyll, phycobilins, and carotenoids) can increase the photosynthetic rate and lipid productivity with reduced energy consumption.

3.3.2. Effect of Temperature

The temperature has a vital effect on microalgae growth and lipid production (Table 4). Temperature can operate as a stress factor that can bump up the reactive oxygen species level and stimulate neutral lipids, i.e., TAGs [68]. Biochemical pathways for lipid synthesis and stock up are regulated by enzymes that are very sensitive to thermal variations [69]. Converti et al. [42] investigated thermal variation (20 to 25 °C) with respect to lipid content for *N. oculata* and *C. vulgaris*. They observed that a rise in temperature leads to a drop in lipid content in *C. vulgaris* and vice versa, whereas for *N. oculata* a rise in temperature led to enhanced lipid production by 2-fold (7.90–14.92%). Subhash et al. [70] observed a 5-fold rise in neutral lipid at a high temperature of 30 °C in a mixed microalgae culture for wastewater treatment. Bohnenberger and Crossetti [71] reported that for *Monoraphidium consortiums* and *Desmodesmus quadricauda*, a lower temperature of 13 °C aids in lipid accumulation. Freire et al. [72] observed the highest growth and lipid productivity for *Nannochloropsis limnetica* at 22 °C, whereas for *Heterochlorella luteoviridis*, Menegol et al. [73] observed 40.7% of PUFAs at a temperature of 22 °C, whereas the percentage of saturated fatty acids was increased (52.9%) at 27 °C. Xin et al. [43] reported enhanced lipid build-up at lower temperatures of 10–20 °C than at 25 °C for *Scenedesmus* sp. LX1. Wei et al. [74] reported that the optimal temperature for lipid stock up for *Tetraselmis subcordiformis* was 20 °C and for *N. oculata* 30 °C. Xin et al. [68] reported that almost all of the fatty acids and long-chain species (C16–C18) were saturated at a high temperature of 30 °C for *Scenedesmus* sp. LX1. Analogous results were also reported by Renaud et al. [75] for the microalgae *Rhodomonas* sp., *Cryptomonas* sp., and *Prymnesiophyte* NT19, respectively.

Table 4. Different microalgae responses to the effect of temperature.

Microalgae	Stress Condition	Result	Reference
N. oculata *C. vulgaris*	20–25 °C	Increased temperature decreases lipid content in *C. vulgaris* and increases lipid production for *N.oculata*	[42]
Mixed microalgae culture	30 °C	5-fold rise in neutral lipid	[70]
Monoraphidium consortiums *Desmodesmus quadricauda*	13 °C	Aids in lipid accumulation	[71]
Nannochloropsis limnetica	22 °C	highest growth and lipid productivity was observed	[72]
Heterochlorella luteoviridis	22–27 °C	40.7% of PUFAs at a temperature of 22 °C, whereas 52.9% saturated fatty acids increased at 27 °C	[73]
Scenedesmus sp. *LX1.*	10–25 °C	Enhanced lipid build-up at low temperature	[43]
Scenedesmus sp. *LX1*	30 °C	Most of the fatty acids saturated	[68]

Although the optimal temperature for microalgae growth and lipid production differs from species to species, still, in many microalgae species, high temperature favors saturated fatty acid formation, which is maneuvered by the cell membrane fluidization. The fluidity of phospholipid bilayers is enhanced when the number of double bonds increases [76]. Furthermore, the fatty acid composition of microalgae is also influenced by temperature.

3.3.3. Effect of Carbon Dioxide (CO_2)

Atmospheric CO_2 is another essential component for microalgal growth. CO_2 is a source of carbon for microalgae cell development. Microalgae capture the atmospheric CO_2 in the presence of sunlight to produce biomass and chemical compounds of interest (Table 5). Zhu et al. [8] stated an optimum range of CO_2 necessary for the growth, lipid production, and accumulation within the microalgae. A high concentration of CO_2 helped *Nannochloropsis* species, but for some microalgae species, a high concentration of CO_2 is toxic [77]. Chiu et al. [78] reported lipid productivity of 142 and 82 mg L^{-1} d^{-1} for *N. oculata* when aerated with 2% and 15% CO_2, respectively. Jiang et al. [14] for *Nannochloropsis* sp. observed an increase in biomass productivity (0.39–1.43 gL^{-1}) and growth rate (0.33–0.52 d^{-1}) at 15% CO_2 concentration. *Ettlia* sp. YC001 at 10% CO_2 achieved 3.1 g L^{-1} cell density and 80.0 mg L^{-1} d^{-1} lipid productivity, after 16-days of cultivation [79]. Montoya et al. [80] reported a high concentration of fatty acids and lipid productivity (29.5 mg/L/day) for *C. vulgaris* at a CO_2 concentration of 8% (*v*/*v*). Nakanishi et al. [81] obtained maximum lipid productivity of 169.1 mg/L/day for *Chlamydomonas* sp. JSC4 strain at 4% (*v*/*v*) CO_2 concentration, whereas for the species *D. salina*, growth inhibition was observed at 0.02 mol CO_2/L concentration or higher for *Dunaliella salina*. Bagchi and Mallick [82] reported that at 15% (*v*/*v*) of CO_2 concentration, *Scenedesmus Obliquus* (Turpin) Kützing GA 45 stocked up 850 mg/L of lipid in 16 days. Hui et al. [83] observed a trend of reduced lipid productivity with increased CO_2 concentration while studying the effect of varying CO_2 concentration (0.03–20%) in *Tribonema minus*. At 2% CO_2 aeration, maximum total lipid content was attained, and at 20% CO_2, total lipid content was at its least. However, the culture aerated at 0.03% CO_2 illustrated the second-lowest lipid content, signifying photosynthesis restraints due to the medium's shortage of carbon sources. Kao et al. [84] cultivated *Chlorella* sp. in synthetic media by aerating with industrial gaseous effluents rich in CO_2. A maximum specific growth rate of 0.827 d^{-1} and lipid production of 0.961 g L^{-1} was obtained. Thus, gaseous effluents can be utilized to enhance biomass and lipid production as well as for CO_2 biofixation.

Table 5. Different microalgae's response to varying CO_2 concentration.

Microalgae	Stress Condition	Result	Reference
N. oculata	2% and 15% CO_2	Lipid productivity of 142 and 82 mg L^{-1} d^{-1}	[78]
Nannochloropsis sp.	15% CO_2	Increased biomass productivity (0.39–1.43 gL^{-1}) and growth rate (0.33–0.52 d^{-1})	[14]
Ettlia sp. *YC001*	10% CO_2	3.1 g L^{-1} cell density and 80.0 mg L^{-1} d^{-1} lipid productivity	[79]
C. vulgaris	8% CO_2	29.5 mg L^{-1} d^{-1} lipid productivity	[80]
Chlamydomonas sp. *JSC4*	4% CO_2	169.1 mg L^{-1} d^{-1} lipid productivity	[81]
Scenedesmus Obliquus	15% CO_2	850 mg/L of lipid in 16 days	[82]
Tribonema minus	(0.03–20%) CO_2	Reduced lipid productivity with increased CO_2	[83]

Ying et al. [85] studied pH changes at different CO_2 concentrations, leading to drastic pH changes which destroy enzymes involved in photosynthesis. The availability of a high level of H_2CO_3, transformed from the unused CO_2, lowered the media's pH, creating a hostile condition for the growth of microalgae. Additionally, the lowered pH hinders lipid synthesis's carbon assimilation due to the lowered bicarbonate concentration [8]. pH changes in microalgae cause diverse biochemical responses. In *Chlorella*, high pH

hindered the cell division cycle, provoked the discharge of autospores and initiated TAG utilization [86].

CO_2 concentration for microalgal growth and lipid accumulation is species-specific. In short, the increase in CO_2 could help in the production and stock up of lipids in the microalgae for some species, but it might not be the same for other microalgae species. Supplying additional CO_2 during phycoremediation of wastewater helps in lipid content enhancement [87]. In addition to CO_2 concentration, it is crucial to estimate the optimum pH range for optimal biomass growth and lipid stock up in microalgae. When microalgae are provided with high CO_2 concentration, a portion of the carbon is utilized for photosynthesis, whereas the remaining carbon is transformed into carbonic acid (H_2CO_3). H_2CO_3 causes acidification changing metabolic pathways.

3.3.4. Effect of Nutrients

Microalgae require nutrients (nitrogen, phosphorus, iron, magnesium, sulfur, and silicon) for photosynthesis, respiration, cell division, intracellular transportation, and protein synthesis. Microalgae tend to stock up lipids during nutrient stress (Table 6). Tran et el. [88] investigated the effect of nitrogen deficiency in *Chlorella vulgaris.* They observed that *Chlorella vulgaris* accumulates a higher amount of lipids (TAGs). Converti et al. [42] reported that under nitrogen deficiency, the lipid yield enhanced to 15.31% for *Nannochloropsis oculata* and 16.41% for *Chlorella vulgaris*. Nitrogen deficiency in *Nannochloropsis* sp. can synthesize more TAGs and reduce polar lipids [89]. *Nannochloropsis* sp. F&M-M24 illustrated maximum lipid productivity of 204 mg L^{-1} d^{-1} at nitrogen-deficient condition [90], whereas *N. oceanica* DUT01 illustrated maximum lipid productivity of 31 mg L^{-1} d^{-1} under nitrogen-rich conditions [45]. Other studies conducted by Hsieh and Wu [91], Praveenkumar et al. [92] and Li et al. [93] also reported similar results for various species of microalgae. Many previous studies examined that in most microalgae species nitrogen deficiency enhanced lipid accumulation (30–70%) within 7–20 days [94]. Nitrogen deficiency for 9 days helped *Monallantus salina to* attain the maximum lipid concentration of 72%. *Botryococcus braunii* attained 61.4%, *Chlorella* vulgaris attained 57.9% and *Nannochloropsis* sp. attained 55% lipid production during their lifetime, whereas *Scenedesmus* sp. and *Chlorella sorokiniana could not attain* maximum lipid content even after 7 and 14 days. Adams et al. [95] demonstrated that *Chlorella vulgaris* and *Chlorella oleofaciens* have more potential as biofuel feedstock species than *Chlorella sorokiniana*, *Neochloris oleoabundans*, *Scenedesmus dimorphus*, and *S. naegleii* as they react to slight stress, thus growing and accumulating lipids simultaneously. Lin and Lin [96] reported the highest biomass productivity in *Scenedesmus rubescens* by a mixture of urea and sodium nitrate.

Tao et al. [97] observed differences in lipid content and fatty acid composition for the microalgae species *Chlorococcum ellipsoideum*, *Chlorococcum nivale*, *Chlorococcum tatrense*, and *Scenedesmus deserticola* due to nitrate and urea deficiency. Yang et al. [98] demonstrated a significant increase in fatty acid yield for *Chlamydomonas reinhardtii* when the growth media were deficient in phosphorus or nitrogen. Phosphorus deficiency displays minor effects on the photosynthetic physiology and protein content of *Chlamydomonas reinhardtii* compared with nitrogen deficiency, and microalgae lessen the number of ribosomes to uphold the protein synthesis or polyphosphates storage [99]. Cordeiro et al. [100] studied the effects of phosphorus and nitrogen levels on the growth of species of *Microcystis panniformis* and *Microcystis novacekii*. They reported that the lipid accumulation had an inverse and direct correlation with nitrogen (35.8%) and phosphorus concentration (31.7%) for *Microcystis panniformis* and *Microcystis novacekii*, respectively. At the same time, Mata et al. [101] observed that increasing the nitrogen concentration up to 10-fold led to increased lipid productivity (47.4 mg/L/day) and content (33.5%) for *Dunaliella tertiolecta*. Xin et al. [68] in *Scenedesmus* sp. reported maximum lipid accumulation of 53% under phosphate deficiency (0.1 mg L^{-1}). Microalgae stock up phosphorus as polyphosphate during adequate nutrient conditions, which is then used up during nutrient-deficient conditions.

Microalgae have verified their efficiency and effectiveness in enduring a high concentration of micronutrients. Various studies have demonstrated the increase in the lipid content of some microalgae species due to micronutrients stress. Liu et al. [102] demonstrated 3–7 folds enhanced lipid accumulation in *Chlorella vulgaris* under higher iron concentration than the control. Baky et al. [103] found enhanced lipid yield (28.12%) with an increase in $FeCl_3$ concentration of 20 mg L^{-1} when *Scenedesmus obliquus* was cultivated in N-9 medium. Singh et al. [46] observed that a low iron concentration of 3 mg L^{-1} decreased the lipid content and productivity of *Ankistrodesmus falcatus*, whereas enhanced lipid content and productivity were observed when iron concentration (6 mg L^{-1}) increased. Ren et al. [104] studied the effect of Fe^{3+} (0–0.12 g/L), Mg^{2+} (0–0.73 g/L), and Ca^{2+} (0–0.98 g/L) on lipid accumulation in *Scenedesmus* sp. They reported that the addition of EDTA (0–1 mg/L) during cultivation increased total lipid content up to 28.2% and lipid productivity up to 29.7%. Gorain et al. [105] observed enhanced lipid content (1.44-fold) when 100 mg L^{-1} magnesium was added in the media. Battah et al. [94], using manganese chloride ($MnCl^2$) at a concentration of 2 µM, 10 µM, and 12 µM, evaluated the effect of Mn^{2+} and Co^{2+} on the lipid content of *C. vulgaris*. They observed that $MnCl_2$ at 2 µM, 10 µM, and 12 µM concentration increased lipid content significantly by 14%, 16%, and 15%, respectively. In addition to that, Battah et al. [106] reported a 25% increase in lipid content when cobalt nitrate was added in varying concentrations. Liu et al. [102] reported a 56.6% increase in the total lipid content in *C. vulgaris* at five varying Fe^{3+} concentrations.

Nutrient stress is a practical and promising strategy exploited by researchers and industries to modify and regulate the biochemical pathways for lipid production and accumulation in microalgae [107]. During the early stages of microalgal growth, nutrient-rich media lead to enhanced biomass productivity. However, deficiency of nutrients during the later stages of growth has detrimental effects, leading to the lipid stock up. During nitrogen deficiency, most microalgae exhibit enhanced lipid synthesis (TAGs) and protein biodegradation. The lipid enhancement mechanism induced by nutrient stress is not understood properly. Yet, nitrogen deficiency stimulates acyl hydrolase and phospholipid hydrolysis, demonstrating a reduction in the cellular content of thylakoid membranes and protein synthesis, ultimately reducing cell growth [108,109], thereby compromising the microalgal growth, biomass yield, and consequently affecting the lipid productivity [110]. Many studies investigated different species of microalgae for various nutrient deficiencies. In the growth media, nitrogen may be supplied in the form of nitrates, urea, and ammonium salts. The utilization of these nitrogen forms by microalgae might differ. Ammonia can be directly converted to amino acids, thus microalgae use it more efficiently than the other forms of nitrogen [43,111]. Changing the nitrogen source might cause a change in the amount of lipid accumulation and fatty acid composition. Metal ions also have numerous physiological functions influencing the metabolism and lipid accumulation of microalgae. Ca^{2+} is engaged in the signaling of environmental and developmental stimuli. Mg^{2+} is involved in triggering and mediating various biochemical reactions, i.e., regulating carbon fixation in chloroplasts in the Calvin cycle. Increasing Mg^{2+} can help Acetyl-CoA carboxylase, the key regulator of fatty acid synthesis, to enhance the neutral lipid content in microalgae.

3.3.5. Effect of Salinity Stress

Salinity stress in microalgae causes a difference in osmotic pressure, generating a stress response that alters their metabolic activity [112]. Different microalgae's response to salinity stress has been summarized in Table 7. Rao et al. [113] illustrated a 1.7–2.25-fold increase in palmitic acid's relative quantity and a 2-fold increase in oleic acid at high salinity concentration (34 mM and 85 mM) for *Botrycococcus braunii*. Sharma et al. [114] reported that varying salt concentration in the microalgal growth medium increases the total lipid content and alters the lipid composition. Bartley et al. [115] studied the effect of salinity stress on the growth of *Nannochloropsis salina*. They examined it at a salt concentration of 34, 46, and 58 PSU. They reported the highest total fatty acid content of 36% dry tissue mass at 34 PSU.

Salama et al. [44] observed the maximum lipid content of 37% and 34% for *Chlamydomonas Mexicana* and *Scenedesmus obliquus*, respectively, at a 25 mM NaCl concentration. They also reported the dominant fraction of fatty acids as linoleic acids (41%) and oleic acids (41%). Figler et al. [116] reported on the halotolerant species *Coelastrum morus*, which could thrive at 1000 mg L^{-1} NaCl and remove a significant amount of nitrate in media with different N:P ratios and salt concentrations, although the growth of the species was negatively affected.

Table 6. Different microalgae responses to nutrient stress.

Microalgae	Stress Condition	Result	Reference
Nannochloropsis oculata *Chlorella vulgaris*	Nitrogen deficiency	15.31% lipid yield for *N. oculata* and 16.41% for *C. vulgaris*	[42]
Nannochloropsis sp. *F&M-M24*	Nitrogen-deficient condition	Lipid productivity of 204 mg L^{-1} d^{-1}	[90]
N.oceanica *DUT01*	Nitrogen-rich condition	31 mg L^{-1} d^{-1} lipid productivity	[45]
Microcystis panniformis *Microcystis novacekii*	Phosphorus and nitrogen	Lipid accumulation had an inverse and direct correlation with nitrogen and phosphorus concentration	[100]
Dunaliella tertiolecta	Nitrogen	10 folds increased nitrogen led to lipid productivity of 47.4 mg L^{-1} d^{-1} and content of 33.5%	[101]
Chlorella vulgaris	High iron	3–7 folds enhanced lipid accumulation	[102]
Ankistrodesmus falcatus	3–6 mg L^{-1} iron	3 mg L^{-1} decreased the lipid content and productivity whereas 6 mg L^{-1} enhanced lipid content and productivity	[46]

Table 7. Different microalgae responses to salinity.

Microalgae	Stress Condition	Response	Reference
Botrycococcus braunii *N. oculata*	Salinity concentration of 34 mM and 85 mM	1.7–2.25-fold increase in palmitic acid and 2-fold increase in oleic acid at 34 mM and 85 mM for *B. braunii*. For *N. exican*, rise in temperature led to an enhanced lipid production by 2-fold	[113]
Nannochloropsis salina	Salt concentration of 34, 46, and 58 PSU	Highest total fatty acids content of 36% dry tissue mass at 34 PSU	[115]
Chlamydomonas mexicana *Scenedesmus obliquus*	25 mM NaCl	Maximum lipid content of 37% and 34% for *C. mexicana* and *S. obliquus*	[44]
C. vulgaris *Acutodesmus obliquus*	0.4 M NaCl	Highest growth and lipid productivity was observed	[117]
Acutodesmus dimorphus	200 mM NaCl	33.40 ± 2.29% lipid accumulation	[118]
Chlorella sorokiniana *CG12(KR905186)* *Desmodesmus GS12(KR905187)*	NaCl, KCl, $MgCl_2$ and $CaCl_2$	$CaCl_2$ improved up to 40.02–44.97% in *Chlorella sorokiniana* CG12(KR905186) and *Desmodesmus* GS12(KR905187)	[43]

Pandit et al. [117] reported that the maximum amount of lipids was 49% and 43% for *C. vulgaris* and *Acutodesmus* obliquus, respectively, at a concentration of 0.4 M NaCl, whereas Chokshi et al. [118] observed 33.40 ± 2.29% lipid accumulation at 200 mM NaCl for Acutodesmus dimorphus, which enhanced up to 43% when salinity stress extended up to 3 days. Srivastava et al. [119] studied the effect of lipid accumulation in microalgae with different salts (NaCl, KCl, $MgCl_2$ and $CaCl_2$). They reported that $CaCl_2$ had the maximum effect on lipid production, improving up to 40.02% and 44.97% in *Chlorella sorokiniana* CG12(KR905186) and *Desmodesmus* GS12(KR905187), respectively, thereby indicating that Ca^{2+} demonstrates an authoritative function in cell signaling under salinity stress leading to an enhancement in lipid synthesis.

Hence, the addition of salts is a simple, efficient strategy to enhance lipid accumulation while reducing non-target microalgae invasion, as salts perform a vital function in the physiological and biochemical pathways of growth and fatty acid metabolism in microalgae.

4. Integration of Wastewater Treatment, Enhanced Biomass, and Lipid Production Strategies

Microalgae cultivation for simultaneous lipid production and wastewater treatment is a sustainable and economical route (Figure 2). Various sources of wastewater are utilized for the cultivation of microalgae. The various sources of wastewater include but are not limited to; domestic wastewater [120], industrial and agro-industrial wastes [121], swine wastewater [122], and stabilization lagoon from sanitation facilities [123]. Several previous studies have demonstrated either biomass productivity or enhanced lipid productivity in addition to wastewater treatment. Nevertheless, many studies now integrate wastewater treatment and enhanced biomass and lipid production strategies. Fields et al. [124] stated that high lipid productivity could be attained by a high concentration of inorganic carbon and nutrient stress. When light is sufficient, and nitrogen is deficient, photosynthetic carbon fixation continues while growth ceases. This, consequently, enhances the C:N ratio and TAG productivity.

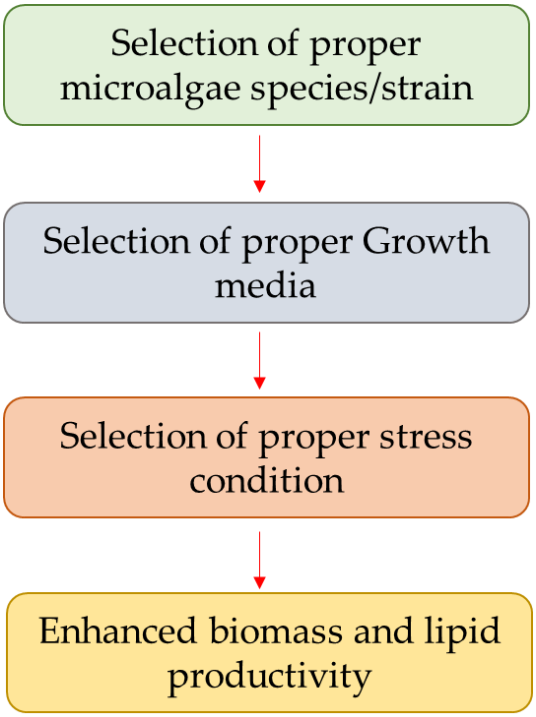

Figure 2. Showing the stepwise selection of proper conditions for enhanced biomass and lipid productivity.

Zhou et al. [125] identified different microalgae capable of growing in concentrated municipal wastewater at a light intensity of 50 μmol m^{-2} s^{-1}. They observed that *Chlorella* sp., *Heynigia* sp., *Hindakia* sp., *Micractinium* sp. and *Scenedesmus* sp. could adapt to the concentrated municipal wastewater. Additionally, high growth rates (231–275 mg/L/d) and higher lipid content (28.9–33.5%) compared with other species were witnessed. They concluded that the microalgae that showed high biomass productivity and lipid content could acclimatize themselves to the concentrated municipal wastewater and lighting conditions provided for their growth, lipid enhancement, and nutrient removal. In another study, Li et al. [126] investigated the effect of light intensity on biomass accumulation, wastewater treatment, and biodiesel productivity for *Chlorella kessleri* and *Chlorella protothecoide*. The optimum light intensity for *C. kessleri and C. protothecoide* was 120 μmol m^{-2} s^{-1} and

30 μmol m^{-2} s^{-1}, respectively. They reported the highest biodiesel content of 24.19% and 19.48% *C. kessleri* and *C. protothecoide*, respectively, with the main components being 16-C and 18-C FAME.

Cabanelas et al. [127] evaluated the growth of microalgae using domestic wastewater amended with glycerol. The best results were attained with the highest glycerol supplementation (50 mM) when aerated with 2.5% CO_2, photoperiod of 12:12 light/dark cycles, the luminance of 174 μE/m^2/s and incubation at 25 ± 1 °C. At this condition, *Chlorella vulgaris* and *Botryococcus terribilis* showed biomass productivity of 118 and 282 mg L^{-1} d^{-1}, which produced about 18 and 35 mg L^{-1} d^{-1} lipids, respectively.

Aketo et al. [29] observed the lipid productivity of various microalgal strains in municipal wastewater. They reported *Parachlorella kessleri* NKG021201 removed 99% nitrogen and 82% phosphorous from the wastewater along with 101 ± 1 mg/L/d biomass productivity and 39 ± 1% lipid content. *P. kessleri* NKG021201 was cultured again at optimal culture conditions in municipal wastewater to further enhance the biomass productivity and lipid content. The biomass productivity and lipid content enhanced to 147 ± 1 mg/L/d and 56 ± 1 mg/L/d, respectively, when the culture condition was changed to 1% NaCl, 0.8 L/L/min aeration with 4% CO_2, 30 °C and continuous illumination at 200 μmol/m^2/s. Singh and Ummalyma [128] observed enhanced biomass (450 mg L^{-1}) and lipid yield of 129 mg L^{-1} (28%) when *Chlorococcum* sp. *SL7B* was grown in river water contaminated with pharmaceutical effluent. The *Chlorococcum* sp. *SL7B* was maintained at 26 ± 2 °C, 40.5 μmol photons m^{-2} s^{-1} at a light/dark cycle of 14:10 h. They concluded that microalgae grown on polluted river water exhibited enhanced biomass and lipid production along with organic pollutant removal.

The utilization of microalgae for the treatment of wastewater is known as phycoremediation. Usually, the wastewater stream has a high content of nutrients and organic matter, but wastewater sources have varying nutrient concentrations which may be toxic to microalgae. Collos and Harrison [129] examined the effect of nitrogen concentration from ammonia on six microalgae classes. For *Chlorophyceae* they exhibited an optimal concentration of 7600 μM and toxic concentration of 39,000 μM. Improvement in nutrient tolerance can be achieved by strains acclimation in culture media. Moreover, they concluded that further research is necessary as ammonium and ammonia toxicity can vary with other parameters (light, pH, temperature). Silambarasan et al. [21] reported that for the consortium of *Chlorella* sp. and *Scenedesmus* sp. when grown in 75% diluted wastewater, the highest biomass of 1.78 g L^{-1} and lipid content of 34.83% dry cell weight were obtained. The reported algal consortium was proficient in removing 96% nitrate removal, 98% ammonium, 95% phosphate, and 94% total nitrogen from the 75% diluted wastewater. Han et al. [22] observed that 0.33–0.38 g L^{-1} of total lipid was yielded by *Scenedesmus obliquus* when cultivated in municipal wastewater along with 98.9–99.8% of nitrogen removal and 83.1–97.6% of phosphorus removal. *Scenedesmus obliquus* was supplied with 14,500 lx light intensity, 97 mg L^{-1} nitrogen, 11 mg L^{-1} phosphorus, and 9.8% CO_2.

Several studies used pretreated or raw industrial wastewater for microalgae growth [123,130]. However, a few studies also suggested that microalgae may be utilized as a tertiary treatment to eliminate residual organic and inorganic effluent contamination [131]. Either way, both wastewater treatment and lipid concentration in microalgae can be obtained.

Microalgae biorefineries corresponds to an effective green strategy for synchronous wastewater treatment and bioenergy production [132–134]. Silkina et al. [135] developed a bio-refinery approach cultivating up to 1.5 m^3 *Nannochloropsis oceanica* biomass in pilot scale where they witnessed 99% of nitrogen and phosphorus uptake by microalgal cultures. A similar pilot scale biorefinery approach was conducted with *Nephroselmis* sp. KGE8, *Acutodesmus obliquus* KGE 17 and *Acutodesmus obliquus* KGE32 microalgae [136]. In a pilot scale study of integrated biofuel production and wastewater treatment, the process simulation provided an estimated 82.77–140.58 tons/ha/year CO_2 sequestration with 63–107 tons/ha/year potential biomass production. The integrated process was reported

to significantly enhance the energy balance and economics of the wastewater treatment plant [137].

Therefore, it can be concluded that a proper microalgae strain should be acclimated in wastewater integrated with the favorable lipid enhancement strategies and culture conditions to simultaneously treat wastewater and enhance biomass and lipid productivity for biofuel production.

5. Conclusions and Future Recommendation

Microalgae are efficient organisms for wastewater treatment, and when combined with proper culture conditions, humankind can reap benefits such as high biomass and lipid productivity. Diverse microalgae have diverse optimal culture conditions. It is essential to comprehend the intricacies and flexibilities of the culture condition. Synchronizing the culture condition can facilitate maximizing biomass production and lipid accumulation in conjunction with wastewater treatment. It is crucial to adopt a less expensive and efficient sustainable culture media to reduce cultivation costs. Thus, this review provided insight on the influence of culture media and conditions to address synchronous microalgae biomass and lipid enhancement and phycoremediation. Most of the reviewed studies presented are investigations either on lipid enhancement or wastewater treatment by microalgae; there are very few studies available on synchronous microalgal lipid enhancement and wastewater treatment. Laboratory and pilot scale examinations and economic feasibility analyses to address synchronous microalgae biomass and lipid enhancement are essential to substantiate its application.

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