Biotechnological Approaches for Biomass and Lipid Production Using Microalgae Chlorella and Its Future Perspectives

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Introduction

Microalgae have recently drawn considerable attention for their high potential to produce valuable compounds as well as their applications in biodiesel production, phycoremediation, and dietary supplements. As a source of bioenergy raw materials that can be used to produce biofuels, microalgae are a unique bioresource that has been proposed as an effective agent for biomass production. Several advantages of microalgae include faster growth, usage of non-arable land, recovery of nutrients from wastewater, efficient CO₂ capture, and high amount of biomolecules that are valuable for humans. Microalgae Chlorella spp. are a large group of eukaryotic, photosynthetic, unicellular microorganisms with high adaptability to environmental variations. Over the past decades, Chlorella has been used for the large-scale production of biomass. In addition, Chlorella has been actively used in various food industries for improving human health because of its antioxidant, antidiabetic, and immunomodulatory functions. However, the major restrictions in microalgal biofuel technology are the cost-consuming cultivation, processing, and lipid extraction processes. Therefore, various trials have been performed to enhance the biomass productivity and the lipid contents of Chlorella cells. This study provides a comprehensive review of lipid enhancement strategies mainly published in the last five years and aimed at regulating carbon sources, nutrients, stresses, and expression of exogenous genes to improve biomass production and lipid synthesis.

Keywords: Chlorella, biotechnology, lipids, microalgae, biomass, phycoremediation

Heavy reliance on fossil fuels has been associated with increased climate disasters. As an alternative, microalgae have been proposed as an effective agent for biomass production. Several advantages of microalgae include faster growth, usage of non-arable land, recovery of nutrients from wastewater, efficient CO₂ capture, and high amount of biomolecules that are valuable for humans. Chlorella spp. are a large group of eukaryotic, photosynthetic, unicellular microorganisms with high adaptability to environmental variations. Over the past decades, Chlorella has been used for the large-scale production of biomass. In addition, Chlorella has been actively used in various food industries for improving human health because of its antioxidant, antidiabetic, and immunomodulatory functions. However, the major restrictions in microalgal biofuel technology are the cost-consuming cultivation, processing, and lipid extraction processes. Therefore, various trials have been performed to enhance the biomass productivity and the lipid contents of Chlorella cells.

This study provides a comprehensive review of lipid enhancement strategies mainly published in the last five years and aimed at regulating carbon sources, nutrients, stresses, and expression of exogenous genes to improve biomass production and lipid synthesis.

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Chlorella belongs to the Chlorophyta division and consists of small, non-motile, spherical unicellular microalgae with a single chloroplast [10]. Chlorella mainly lives in freshwater, but a few species are also found in the marine environment. They are autospores and mainly reproduce asexually by mitosis [11]. Chlorella species have been used as a bioresource because of their valuable molecules. Although they were initially considered a food resource owing to their high protein content [12], they have more recently been used for biofuel production [13]. Chlorella species have high adaptability to environmental variations [14], and these characteristics make Chlorella the most effective microalgae for the generation of bioresources. In addition, Chlorella species can survive and accumulate products using wastewater, making them an exciting target of study in the phycoremediation field [15, 16].

For example, lipids accumulated by microalgae can be used as feedstock for biodiesel production, and microalgal oils can be used in the food industry [17, 18]. Many studies have shown the importance of cultivation conditions for microalgal growth and lipid accumulation. Nutrients [19, 20], high salinity [21, 22], metal ions [23], light intensity, temperature, pH, and abiotic/biotic treatments are regarded as critical parameters for microalgal growth and lipid accumulation. This review presents updated research on Chlorella biomass and lipid production, published mainly in the last five years, and discusses the subsequent progress and perspectives.
Nutrients
Carbon Source for Cultivation of Chlorella

Photoautotrophic growth of microalgae requires inorganic carbon as a carbon source for growth, which relies on light as a sole energy source. The application of organic carbon sources can be divided into two types depending on light’s presence (mixotrophic) or absence (heterotrophic). Chlorella strains can grow under photoautotrophic, heterotrophic, and mixotrophic conditions, allowing them to shift in response to changes in the environment. Many studies have been performed to reveal the cultivation method with high efficiency under either photoautotrophic, mixotrophic, or heterotrophic conditions [24-27].

Photoautotrophic Mode

In photoautotrophic cultivations, the only source of carbon for photosynthesis comes from the available atmospheric CO₂ (Fig. 1). The photobioreactor system is capable of photoautotrophic cultivation of Chlorella, and several studies investigated the ability for lipid production with the combination of photoautotrophic cultivation and nitrogen depletion, which will be discussed later. Amaral et al. optimized photoautotrophic cultivation of Chlorella in a photobioreactor with statistical analysis, and increased the biomass and lipid productivity of C. minutissima at least 1.42-fold and 2.43-fold (5.72 mg/l/day), respectively, in a medium with reduced nutrient availability [28]. Singh et al. developed a two-stage photoautotrophic lipid production strategy in a sintered disk photobioreactor [29]. Initially, C. pyrenoidosa was incubated with sufficient nutrients and further treated with nitrogen starvation for lipid induction, resulting in 410 mg/l/day lipid productivity [29]. This lipid productivity is higher than those obtained from different photoautotrophic cultivation strategies of different algal strains (Table 1 in [29]). Investigating proper light intensity and CO₂ levels is also essential in photoautotrophic cultivation. C. sorokiniana AM-02 was cultured under high photosynthetic photon flux density (PPFD) conditions and CO₂ gas levels. The preferred high PPFD and optimal CO₂ levels were 1,000-1,400 μmol photons/m²/s and 0.5-2.0% (v/v), respectively [30]. According to our research, not so many reports intend to improve the photoautotrophic condition for Chlorella cultivation within these five years due to low biomass and lipid productivity compared to heterotrophic or mixotrophic conditions.

Heterotrophic Mode

Although microalgae can utilize inorganic carbon sources for photosynthesis, the biomass productivity of microalgae is low and limited [31]. Biomass productivity can be improved under heterotrophic conditions compared to the basal photoautotrophic culture conditions. Morowvat et al. optimized the growth conditions of naturally isolated C. vulgaris strain in BG-11 medium in a flask and bioreactor. The total biomass and lipid content in heterotrophic culture with glucose were improved 3.5-fold and 9.3-fold, respectively, compared to the basal photoautotrophic culture condition in the shake flask experiment [32]. In the bioreactor experiment, total biomass and lipid concentration or density also increased to 4.95 and 2.18 g/l, respectively, during five days of the experiment compared to its basic photoautotrophic culture [32]. Kim et al. developed heterotrophic cultivation conditions using statistical assessment to explore the full potential of Chlorella sp. HS2, which was isolated for the ability to achieve extraordinary culture density (5.91 g/l) and biomass productivity (656.7 mg/l/day) under photoautotrophic conditions [33]. The cultivation with a 5-L fermenter under heterotrophic conditions using glucose resulted in significantly improved biomass productivity (5.37 g/l/day), and total lipid productivity (0.86 g/l/day) was achieved [33]. Their trial was vastly superior to the performance in most previous works involving the heterotrophic fermentation of green algae (Table 3 in [33]). Thus, heterotrophic cultivation allows us to produce...
**Table 1. Chlorella biomass and lipid productivity using different carbon sources.**

| Mode          | Carbon source  | Carbon source | Strain                          | Medium       | Volume | Culture volume | Biomass Unit | Lipid Unit | Ref.                  |
|---------------|----------------|---------------|---------------------------------|--------------|--------|----------------|---------------|-------------|-----------------------|
| Autotrophic   | CO2            | Nitrogen      | C. minutissima                 | Guillard F/2 | 62.5   | mg/l/d         | 5.72          | mg/l/d     | [28] Amaral et al., 2020 |
| Autotrophic   | CO2            | Nitrogen      | C. pyrenoidosa 2738             | Fog media    | 5      | g/l/13d        | 410           | mg/l/d     | [16] Naskarkar et al., 2019 |
| Autotrophic   | CO2            | -             | C. sorokiniana AM-02           | BMM         | 2.4    | g/l            | NA            | mg/l/d     | [30] Zaganshina et al., 2020 |
| Heterotrophic | Glucose       | 10 g/l glucose| C. vulgaris AB MCCS 130         | BG11         | 2L     | 265 mg/l/d    | 118 mg/l/d    | [32] Motowrat et al., 2019 |
| Heterotrophic | Glucose       | 10 g/l glucose| Chlorella® sp. HS2              | BG11         | 3L     | 5370 mg/l/d   | 860 mg/l/d    | [33] Kim et al., 2019 |
| Mixotrophic   | Glucose       | 15 g/l glucose| C. vulgaris KNUA104             | BG11         | ?      | 2.98 mg/l/d   | 68.80% DCW    | [38] Yun et al., 2021 |
| Heterotrophic | Glucose       | 20 g/l glucose| C. vulgaris CCALA 256           | BBM         | 2L     | NA            | 32.70% DCW    | [55] Canelli et al., 2020 |
| Mixotrophic   | Wastewater    | 25% Sweet     | C. vulgaris strain UTEX-2714    | TAP         | 150 ml | 6.1 g/l       | 383 mg/l/d    | [39] Ward et al., 2019 |
| Mixotrophic   | Wastewater    | Acetate       | C. pyrenoidosa FACHB-1216       | BG11         | 800 ml | 134 mg/l/d    | 42.04 mg/l/d  | [54] Li et al., 2022 |
| Mixotrophic   | Wastewater    | Acetate       | C. sorokiniana 211-32           | BG11         | 300 ml | 40 mg/l/d     | 13.48 mg/l/d  | [41] Liu et al., 2018 |
| Mixotrophic   | Wastewater    | Glycerol      | C. pyrenoidosa                  | -           | 3.5 L  | 1.28 g/l      | 50.76% DCW    | [45] Rana et al., 2021 |
| Heterotrophic | Glucose       | 30% Palm      | C. vulgaris strain UTEX-395     | BBM         | 2L     | 141 mg/l/d    | 32.70% DCW    | [55] Canelli et al., 2020 |
| Heterotrophic | Wastewater    | Food waste    | Chlorella® sp. GY-H4            | -           | 2L     | 6.9 g/l       | 1.8 g/l       | [86] Wang et al., 2020 |
| Heterotrophic | Wastewater    | 30% Palm      | C. sorokiniana CY-1             | -           | 7.02 L | 409 mg/l/d    | 14.43% DCW    | [81] Cheah et al., 2020 |
| Heterotrophic | Wastewater    | Sugarcane     | C. protothecoides               | -           | 7L     | 10.7 g/l      | 16.80% DCW    | [83] Chen et al., 2019 |
| Heterotrophic | Wastewater    | Forest        | C. vulgaris NIOCCV              | -           | 4L     | 264 mg/l/d    | 100.54% DCW   | [85] Jain et al., 2019 |
| Mixotrophic   | Wastewater    | Seafood       | Chlorella® sp.                  | -           | 350 ml | 77.7 mg/l/d   | 20.4 mg/l/d   | [86] Gao et al., 2018 |
| Mixotrophic   | Wastewater    | Tannery       | C. vulgaris                    | -           | 300 ml | 7.25 mg/l/d   | 25.40% DCW    | [87] Suranya et al., 2019 |
| Mixotrophic   | Wastewater    | Butyric acid  | C. pyrenoidosa                 | -           | 200 ml | NA            | 25.40% DCW    | [89] Hu et al., 2018 |

**Notes:**
- CO2: Carbon dioxide
- ACS: Autotrophic carbon source
- HCS: Heterotrophic carbon source
- MCS: Mixotrophic carbon source
- CB: Culture biomass
- SW: Sea water
- W: Wastewater
- SO: Sugar
- PB: Palm bagasse
- SB: Sea bottom
- BBM: Brine broth medium
- TAP: TAP medium
- BG11: BG11 medium
- MCCS: Marine complex medium
- TAP: TAP medium
- UTEX: University of Texas (Austin)
- NIOCCV: National Institute of Oceanography (New Delhi, India)
- NRMCF0128: National Research Center for Marine Science and Technology (Egypt)
- VIT_SDSS: VIT (Vanavil, India)
- NIMCF0128: National Institute of Marine Science and Technology (Egypt)
- VIT_SDSS: VIT (Vanavil, India)
- P. pringsheimii: Pringsheimia species
- DCW: Dry cell weight
- Ref.: Reference
much higher biomass and lipid yields than photoautotrophic mode. However, specialists have not concluded which option, photoautotrophic or heterotrophic, is the most economical. Recent reports discussed possible

Table 1. Continued.

| Mode               | Carbon source | Carbon   | Strain         | Medium     | Culture volume | Biomass Unit | Lipid Unit | Ref.       |
|--------------------|---------------|----------|----------------|------------|----------------|--------------|------------|------------|
| Autotrophic        | Wastewater + CO₂ | Real swine wastewater (RSW) + 3% CO₂ | C. vulgaris MBFJNU-1 | - | 3000 L | 478.5 mg/l/d | 9.1 mg/l/d | [90] Xie et al., 2022 |
| Mixotrophic        | Wastewater    | 2000 mg/l COD | Chlorella sp | - | 225 ml | 288.84 mg/l/d | 104.89 mg/l/d | [91] Zhu et al., 2017 |
| Heterotrophic      | Sucrose + yeast | 10 g/l sucrose | C. pyrenoidosa | BG11 | 100 ml | 2290 mg/l/10d | 124.3 mg/l/10d | [104] Kilian et al., 1996 |
| Mixotrophic        | Sucrose + yeast | 1% sucrose | + Cryptococcus sp | 2930 | 165.4 |
| Heterotrophic      | Sucrose + yeast | 1% sucrose | + Rhodotorula glutinis | 340 mg/l/d | 29.70% DCW |

Detailed conditions of carbon treatments for the accumulation of lipids in Chlorella.

*indicates value estimated from figure images.

Table 2. Chlorella biomass and lipid productivity using different nitrogen sources.

| Nitrogen Source | Nitrogen | Strain         | Medium     | Culture volume | Growth rate | Unit Biomass Unit | Lipid Unit | Protein Unit | Ref.       |
|-----------------|----------|----------------|------------|----------------|-------------|-------------------|------------|--------------|------------|
| Nitrite         | Nitrite 9% (Nitrite + Nitrate) | C. vulgaris | B3N | 50 ml | 1.3 day | NA | NA | [64] Pozzobon et al., 2021 |
| Nitrate         | Nitrite 20-100% (Nitrite + Nitrate) | C. vulgaris | Jaworsky | 8 L | NA | 0.18 g/l | 0.12 | 12.29% DCW | 50.80% DCW |
| Nitrate+ Nitrite | Nitrate | Chlorella sp | mBG11 | 180 ml | NA | 342.5 mg/l | 357.5 | 19.99 | [66] Muthu et al., 2011 |
| Nitrate+ Nitrite | Nitrate | C. pyrenoidosa | FACHB-9 | 100 ml | 340 mg/l/d | 29.70% DCW |
| Nitrate+ Nitrite | Nitrate | C. pyrenoidosa | FACHB-9 + Rhodotorula glutinis | 11.5 | 5.06 |

Detailed conditions of nitrogen treatments for biomass, lipid and protein productions in Chlorella.

* indicates value estimated from figure images. Numbers discussed in the text are in bold.
future scenarios in which the cost of heterotrophic production of microalgae on an industrial scale would be comparable to autotrophic production [34].

### Mixotrophic Mode

In the mixotrophic mode, photoautotrophic metabolism is integrated with heterotrophic metabolism. Recent studies successfully increased the *Chlorella* biomass and lipid productivity by using various organic carbon sources for cultivation, such as glucose, acetate, or glycerol (Fig. 1). Of these, many previous publications concluded that glucose is an efficient trigger to increase biomass productivity of the microalgae [35-37]. Recently, Yun *et al.* evaluated the applicability and usability of 10 g/l glucose as an organic carbon source for *C. vulgaris* and *C. sorokiniana* under heterotrophic and mixotrophic conditions [38]. As a result of optimization of culture conditions, mixotrophic conditions provided the highest lipid content (68.80%) in *C. vulgaris* KNUA104 and the highest biomass production (4.73 mg/l/day) in *C. sorokiniana* [38]. Ward and Rehmann optimized various nutrients for mixotrophic cultivation, including glucose, sodium nitrate, and magnesium sulfate, by the response surface methodologies, which can evaluate complex relationships, resulting in overall lipid productivity of 383 mg/l/day with 18.8 g/l glucose as a carbon source [39]. Thus, glucose seems to be a promising candidate as a carbon source for the mixotrophic cultivation of algal cells.

Acetic acid is preferentially adsorbed by the microalgal cells and directly converted into acetyl-CoA, achieving higher efficiency of lipid production. León-Vázquez *et al.* used 100 mM (6 g/l) acetic acid from the oxidized wine waste lees for mixotrophic cultivation of *C. sorokiniana*, and lipid productivity was 193.37 mg/l/day [40]. Liu *et al.* used 10 g/l sodium acetate as organic carbon for mixotrophic cultivation of *C. pyrenoidosa* to obtain the maximum production of total lipid [41].

Chlorella cultivation in a mixotrophic mode with glycerol enhanced the overall biomass concentration and lipid accumulation [42-44]. Recently, Rana and Prajapati showed that supplementation of glycerol (3 g/l) in synthetic wastewater (SWW) could enhance lipid accumulation (30.76% dry weight basis) in *C. pyrenoidosa* compared to control (without glycerol, 13.16% dry weight basis) [45].

Chai *et al.* compared the effect of four monosaccharides (glucose, fructose, galactose, and xylose) on Chlorella growth. Chlorella medium with fructose promoted *C. sorokiniana* growth to a much lesser extent than glucose, whereas supplementation with galactose had no effect, and supplementation with xylose inhibited growth [46].

The question is which carbon source will provide the best lipid productivity. Glucose is first catabolized into glucose-6-phosphate and converted to pyruvate through an anaerobic glycolysis process. Furthermore, it is converted into acetyl-CoA, which is subsequently utilized in the TCA cycle for energy production or as a precursor for fatty acid synthesis (Fig. 1); therefore, both biomass and lipid production can be accelerated. On the other hand, acetate is a simple substrate necessitating only one or two activation steps at the expense of one ATP molecule to produce acetyl-CoA [47]. Perez-Garcia et al. evaluated eleven known carbon sources for the cultivation of *C. vulgaris* (Beij.) and found that the best growth rate was provided by acetate cultivation and the second by glucose cultivation [35, 48]. In principle, the uptake of glucose and ammonium during the mixotrophic growth would decrease pH [49, 50], while acetate consumption and photosynthesis increase pH [51, 52]. To solve this problem, Xie *et al.* proposed Glucose-Acetate-Phosphorus (GAP) medium, which can maintain pH during cultivation [53], and might be a promising alternative for mixotrophic cultivation.

### Heterotrophic Mode vs. Mixotrophic Mode

Some researchers compared the effects of conditions in heterotrophic and mixotrophic cultivation modes for *Chlorella* biomass or lipid productivity. Li *et al.* reported that heterotrophic cultivation produced the maximum biomass productivity of 134.9 mg/l/day and maximum lipid productivity (42.4 mg/l/day), which were much higher than those under mixotrophic condition [54]. Canelli *et al.* found that the heterotrophic mode maximized lipid productivity (32.7% DCW), and the mixotrophic condition induced a nutritionally favorable fatty acid profile and higher concentrations of carotenoids and phenolics with less lipid quantity (24.2% DCW) [55]. Applying light to heterotrophic cultivation (mixotrophy) might induce oxidative stress, increasing the carotenoid content [56]. On the other hand, Yun *et al.* reported that *Chlorella* biomass productivity and lipid yield under mixotrophic conditions (3.02 g/l/day and 1.86 g/l, respectively) were higher than the heterotrophic conditions (1.78 g/l/day, 0.54 g/l, respectively) [38].

It remains a question as to which trophic mode is best for growing *Chlorella* on a global industrial scale. The Chlorella market is mainly segmented into autotrophic and heterotrophic, and the heterotrophic segment is expected to grow at the highest compound annual growth rate (CAGR) during the forecast period [57]. It is mainly used due to its higher cell concentration, higher productivity, low risk of contamination, lower water consumption, lower space usage, and purity of biomass.

### Nitrogen Source for Cultivation of Chlorella

Nitrogen is one of the essential nutrients for microalgal cultivation. Nitrogen can be delivered in various forms to the culture, such as nitrate NO$_3^-$, nitrite NO$_2^-$, ammonium NH$_4^+$, and urea CO(NH$_2$)$_2$. Although ammonium (NH$_4^+$) can be directly assimilated into amino acids via the GS/GOGAT cycle [58, 59], nitrate (NO$_3^-$) needs to be reduced to nitrite (NO$_2^-$) in the cytosol, after which it is immediately reduced to ammonium in chloroplasts or plastids [60] (Fig. 2). Thus, ammonium is more efficient than nitrate as a nitrogen source. However, ammonium can be toxic to many organisms, particularly plants and oxygenic photosynthetic microorganisms [61, 62]. Here, we mentioned recent trials to investigate the nitrogen sources that allow better cultivation and growth of *Chlorella*. 

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Nitrate vs. Nitrite

Pozzobon et al. cultivated C. vulgaris using mixed nitrate and nitrite as a nitrogen source to optimize the ratio of nitrate to nitrite. Nitrite addition triggered a growth rate inhibition, and nitrite uptake remained constant at a low level [63]. On the other hand, nitrate uptake rate was correlated with nitrate content in the culture medium [63]. Mutlu et al. previously showed that cultivations with nitrate produced higher protein content (51%) compared to those with nitrite (41%) [64]. Recently, different Chlorella strains also reported better growth with nitrate supplementation [65].

A few studies showed better lipid productivity using nitrite as a nitrogen source. Zhan et al. previously reported that nitrite-nitrogen (NO2-N) was the best among the nitrogen sources for the HQ growth and lipid accumulation potential of Chlorella sp. Nitrate-nitrogen (NO3-N) and urea-nitrogen (Urea-N) also contributed to algal growth and lipid accumulation potential at a lesser level, but ammonium nitrogen (NH4-N) and N-deficiency instead caused inhibitory effects in this Chlorella strain [66]. Li et al. also reported that 200 μmog/l nitrite provided 3.0 mg/l/day lipid productivity in Chlorella sp. L38. By contrast, the average lipid productivity in the medium containing 200 μmog/l nitrite + nitrate resulted in 1.15 mg/l/day [65]. Such increases in lipid content in nitrite medium in these studies might be due to the induction of nitrogen deficiency condition, which is beneficial for lipid accumulation [67], since nitrite seems to be barely taken up by most Chlorella cells [63].

Urea

The consumption of either nitrate or ammonium by microalgae causes a change in medium pH as they grow. Davis et al. reported that glycine and urea were organic nitrogen sources without a drastic increase in pH fluctuations in the medium [68]. Several reports showed that Chlorella species could grow faster in urea than in nitrate and ammonium as nitrogen sources. When urea was the nitrogen source, the highest dry cell weight (2.86 g/l), biomass productivity (345 mg/l), and specific growth rate (1.903/day) were obtained in Chlorella sp. GN1 culture [69]. Additionally, Nayak et al. reported that urea best promoted the biomass production, specific growth rate, and biomass productivity of Chlorella sp. HS2 among all nitrogen sources [70].

Ammonium

Ammonia nitrogen includes the ionized (ammonium, NH4+) and unionized form (ammonia, NH3, toxic to aquatic organisms). Unlike nitrate NO3-, when ammonium NH4+ is utilized, microalgae spend less energy on its assimilation, and ammonium is directly incorporated into amino acids [71]. However, excessive amounts of ammonium are toxic to algae due to the damaging effects on photosynthesis [61, 72]. This is because ammonium directly induces photodamage to PSII rather than affecting the repair of photodamaged PSII [73, 74].

Chlorella can use ammonium for growth, making it possible to use this alga for bioremediation to remove ammonium [75]. Ziganshina et al. reported that the highest growth rate (1.26/day) was observed in modified Bold's basal medium (BBM) with ammonium, while the growth rate in BBM with nitrate was only 1.07/day [76]. Thus, although Chlorella strains seem tolerant to ammonium, the degree of growth inhibition by ammonium varies between the strains [71, 77, 78]. Wang et al. compared the tolerance of green algae to ammonium using ten Chlorella strains. As a result, FACHB-1563 had the highest tolerance to ammonium among all strains tested, suggesting that FACHB-1563 might be able to remove excess ammonium from wastewater for bioremediation [74]. Thus, different nitrogen sources have different effects on the physiological indexes of Chlorella strains.

In photosynthetic eukaryotes, nitrogen assimilation is performed by nitrate or nitrite transports. From the structural point of view, three families of proteins are involved in nitrate or nitrite transport in microalgae: NRT1 (nitrate NO3- transporter), NRT2 (nitrate NO3-, nitrite NO2- transporter), and NAR1 (nitrite NO2- transporter)
Interestingly, although Chlorella sp. NC64A has a complete set of all the genes needed for nitrate assimilation, the strain can use ammonium or amino acids but not nitrate or nitrite. Additional complexity is the genetic variation of nitrogen assimilation genes among Chlorella strains. Chlorella NC64A contains two NRT1 and two NRT2 genes, but C. paradoxa conserves two NRT2 genes (Table 1 in [58]). Hence, we must carefully select nitrogen sources depending on the Chlorella strains used and the purpose of cultivation based on genetic information.

**Phycoremediation: Wastewater as a Nutrient Source for Cultivation of Chlorella**

Phycoremediation refers to remediation with the help of algae. Using wastewater to grow microalgae as a nutrient source would decrease the cultivation costs and purify polluted water. Food waste also represents a valuable carbon source for algal cultivation and can improve the production of microalgal biomass and valuable oleochemicals. Arora and Philippidis utilized 25% sweet sorghum bagasse (SSB) hydrolysate and achieved the highest biomass and lipid productivity (3.44 g/l and 120 mg/l/day, respectively) under mixotrophic conditions compared to heterotrophic and phototrophic conditions [80]. Wang et al. used 10 g/l glucose from food waste hydrolysis for mixotrophic cultivation of Chlorella sp. GY-H4, resulting in a 6.1 g/l biomass yield with a 2.5 g/l lipid yield [81]. The cultivation of C. sorokiniana in palm oil mill effluent (POME) using a novel designed photobioreactor has brought enhancement in biomass production (409 mg/l/day), excellent lipid content (14.43%, DCW), as well as effective POME remediation [82]. Chen et al. investigated the effects of sugarcane bagasse hydrolysate (SCBH) carbon sources on cell growth and fatty acid accumulation in Chlorella protothecoides. With the medium containing SCBH (20 g/l sugar concentration), the highest biomass and fatty acid yield were 10.7 g/l and 0.55 g/l, respectively, which was significantly higher than that in the culture using glucose [83]. Taken together, utilizing these food waste hydrolysates seems to create potential industrial applications for sustainable Chlorella biomass and lipid production.

Using forest residues for biofuel production has attracted interest due to the generation of additional revenue and reduction of greenhouse gas emissions. Vyas et al. utilized cellulose-rich pretreated solids from spruce biomass to grow and produce lipids in oleaginous microalgae. They cultivated microalgae in a medium containing (20 g/l) glucose obtained from spruce hydrolysate, which resulted in the production of biomass (8.28 g/l at C/N 60) and lipid synthesis (3.61 g/l at C/N 60) after 72 h of cultivation [84].

The commercial seafood processing industry generates large quantities of solids and wastewater. Seafood processing wastewater (SPW) usually contains high concentrations of nutrients, indicating that SPW could be an alternative nutrient source for microalgae cultivation. Jain et al. cultivated C. vulgaris in SPW under mixotrophic conditions [85]. The biomass productivity and lipid content accounted for 264.58 mg/l/day and 38% (DCW), respectively, at a 10% CO2 supply [85]. Gao et al. treated Chlorella sp. with aerated seafood processing wastewater, and higher biomass productivity (77.7 mg/l/day) and higher lipid productivity (20.4 mg/l/day) were obtained compared to those in SPW [86], indicating that the aeration pretreatment is essential to reduce the amount of toxic unionized ammonia in SPW.

Sewage wastewater treatment with microalgae cultivation is an eco-friendly process. Saranya and Shanthakumar evaluated the remediation of combined sewage and tannery effluent under different dilutions. The maximum biomass yield was achieved at 20% tannery effluent and 80% sewage effluent (20% tannery effluent diluted with sewage), resulting in 3.25 g/l and 2.84 g/l in C. vulgaris and Pseudochlorella pringsheimii, respectively. Between the two species, P. pringsheimii showed high lipid accumulation potential of 25.4% (dry weight basis) compared to C. vulgaris (9.3%) at 20% tannery effluent diluted with sewage (Fig. 3 in [87]). Azam et al. investigated the
production of Chlorella biomass and nutrient removal efficiencies with a 50% concentration of open sewage contaminated channel wastewater (OSCCW), which contributed to the biomass (60.1% and 56.5% g/l) and lipid content (20.8 and 17.5 mg/l/day) in C. vulgaris and C. pyrenoidosa, respectively [88]. Anaerobic hydrolysis and acidification of complex organic wastes are common wastewater treatment methods. There are three widely recognized fermentation types in a mixed culture of acido genesis: butyric acid type, propionic acid type, and ethanol type. Of these, the highest lipid content (25.4% DCW) of Chlorella sp. UJ-3 was achieved in the butyrate-type fermentation, and the fatty acid compositions were considerably different for these three fermentation systems [89].

Using livestock wastewater for microalgal cultivation seems to be another alternative solution. C. vulgaris MBFJNU-1 in natural swine wastewater (RSW) with 3% CO$_2$ resulted in the highest microalgal biomass (478.5 mg/l/day) and lipid (9.1 mg/l/day) productivities [90]. The livestock waste compost medium with 2,000 mg/l COD provided an optimal nutrient concentration for Chlorella sp. cultivation, where the highest productivities of biomass (288.84 mg/l/day) and lipid (104.89 mg/l/day) were achieved [91].

It is worth mentioning that free ammonia in wastewater has been demonstrated as the primary stress factor suppressing microalgal activities. Gao et al. reported that aerated pretreatment of SPW reduced the amount of toxic unionized ammonia to solve this problem. At the same time, most of the nutrients were retained in the wastewater [86]. An aerated biological filter (BAF), which is standard technology for aerobic biological treatment of wastewater, contains a granular media that is a collection of closely packed solid particles surrounded by a liquid media and can provide large surface areas for biofilm development [92]. BAFs have been successfully used in the traditional nitrification and denitrification processes [93]. Qin et al. investigated the effect of pretreatment with a BAF on microalgae culture with dairy liquid digestate. They found that the BAFs can rapidly nitrify ammonia nitrogen, eliminating ammonia inhibition for C. pyrenoidosa [94].

The contaminated biomass generated during phytoremediation poses a threat to our environment. Therefore, proper management is essential to dispose of the wastes to prevent them from further entering the food chain. The use and safe disposal of algal biomass after phytoremediation has been addressed by some researchers. For instance, the integration of algal bio-fertilizer production is recently gathering attention only when we use wastewater with a high level of safety to obtain pollutant-free biomass, such as wastewater from food or feed industries [95]. On the other hand, polluted water limits the application of the algae biomass produced [96]. In general, the microalgae biomass from contaminated waters can be used to produce alternative energies, including biodiesel, bioethanol, and biomethane. Additional experimentation and validation are required before the exploitation of such biomass for industrial or domestic use.

Co-Cultivation with Bacteria, Yeast, or Other Microalgae

Co-cultivation of algae and bacteria can enhance the efficiency of nutrient utilization in wastewater, and the growth rate of microalgal cells can be improved [97]. Since various microorganisms are present in wastewater, investigating the symbiotic systems existing between microalgae and bacterial communities is necessary for developing wastewater treatment technologies. Shen et al. proposed a symbiotic microalgal-bacterial system using C. vulgaris and Pseudomonas putida. They revealed that the cell density of the microalgal-bacterial system was considerably increased compared to that of monoculture microalgae [98]. Moreover, Liu et al. reported that Chlorella sp. HL and three indigenous bacteria (Brevundimonas, Chrysobacterium, and Pseudomonas) had synergistic effects on nutrient removal [99].

Generally, the biomass produced through microalgae cultivation is harvested using processes such as centrifugation and filtration [8]. To reduce harvesting costs and remove nitrogen and phosphorus from wastewater, the floc formed by bacteria can be applied to microalgal biomass harvesting [100]. In the study by Kim et al., three bacterial strains (Melaminivora jejuensis, Comamonas flocculans, and Escherichia coli) were inoculated into a medium to form a floc with the microalgal strain C. sorokiniana. Among the bacterial strains tested, M. jejuensis formed the largest floc with C. sorokiniana, with the highest sedimentation ability. Furthermore, the M. jejuensis co-culture improved biomass and lipid productivity compared with the pure algal culture [101].

Disaccharides or polysaccharides, such as sucrose and lactose, are difficult to utilize for microalgae under heterotrophic conditions [35,102]. Some yeasts extracellularly hydrolyze sucrose into monosaccharides. Since the sucrose hydrolysis rate is much higher than the monosaccharide uptake rate, the monosaccharide is accumulated in the culture [103, 104]. Wang et al. developed a co-culture system that incubates Chlorella with yeast Rhodotorula glutinis placed on immobilized beads to enhance algal growth using sucrose [105]. Tian et al. found that a co-culture system can enhance Chlorella growth using sucrose at both heterotrophic and mixotrophic modes when mix-cultured with yeast Cryptococcus sp. [106].

Hu et al. revealed that the co-cultivation system among different species of algae improved their growth. When the growth of C. vulgaris and a unicellular green algae Scenedesmus dimorphus in the landfill leachate was compared, the co-culture biomass in 10% landfill leachate demonstrated improved nutrient utilization efficiency in microalgae [107].

Using Nanoparticles for Chlorella Culture

Nanotechnology is currently a hot topic for its various applications and prospects for providing solutions to the various needs of many industries [108]. Nanoparticle application in microalgae for enhanced lipid production is an ongoing task that contributes to biodiesel production (reviewed in [109]). For example, magnesium, zinc, or lead nanoparticles induced a higher lipid content than non-metal exposed medium in C. vulgaris, accounting for
3.93-fold, 3.33-fold, or 2.07-fold increases, respectively [110]. Vashist et al. attempted to improve lipid production using silica-coated magnetic nanoparticles. As a result, silica-coated magnetic nanoparticles induced 4-fold lipid production (98 mg/l) compared to the control (28 mg/l) [111]. Moreover, MgSO₄ was evaluated as a magnesium source for lipid production by C. vulgaris. The application of MgSO₄ nanoparticles was found to improve lipid production [112]. Thus, metal nanoparticle exposure in Chlorella might impact various physiological or molecular changes, thereby increasing the growth rate, biomass, and lipid production.

**Various Stress Factors for Lipid Accumulation in Chlorella**

Under favorable growth conditions, Chlorella produces large amounts of biomass with essential lipid contents. Further induction of lipid biosynthesis by environmental stresses is a crucial step for lipid production using Chlorella. There has been a wide range of studies to identify and develop efficient lipid induction techniques in microalgae, such as nutrient stress (e.g., nitrogen, phosphorus, sulfur starvation), osmotic stress, light, pH, temperature, heavy metals, and other chemicals (Fig. 3A).

**Nutrient Starvation for Lipid Accumulation**

Nitrogen is one of the essential nutrients for the growth of microalgae. Nitrogen deprivation in microalgae is widely studied during cultivation to induce lipid productions (Table 1 in [113]). Several studies have employed a frequently used approach for increased lipid production consisting of a combination of the biomass (favorable medium) and lipid induction phase (limited nutrient medium). A commonly used two-stage strategy has been adopted for lipid induction, in which the algal cultures are harvested by centrifugation after the biomass production phase, followed by incubation in a fresh nutrient-deficient medium for the lipid induction phase [70, 114]. Due to a time- and cost-consuming harvesting process before the lipid induction stage, recently, a single-stage strategy has been getting attention, wherein nitrogen concentration in the media is adjusted to improve the overall lipid productivity. Farooq et al. investigated the effect of four initial nitrogen concentrations (1-, 2-, 6-, and 10-mM nitrate) on lipid yield, CO₂ fixation rate, and water quality for further reuse after first cultivation. They concluded that the initial 6 mM nitrate was found optimum for the growth and overall lipid productivity of C. vulgaris [115]. Cho et al. observed that the effects of the initial concentrations of nitrate in the medium varied between 2.5 to 15 mM on biomass generation and lipid production of Chlorella sp. ABC-001, a newly isolated strain with advantageous characteristics for CO₂ fixation and biofuel production [116]. The lipid content showed the highest value of 47.4% (DCW) with 2.5 mM nitrate, whereas the highest biomass productivity of 0.422 g/l/day was achieved under a nitrogen-rich condition (15 mM nitrate) [116].

In addition to nitrogen starvation, various nutrient starvation methods also achieved lipid stimulation in Chlorella [19, 117-119]. Recently, Sakarika and Kornoros optimized culture conditions for lipid accumulation under sulfur limitation, resulting in maximum total lipid content of 53.43% (DCW) [120]. Compared to nitrogen starvation, a few reports have explored the lipid contents with other nutrient-limited cultivation methods, suggesting that nutrient starvation stresses might be a powerful strategy to boost lipid production in Chlorella (Fig. 3B).

**Various Stresses for Lipid Accumulation**

Dong et al. compared the effects of various stress factors on the growth and lipid production of C. pyrenoidosa. Their results show that the growth of C. pyrenoidosa was inhibited under stress conditions, but the intracellular lipid content was significantly increased. After 120 h, the greatest lipid content was under the condition of nitrogen deficiency (47.10% DCW) compared to the conditions of phosphorus deficiency (36.53% DCW), high light (34.44% DCW), high salt (28.75% DCW), and control (25.14% DCW) [121]. Gour et al. selected the best salinity conditions for better growth, biomass accumulation, and lipid productivity of microalgae. Chlorella sp. showed the maximal lipid content of 32.19% DCW and lipid productivity of 10.27 mg/l/day (1.52- and 2.64 g/l/day, respectively). Thus, heat shock was successfully adopted in the novel Chlorella sp. HS2 cultivation for lipid induction [123]. Treatment with Brefeldin A (BFA), a chemical inducer of ER stress, triggers lipid droplet formation within 4 h in two varieties of C. vulgaris [124]. Zhang et al. treated C. pyrenoidosa with ferrous ions to induce reactive oxygen species (ROS) via the catalytic decomposition of hydrogen peroxide (Fenton reaction), increasing the total lipid content [125]. Abscisic acid (ABA) treatment enhanced the lipid accumulation in Chlorella sp. FACHB-8 strain, changing fatty acids from unsaturated to saturated fatty acids, which were suitable for diesel application [126]. Pyrene (polycyclic aromatic hydrocarbon) is an anthropogenic organic pollutant in various ecological units. Jaiswal et al. used pyrene pollutants (50-500 ppm) to evaluate the impact on metabolites and the induction of lipid biosynthesis to produce renewable biodiesel. They concluded that pyrene concentration at 230 ppm caused 1.24-fold higher lipid biosynthesis compared to the control medium [127].

Research in magnetic fields has significantly affected the growth and production of proteins, carbohydrates, and lipids in microalgae. The magnetic field has been shown to have significant effects on the growth and production of proteins, carbohydrates, and lipids with C. kessleri [128], Chlorella fusca [129], and C. vulgaris [130]. Costa et al. investigated the influence of different intensities and exposure times of magnetic fields on the stimulation of lipid synthesis by the microalga C. homosphaera. Lipid productivity reached 39.5 mg/l/day (1.52-
fold) with the magnetic fields (30 mT, 1 h/day) with a slight reduction in biomass productivity [131]. Baldev et al. optimized conditions of a magnetic flux density and yielded a maximum dry cell weight of 0.61 g/l, two-fold higher than the normal condition, with lipid content of 55.2% DCW, suggesting the enhancement of growth and lipid of *C. vulgaris* by magnetic fields [132].

### Genetic Engineering of *Chlorella* for Better Lipid Production

As discussed in Yang et al. [133], *Chlorella* transformation methods are currently considered the most severe limitation in *Chlorella* genetic engineering. Here, we introduce the latest successful examples of genetic engineering of *Chlorella* for better lipid production (Fig. 3A). Transcription factor engineering to regulate multiple genes has shown promise in the field of microalgae genetic engineering. Overexpressing *HSBZIP1*, encoding a C-type bZIP transcription factor, in *Chlorella* sp. HS2 increased fatty acid production (up to 2.13-fold) compared to control [134]. *LEAFY COTYLEDON1* (*LEC1*) is a central regulator that controls many aspects of seed development, including the maturation phase during which seeds accumulate storage macromolecules and embryos acquire the ability to withstand desiccation in *Arabidopsis thaliana* [135]. Overexpression of *Arabidopsis LEC1* in *C. ellipsoidea* enhanced the total fatty acid content (1.33-fold) and total lipid content (1.30-fold) with upregulation of key genes in the lipid synthesis pathway, such as *ACCase*, *PDAT1*, and *DGAT1* [136]. In the study of Tokunaga et al., the candidate DOF transcription factor was endogenously overexpressed in *C. vulgaris* to improve neutral lipid production, resulting in the production of 1.5-fold higher neutral lipid content compared to control cells in *C. vulgaris* [137]. These studies provide increasing lipid content by introducing exogenous or endogenous transcription factors in *Chlorella*. The carbonic anhydrases (CA) can catalyze the rapid conversion of carbon dioxide to bicarbonate and play a key role in CO2 transfer for cell respiration and photosynthesis [138]. To increase the solubility of CO2, You et al. introduced carbonic anhydrase fused with dockerin to immobilize protein on the surface of *C. vulgaris*. As a result, *C. vulgaris* showed 1.6-fold rapid growth and 1.7-fold lipid production, suggesting that the CA complex can enhance CO2 fixation [139]. Although the success rate of heterologous gene expression remains relatively low, *Chlorella* strains harbor significant advantages for biomass and lipid production. Further improvement of *Chlorella* transformation techniques remains to be developed to provide *Chlorella* biomass as feedstock for oils, antioxidants, or other bioactive benefits by genetic engineering.

### Potential Applications of *Chlorella*

According to a market report of the Research and Market, the *Chlorella* market was worth $269.6 million in 2021, and it is expected to grow at a CAGR of 6.3% from 2021 to 2028 to reach $412.3 million by 2028 [140]. In addition, Future Market Insights reported that the global *Chlorella* market is expected to reach a market value of $198.5 million in 2022 and ultimately $427.7 million by registering a CAGR of 8% in the forecast period 2022-2032 [141]. According to another report, the global *Chlorella* market was estimated at $263.49 million in 2021. It is projected to reach $431.91 million by 2028, exhibiting a CAGR of 7.32% during the forecast period [142]. This acceleration of the *Chlorella* market growth over the forecasted period might be due to the rapidly increasing numbers of the vegan population and health-conscious consumers. According to the market report above, a high number of key participants now compete in the *Chlorella* industry. Currently, global players such as Sun Chlorella Corp. (Japan, https://www.sunchlorella.com), Vedan Enterprise (Taiwan, https://000527.vedan.com), FEMICO (Taiwan, http://www.femico.com.tw), and Taiwanese Chlorella Manufacturing Co. (Taiwan, https://www.taiwanchlorella.com) together account for a considerably large market, where *Chlorella* is mainly sold as a dried powder, capsules, or pressed pills [58, 143]. Nutraceuticals and dietary supplements are expected to command the largest share and fastest growth in the *Chlorella* market [143].

### Valuable Compounds from *Chlorella*

Omega-3 and omega-6 fatty acids are essential to human health, and we must consume them through food; therefore, they are called *essential fatty acids*. Omega-3 polyunsaturated fatty acids (PUFAs) include α-linolenic acid (18:3), eicosapentaenoic acid (20:5; EPA), and docosahexaenoic acid (22:6; DHA), which are efficient at preventing cardiovascular diseases in humans, due to their characteristics that alter membrane fluidity and increase the solubility of CO2, You et al. introduced carbonic anhydrase fused with dockerin to immobilize protein on the surface of *C. vulgaris*. As a result, *C. vulgaris* showed 1.6-fold rapid growth and 1.7-fold lipid production, suggesting that the CA complex can enhance CO2 fixation [139]. Although the success rate of heterologous gene expression remains relatively low, *Chlorella* strains harbor significant advantages for biomass and lipid production. Further improvement of *Chlorella* transformation techniques remains to be developed to provide *Chlorella* biomass as feedstock for oils, antioxidants, or other bioactive benefits by genetic engineering.

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Flavonoids, secondary metabolites of plants, are involved in defense against pathogens, photosynthesis, and growth [148]. Also, flavonoids are in many human foods because of their antioxidant capacity, which can prevent ROS formation in cells [149]. Yadavalli et al. revealed that *C. vulgaris* and *C. pyrenoidosa* contain 138 mg/ml and 118 mg/ml of flavonoids, respectively, and these *Chlorella* species contain quercetin, catechin, and p-coumaric acid [150].

The demand for natural colorants has significantly increased due to health and environmental issues [151]. Because of the fast growth rates and diversity of pigments, microalgae have attracted great interest as a natural colorant. *Chlorella* species contain various types of pigment, including astaxanthin (red), β-carotene and violaxanthin (orange), lutein (yellow), chlorophyll-a and chlorophyll-b (green), and phaeophytin-a (green-gray) [152, 153]. The amount of pigment in *Chlorella* can change by various factors, such as photoperiod, light intensity, carbon source, nitrogen source, and nanoparticles [64, 153-156].

Carotenoids have two important functions in human health as antioxidants and a precursor of vitamin A.
However, humans cannot synthesize carotenoids in the de novo pathway, so it is important to consume foods containing carotenoids [158]. Carotenoids are divided into two classes, carotenes and xanthophylls [157]. Many Chlorella species contain xanthophylls. C. protothecoides, C. sorokiniana, and C. vulgaris contain lutein [159], C. zofingiensis contains astaxanthin [160], and C. luteoviridis contains zeaxanthin [161]. Also, C. vulgaris, C. sorokiniana, and C. ellipsoidea contain β-carotene, the precursor of vitamin A [162-164].

C. vulgaris contains eleven essential vitamins that humans cannot synthesize, including vitamin A, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B6 (pyridoxine), vitamin B9 (folate), vitamin B12 (cobalamin), vitamin C, vitamin E, and vitamin K [165-168]. Also, minerals promote several biological functions in the human body. Calcium, iron, magnesium, sodium, potassium, zinc, copper, and manganese can be found in C. vulgaris [166, 167]. Thus, Chlorella supplementation can provide various vitamin and mineral sources.

Clinical Trial of Chlorella Nutritional Supplements
Based on the valuable compounds of Chlorella, there are already many clinical trials on how Chlorella affects human health. Chlorella supplementation in humans has been shown to have antioxidant [169], anti-diabetic [170], immunomodulatory [171], and antihypertensive properties [172]. Chlorella intake resulted in noticeable reductions in body fat percentage, total blood serum cholesterol, and fasting blood glucose levels [173]. Chlorella-derived multicomponent supplementation decreases arterial stiffness in young people [174] and middle-aged and senior adults [175]. The consumption of Chlorella increased the level of several dicarboxylic acids in feces and propionate concentrations for individuals with low concentrations of fecal propionate [176]. These studies suggest that Chlorella-derived compounds might provide substitutes for synthetic compounds or drugs.

Bioplastic Production
Bioplastics are being actively studied to eliminate the dependency on fossil fuels to produce plastics and avoid endocrine disruptors [177], and some Chlorella species were used in this field. C. pyrenoidosa can accumulate 27% poly hydroxybutyrate (PHB), a type of poly hydroxy alkanoate that is categorized as biodegradable bioplastic [178]. C. fusca accumulates 17.4% PHB with the addition of xylose [179].

Taste Aspect of Chlorella for Plant-Based Alternatives
Chlorella is one of the most nutrient-dense superfoods on the earth. Chlorella products contain high-quality and high-quantity proteins (C. pyrenoidosa, 57%; C. vulgaris, 51%-58%) [180, 181]. Although Chlorella is rich in proteins, vitamins, minerals, and dietary fiber, it has been reported to have a rather bland flavor profile dominated by “grassy, vegetable, cucumber” aromas [182]. Thus, most consumers add Chlorella powder as an ingredient or take Chlorella tablets. Recently, Coleman et al. analyzed the taste of eight phototrophic microalgae, including C. vulgaris, to be used as flavor ingredients in plant-based seafood alternatives. According to their odor evaluation of eight microalgae, C. vulgaris has the highest earthy odor (beetroot, stale, musty odor) among the algal species they analyzed [183]. Their analysis of the chemical aroma profiles revealed that C. vulgaris contains higher odor activity values of aldehydes (malty/nutty/coffee) and benzaldehydes (nutty/almond) compared to the other microalgae and seafood. Although C. vulgaris seems to have a relatively low seafood aroma due to a lack of dimethyl sulfide, different taste evaluations disclosed that C. vulgaris has an intermediate level of umami (8 g MSG/100 g DW) among the algae examined with less bitterness [183]. Thus, bioengineering strategies to produce Chlorella strains or develop culture conditions to reduce their off-taste (earthy odor) seems necessary to use Chlorella as a flavor ingredient or plant-based seafood alternative.

Indeed, several companies have recently developed Chlorella strains to improve its taste, such as white and honey Chlorella (Allmicroalgae, Portugal, https://www.allmicroalgae.com), Duplaco Gold (Duplaco, Netherlands, https://duplaco.com), white Chlorella (Aliga Microalgae, Danmark, https://www.aliga.dk), and Chlorella Colors (Algenuity, England, https://www.algenuity.com). Thus, Chlorella seems to be a promising candidate for meeting the food needs of the vegan diet and the world’s rapidly growing population.

Future Perspectives and Conclusion
Chlorella, a photosynthetic unicellular microorganism, can accelerate lipid accumulation under various environmental conditions, and has also received significant attention for biofuel production. This review has shown how Chlorella cultivation environments involve Chlorella biomass and lipid productivity, such as cultivation modes, carbon or nitrogen sources, and stress conditions. Heterotrophic cultivation with 10-20 g/l glucose seems to be used for industrial biomass production with many Chlorella strains. Additional glycerol will accelerate lipid production in Chlorella cells. Chlorella cultivation with a light source will induce more antioxidant content. Although Chlorella will assimilate nitrogen from either nitrate or ammonium, ammonium can be assimilated with less energy in Chlorella cells. Moreover, it is worth mentioning that we must carefully select nitrogen sources based on the genetic information of each Chlorella strain. Nitrogen starvation is an efficient environmental pressure for boosting lipid accumulation in Chlorella cells.

The current severe bottleneck includes the high manufacturing cost of Chlorella biomass production, harvesting, and processing. To solve these issues, we need to develop a cost-effective method of culturing Chlorella species, such as Chlorella with high biomass productivity, high photosynthetic efficiency with high density and high tolerance to various harsh environments (pH, temperature, or osmolarity). Also, we need to consider how to increase the production of value-added ingredients that can cover high manufacturing costs. Development of
Chlorella genomic databases might also support genetic engineering approaches for those strain developments. Although Chlorella has come under the spotlight for its potential as a sustainable, nutrient-rich future food solution, it has not yet won over the consumer's taste buds due to its earthy and grassy smell and taste. It is essential to utilize this alga in the food industry while developing innovative applications and culturing methods.

Overall, Chlorella is a valuable alga that has two attractive consumption purposes as a potential source of renewable energy and nutrient-rich food. The knowledge and techniques accumulated in both fields will be utilized to develop innovative applications and culturing methods.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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