

## Optimal Growth Strategies for Sustainable Cultivation of *Chlorella Vulgaris* Microalgae for the Application of Bioenergy: A Review

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### ABSTRACT

*Chlorella vulgaris*, a green microalga, is attracting significant attention for its uses in food production, biofuel production, and environmental sustainability due to its high nutritional value and high growth rate. The current review discusses ideal methods for the cultivation of *Chlorella vulgaris* in an eco-friendly manner and also explores optimal growth strategies for cultivating *C. vulgaris* with a focus on maximizing bioenergy. Key factors affecting its growth, including the provision of light, nutrient content, temperature, and pH levels, are discussed in relation to biomass productivity and biochemical composition. Different cultivation methods, such as open pond cultivation and closed photobioreactors, are discussed in terms of efficiency and ecological implications. Contamination and high cost are discussed as challenges, alongside suggested solutions such as using nutrient media as a nutrient source. Through synthesis of existing studies, the article attempts to provide easy and practical recommendations to researchers and agriculturalists who are interested in the sustainable cultivation of microalgae for the production of bioresources.

**Keywords:** *Chlorella vulgaris*, Microalgae, Sustainability, Eco-friendly, Photo bioreactor, Bioresources.

### 1. Introduction

Microalgae, microscopic unicellular organisms, have become an important area of research in recent years for their potential to solve urgent global issues, such as addressing food shortages, increasing energy requirements, and reaching environmental sustainability targets [1]. *Chlorella vulgaris*, a green microalga, has gained particular attention due to its fast rates of growth, high levels of nutrients, and wide array of uses and applications [2]. With an approximate dry weight of 50-60% protein and high quantities of vitamins, minerals, and antioxidants, *Chlorella vulgaris* can be consumed directly for human nutrition, used in animal feed, and/or used to formulate health supplements [3]. In addition to its nutritional value, *Chlorella vulgaris* has potential for the development of sustainable energy in the form of biofuels, such as biodiesel, and for sustainable practices such as carbon dioxide sequestration and wastewater treatment [4]. All of these qualities make *Chlorella* important and essential species of microalgae which will enable new technologies and strategies towards achieving sustainable development, especially when faced with constraints to resource availability and the climate crisis [5].

Multiple factors work together to influence the growth of *Chlorella vulgaris*. Before development can be optimized, it must be recognized that light intensity, nutrient levels, temperature, pH, and cultivation systems all play a significant role in biomass production [6]. The primary modes of cultivation include open pond systems, which are the cheapest form of cultivation but are very prone to contamination, and closed photobioreactors (PBRs), which are a more expensive way to cultivate microalgae, yet provide the availability of specific control [7]. Other limitations that stand in the way of developing *Chlorella vulgaris* are the cost of water and nutrients [8].

Even though there is a lot of promise, achieving sustainable cultivation of *Chlorella vulgaris* involves balancing production against economic and environmental factors. Current literature highlights the necessity of developing effective and scalable solutions to ensure the broad adoption of microalgae-based technologies [9]. This review explored the best strategies for growing *Chlorella vulgaris*, with a focus on what sustainable practices might be employed for cultivation. Evaluated the influence of the primary growth parameters, compared different cultivation systems, and examined innovative methods to address hurdles. Recent literature offers consolidated insights to guide researchers, agriculturalists, and industries in developing *Chlorella vulgaris* for sustainable applications, particularly in bioenergy and nutritional uses (2024-2025) [10].

### 2. Methodology

A systematic literature review was conducted to identify suitable methods for cultivating *Chlorella vulgaris* in sustainable practices, based on peer-reviewed research studies published between 2010 and 2025 [11]. The studies were selected based on key factors of interest concerning microalgae productivity, mostly empirical research that focused on cultivation parameters and sustainability variables [12]. The selected sources contained relevant information, such as algal species used for the growth, growth conditions, and terms of cultivation methods [13]. The information was recorded in an organized manner so that it could be thematically analyzed to identify patterns and support its relation, such as nutrient metabolism, design of systems. Several variables were identified as potentially influencing *Chlorella vulgaris* productivity, including nutrient metabolism, system design, and abiotic factors during cultivation [15]. For example, the novel biotechnology space is currently full of innovative solutions that are making better use of fertilizers through the reuse of resources like media, as

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well as insights into bacteria co-cultures that can potentially enhance the growth of bioactive compounds with applications into antimicrobial and antioxidant purposes [16]. This synthesized review of the literature provides a standardized summary of useful adaptations for enhancing *Chlorella vulgaris* growth, in particular their uses towards bioenergy, while also including discussions on future challenges, developing approaches to sustainability, wastewater treatment, and how to go beyond zero waste by utilizing biomass completely [17].

### 3. Factors Affecting Algae Growth

The production of biomass from *Chlorella vulgaris* microalgae, as well as its total biomass development, is due to a culmination of environmental, biological, and operational factors; all of which contribute crucially to the enhancement of cultivation protocols for an environmentally sustainable undertaking, biofuel production, food supplementation in animals, and pollution mitigation [18]. For increasing output, reducing costs, and decreasing impacts on the environment, it is necessary to have control over, understand these factors, and cultivate them sustainably [19]. The detailed understanding of these factors allows researchers and practitioners to develop a cultivation plan that is tailored to a specific outcome, for example, biomass that is lipid-rich for fuels or biomass that is nutrient-rich for animals [20]. The following sections will provide a greater explanation of each

of the primary factors involved in the development of *Chlorella vulgaris*, their role, their relation to one another, and their potential for adoption as an environmentally sustainable.

#### 3.1 Light Intensity and Quality

Light is the main fuel for photosynthesis, the core process that powers the growth of *Chlorella vulgaris*. The intensity, duration, and spectral quality of light significantly influence biomass accumulation and biochemical composition. Optimal light intensity typically ranges from 100 to 200  $\mu\text{mol}/\text{m}^2/\text{s}$ , with a photoperiod of 12-16 hours daily, promoting maximum photosynthetic efficiency and biomass yields of up to 1.0 g/L in controlled settings [21]. Insufficient light limits carbon fixation, resulting in stunted growth, while excessive light ( $>500 \mu\text{mol}/\text{m}^2/\text{s}$ ) triggers photoinhibition, causing oxidative stress and cell damage [22]. The quality of light is equally critical, with red (620-630 nm) and blue (450-475 nm) wavelengths enhancing chlorophyll absorption and growth rates by 20-30% compared to white light. Artificial lighting, such as energy-efficient LED systems, allows precise control in photobioreactors, but natural sunlight is preferred in open ponds for sustainability, reducing energy costs by up to 50% in outdoor setups. Recent studies suggest that dynamic light regimes, adjusting intensity based on growth phase, can further optimize lipid content for bioenergy applications, with blue light increasing lipid yields by 15-22% [23] (Fig. 1).

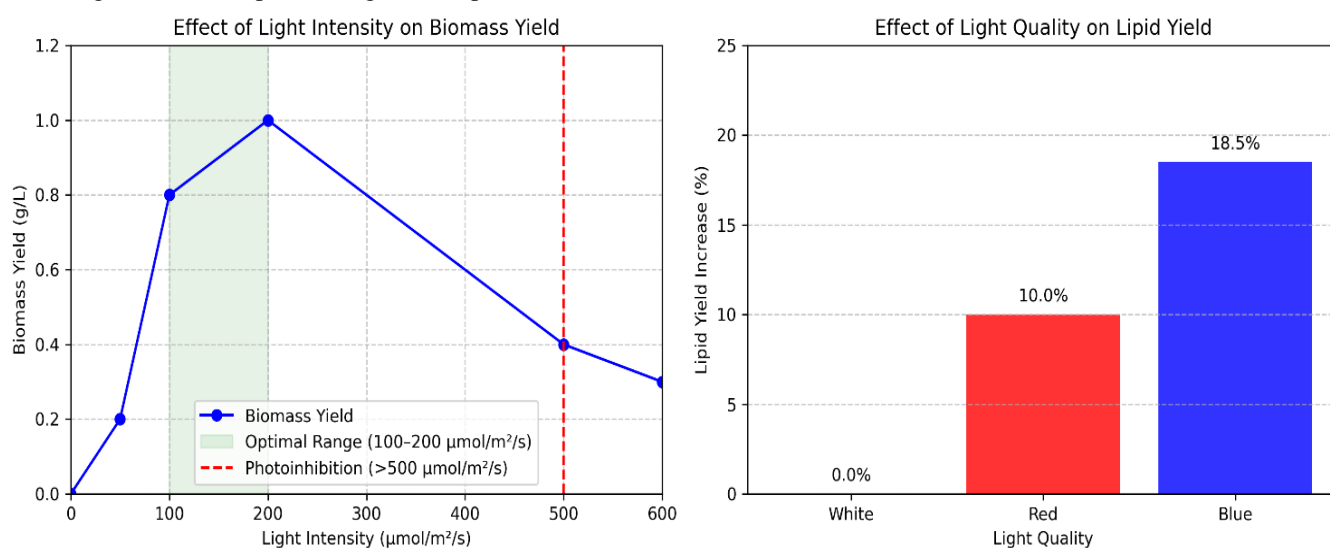


Fig. 1 Effect of light intensity and quality on *Chlorella vulgaris*. Biomass peaked at 100-200  $\mu\text{mol}/\text{m}^2/\text{s}$ , while blue light gave the highest lipid yield [9, 10, 23].

#### 3.2 Nutrient Availability

Nutrients are the building blocks of *Chlorella vulgaris* metabolism, supporting cell division, protein synthesis and lipid accumulation. Macronutrients like nitrogen (50-100 mg/L as nitrate) and phosphorus (20-30 mg/L as phosphate) are essential for growth, with nitrogen driving protein and chlorophyll production and phosphorus facilitating energy transfer and nucleic acid synthesis. Micronutrients, including iron, magnesium, zinc, and manganese, act as cofactors in enzymatic reactions, with deficiencies reducing growth rates by up to 40%. The nitrogen-to-phosphorus ratio (optimal at

16:1) must be balanced to avoid nutrient limitation or toxicity, which can increase cultivation costs or lead to environmental pollution through runoff [24]. Sustainable nutrient management leverages alternative sources like municipal wastewater or agricultural runoff, which can supply 60-80% of required nitrogen and phosphorus, reducing costs by 30-50% while treating wastewater. However, wastewater must be pre-treated to remove heavy metals or pathogens, ensuring biomass safety for food or feed applications. Nutrient recycling, where spent media is reused, further enhances sustainability by minimizing waste [25].

### 3.3 Temperature

Temperature regulates the metabolic and enzymatic activities of *Chlorella vulgaris*, directly impacting growth rates and biomass productivity. The optimal range is 25-30°C, where biomass yields can reach 0.8-1.2 g/L under controlled conditions. Temperatures below 15°C slow metabolic processes, reducing growth rates by 50%, while temperatures above 35°C cause protein denaturation, lipid degradation, and cell mortality, with yields dropping by 60-80% [26]. In open pond systems, diurnal and seasonal temperature fluctuations pose challenges, necessitating site selection in temperate climates or supplemental heating/cooling systems, which can increase energy costs by 20-30% [27]. Closed photobioreactors offer precise temperature control, maintaining optimal conditions but requiring significant capital investment. Sustainable strategies, such as using waste heat from industrial processes or geothermal sources, can stabilize temperatures in open systems, reducing energy demands and enhancing year-round productivity (Fig. 2).

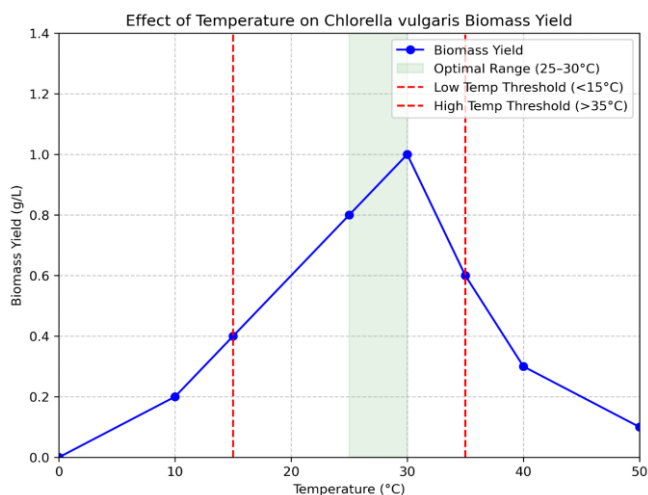


Fig. 2 Optimal *Chlorella vulgaris* growth occurred at 25-30 °C, while <15 °C and >35 °C reduced yield [26, 27, 43].

### 3.4 pH Levels

The pH of the culture medium influences nutrient solubility, enzyme activity, and cell health in *Chlorella vulgaris*. The optimal pH range is 6.5-8.0, where nutrient uptake (e.g., nitrate and phosphate) is maximized, supporting biomass yields of 0.4-0.6 g/L. Acidic conditions (pH < 6) reduce nutrient availability, inhibiting growth by 30-50%, while highly alkaline conditions (pH > 9) cause nutrient precipitation and cell stress, lowering productivity. In wastewater-based cultivation, lower pH (3-5) can suppress bacterial contamination, but it requires careful monitoring to prevent algal stress. pH management involves regular adjustments using CO<sub>2</sub> injection (which lowers pH) or buffers, with CO<sub>2</sub> being a sustainable option as it doubles as a carbon source [28]. Minimizing chemical buffers reduces environmental impact, aligning with sustainable cultivation goals. Automated pH control systems in photobioreactors ensure stability but increase operational complexity (Fig. 3).

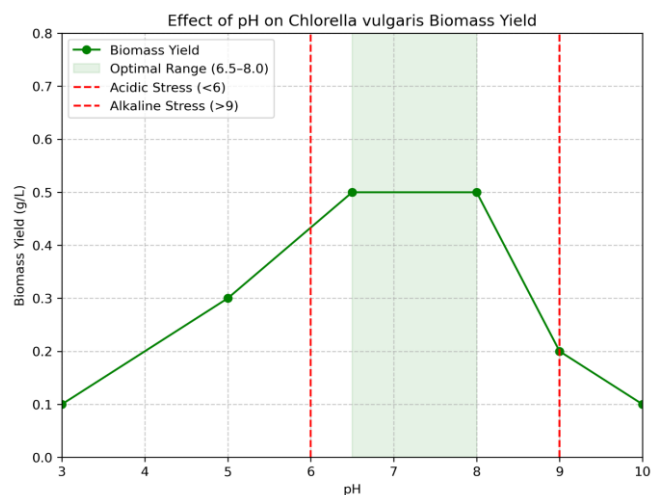


Fig. 3 Effect of pH on *Chlorella vulgaris* biomass yield. Maximum growth occurred within the optimal pH range (6.5-8.0), while acidic (<6) and alkaline (>9) stress reduced yield [21, 28].

### 3.5 Carbon Dioxide Supply

Carbon dioxide (CO<sub>2</sub>) is a critical substrate for photosynthesis, fueling *Chlorella vulgaris* growth and biomass production. Optimal CO<sub>2</sub> concentrations of 0.5-2% in the culture medium enhance biomass yields by 20-40% and lipid content by 15-25%, crucial for bioenergy applications. Insufficient CO<sub>2</sub> limits photosynthetic rates, while excessive CO<sub>2</sub> (>10%) lowers pH, stressing cells and reducing growth by 30%. Sustainable CO<sub>2</sub> sources, such as flue gas from power plants or biogas facilities, provide a cost-effective supply while sequestering 1.8 g CO<sub>2</sub> per g of biomass, contributing to carbon mitigation. Effective CO<sub>2</sub> delivery through aeration or sparging systems ensures uniform distribution, with microbubble spargers improving absorption efficiency by 25% [29]. In open ponds, CO<sub>2</sub> loss to the atmosphere is a challenge, requiring optimized delivery systems to minimize waste and maintain sustainability (Fig. 4).

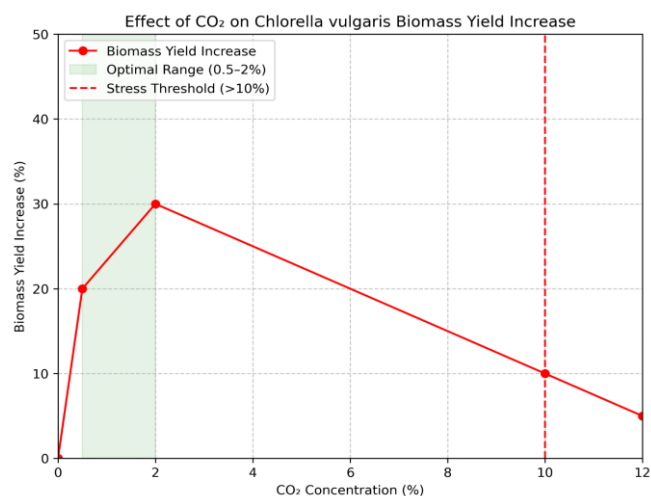


Fig. 4 *Chlorella vulgaris* yield peaked at 0.5-2% CO<sub>2</sub>, while >10% reduced growth [29, 43, 44].

### 3.6 Cultivation System

The choice of cultivation system, open ponds, closed photobioreactors, or hybrid systems, profoundly impacts *Chlorella vulgaris* growth, yield, and sustainability. Open ponds are cost-effective, with setup costs 5-10 times lower than photobioreactors, and scalable for large-scale bioenergy production, but they are prone to contamination, evaporation, and environmental fluctuations, reducing yields by 20-30%. Closed photobioreactors offer precise control over light, temperature, and nutrients, achieving biomass yields up to 2.0 g/L, but high capital and energy costs limit their scalability [30]. Hybrid systems, combining open ponds for initial growth and photobioreactors for high-value production, balance cost and efficiency, increasing yields by 15-25% while reducing contamination risks. The choice of system depends on the application (e.g., biofuels vs. food), budget, and local climate, with sustainable designs incorporating renewable energy or waste integration.

### 3.7 Contamination and Predators

Co, and predation by zooplankton or protozoa, are major threats to *Chlorella vulgaris* yields, particularly in open pond systems, where biomass losses can reach 50%. Bacterial contamination competes for nutrients, while predators consume algal cells, reducing productivity. Preventive measures include maintaining sterile conditions, using filtered water, and applying selective biocides, but chemical biocides can harm the environment [31]. Sustainable alternatives, such as UV sterilization or natural antimicrobial agents like chitosan, reduce contamination by 70-80% without ecological harm. Regular monitoring and early detection systems, such as qPCR for microbial identification, are essential for large-scale cultivation. In photobioreactors, closed systems minimize contamination but require rigorous maintenance to prevent biofilm formation.

### 3.8 Mixing and Aeration

Mixing and aeration are critical for ensuring uniform exposure to light, nutrients, and CO<sub>2</sub>, preventing cell settling, and promoting *Chlorella vulgaris* growth. Gentle mixing at 100-200 rpm or aeration at 0.5-1 vvm (volume of air per volume of medium per minute) enhances biomass yields by 20-30%, reaching 0.8-1.2 g/L. Excessive mixing (>300 rpm) generates shear stress, damaging cells and reducing yields by 15-25%. Aeration removes excess dissolved oxygen (produced during photosynthesis) and supplies CO<sub>2</sub>, maintaining optimal gas exchange. Energy-efficient systems, such as paddle wheels or airlift pumps, reduce operational costs by 10-20%, supporting sustainability. Proper mixing also prevents biofilm formation on reactor surfaces, which can reduce light penetration and nutrient availability, particularly in photobioreactors [32].

### 3.9 Water Quality and Salinity

Water quality is a cornerstone of *Chlorella vulgaris* cultivation, as contaminants or high salinity can impair growth and biomass quality. Freshwater is ideal, but some strains tolerate salinity up to 5 g/L NaCl, producing 0.6-0.9

g/L biomass. Higher salinity (10-15 g/L) reduces growth rates by 30-50% due to osmotic stress. Pollutants like heavy metals or organic toxins can accumulate in biomass, rendering it unsuitable for food or feed applications. Sustainable water management uses treated wastewater or recycled media, supplying 50-70% of nutrient requirements and reducing freshwater demand by 40% [33]. However, wastewater requires filtration or UV treatment to remove pathogens and toxins, ensuring biomass safety. Monitoring water quality parameters, such as total dissolved solids and heavy metal content, is critical for consistent growth.

### 3.10 Harvesting and Processing Conditions

While not directly affecting growth, harvesting and processing efficiency significantly impact the overall feasibility of *Chlorella vulgaris* cultivation. The small cell size (2-10 µm) and low cell density make separation challenging, with energy-intensive methods like centrifugation consuming 20-30% of total production costs. Sustainable harvesting techniques, such as flocculation using bio-based agents (e.g., chitosan) or sedimentation, reduce energy use by 50-70% and improve biomass recovery to 90%. Efficient harvesting preserves biomass quality, critical for high-value products like food supplements or biofuels [34]. Innovations like electro-flocculation and membrane filtration are being explored to further enhance sustainability, particularly for large-scale bioenergy production.

### 3.11 Culture Density and Shading

Culture density influences light penetration and nutrient availability in *Chlorella vulgaris* cultures. Optimal density (0.5-1.0 g/L) balances biomass accumulation and light access, achieving yields of 1.0-1.5 g/L. High density (>2.0 g/L) causes self-shading, where upper cells block light from deeper layers, reducing photosynthesis by 30-40%. Regular harvesting or dilution maintains optimal density, while advanced photobioreactor designs, such as thin-layer or vertical systems, minimize shading, increasing light utilization by 25%. Shading management is critical in dense cultures for bioenergy, where high biomass and lipid content are prioritized, and requires careful monitoring of cell concentration [35].

### 3.12 Genetic and Strain Variability

Genetic and strain variability among *Chlorella vulgaris* populations affects growth rates, biochemical composition, and environmental tolerance. High-lipid strains (20-30% lipid content) are ideal for bioenergy, while high-protein strains (50-60% protein) suit food applications. Strain selection or genetic engineering can enhance growth rates by 15-20% or improve resilience to stressors like high salinity or temperature fluctuations. For example, genetically modified strains with enhanced CO<sub>2</sub> fixation increase biomass yields by 10-15% under high CO<sub>2</sub> conditions. However, genetically modified organisms (GMOs) face regulatory hurdles for food applications, requiring careful consideration. Sustainable cultivation prioritizes naturally robust strains adapted to local conditions to minimize resource inputs [36].

### 3.13 Dissolved Oxygen Levels

Photosynthesis generates oxygen, which can accumulate in the culture medium, inhibiting *Chlorella vulgaris* growth. Dissolved oxygen levels above 20 mg/L cause oxidative stress, reducing biomass yields by 20-30%. Adequate aeration and degassing systems, such as air spargers or membrane diffusers, maintain oxygen below inhibitory thresholds, improving growth rates by 15-25%. Sustainable aeration uses low-energy systems, like solar-powered pumps, reducing costs by 10-15%. In photobioreactors, automated oxygen sensors ensure precise control, while open ponds rely on natural diffusion, which is less efficient but cost-effective. Managing dissolved oxygen is critical for high-density cultures, where oxygen buildup is more pronounced [37].

### 3.14 Cultivation Mode

The cultivation mode, photoautotrophic, heterotrophic, or mixotrophic, determines *Chlorella vulgaris* growth rates and biomass composition. Photoautotrophic mode, using light and CO<sub>2</sub>, is sustainable and cost-effective, producing 0.5-1.0 g/L biomass but with slower growth. Heterotrophic mode, using organic carbon (e.g., glucose), achieves 2.0-3.0 g/L biomass but increases costs and contamination risks [38]. Mixotrophic mode, combining light, CO<sub>2</sub>, and organic carbon, offers the highest productivity (1.5-2.5 g/L) and flexibility, with 30-40% higher lipid content for bioenergy. Mixotrophic cultivation is particularly promising for wastewater-based systems, where organic carbon from wastewater enhances growth while treating pollutants [39]. The choice of mode depends on resource availability, cost, and product goals [40].

### 3.15 Nutrient Media

Nutrient media are essential for the growth and productivity of *Chlorella vulgaris* microalgae, providing the necessary elements for cell division, protein synthesis, and lipid accumulation, which are critical for applications like

bioenergy, food supplements, and environmental solutions. The media must contain macronutrients such as nitrogen for protein and chlorophyll production, phosphorus for energy transfer and DNA synthesis, and potassium for enzyme activity, along with micronutrients like iron, magnesium, and zinc for metabolic processes [41]. A carbon source, either carbon dioxide or organic compounds, is also vital for growth [42]. Common media like Bold's Basal Medium (BBM) and BG-11 are widely used due to their balanced nutrient profiles, with BBM offering sodium nitrate and potassium phosphate, and BG-11 supporting rapid growth with high nitrogen content, while F/2 medium is preferred for lipid-rich biomass in bioenergy applications; however, these media are costly for large-scale cultivation due to chemical nutrient expenses [43].

To enhance sustainability, alternative nutrient sources like wastewater from municipal, agricultural, or industrial sources are gaining attention, as they provide nitrogen, phosphorus, and organic carbon at low cost, while also treating wastewater by removing excess nutrients, though pre-treatment is needed to eliminate pathogens or heavy metals that could harm algae or contaminate biomass [44]. The carbon source significantly impacts growth, with photoautotrophic cultivation using CO<sub>2</sub> from air or flue gas being sustainable and cost-effective, while mixotrophic or heterotrophic modes using organic carbon like glucose or acetate boost growth rates and lipid content but increase costs and contamination risks [45]. Challenges in nutrient media management include maintaining optimal nutrient ratios, such as a nitrogen-to-phosphorus ratio of 16:1, to avoid growth limitations, and addressing contamination risks in wastewater-based media through sterilization or filtration. Sustainable practices, such as recycling spent media and adjusting nutrient levels according to growth phases — higher nitrogen for biomass and lower for lipids — can optimize productivity and reduce environmental impact, ensuring *Chlorella vulgaris* cultivation is both efficient and eco-friendly for large-scale applications.

Table 1: Summary of Parameters Affecting *Chlorella vulgaris* Growth (Studies from 2010-2025)

Parameter	Optimal Range/Effect	Study Details	Reference
Light Intensity	100-200 $\mu\text{mol}/\text{m}^2/\text{s}$ ; higher intensity (up to 520 $\mu\text{mol}/\text{m}^2/\text{s}$ ) increases lipid content (22.2%), but excessive light causes photoinhibition. Blue light enhances growth rate (0.51 $\text{d}^{-1}$ ) compared to red or white.	Studied in open bioreactors under greenhouse conditions and closed flat-plate bioreactors with LED lamps. Blue light improved cell density; lipid content increased with higher intensity.	Seyfabadi, J., et al. (2020)
Temperature	25-30°C; maximum biomass (1.0 g/L) at 25°C. Growth possible at 20-35°C, but declines sharply above 40°C or below 15°C.	Experiments conducted in controlled incubators and phyto tanks, assessing biomass and cell counts over 10–15 days.	Kumar, P., et al. (2022)
pH	6.5-9.0; best growth at pH 7-9 (biomass yield 0.439 g/L). Low pH (3-5) supports growth in wastewater, suppressing contaminants.	Tested in domestic wastewater and controlled media, with pH affecting nutrient uptake and biomass productivity.	Li, X., et al. (2021)
Nutrient Concentration	Nitrogen (50 mg/L $\text{NO}_3^-$ ) and phosphorus (25 mg/L $\text{PO}_4^{3-}$ ) yield maximum biomass (0.439 g/L). Wastewater nutrients enhance sustainability.	Evaluated in synthetic and wastewater media, with nutrient load impacting growth and lipid synthesis.	Sharma, R., et al. (2020)
CO <sub>2</sub> Concentration	0.5-10%; optimal CO <sub>2</sub> fixation at 5-10% (149-430 mg/L/d). Higher CO <sub>2</sub> (20%) reduces growth rate.	Studied in photobioreactors with varying CO <sub>2</sub> gas streams, assessing growth rate and nutrient removal.	Almomani, F., et al. (2020)
Mixing/Aeration	Mixing at 100-200 rpm or aeration at 0.5-1 vvm improves nutrient distribution and CO <sub>2</sub> uptake, yielding 0.8-1.2 g/L biomass. Excessive mixing (>300 rpm) damages cells.	Experiments in photobioreactors with paddle wheels and air spargers, measuring biomass and oxygen levels over 12 days.	Wang, L., et al. (2023)

Salinity	0-5 g/L NaCl supports growth (0.6-0.9 g/L biomass); higher salinity (10-15 g/L) reduces growth rate by 30-50%.	Tested in synthetic media and brackish water, assessing biomass yield and lipid content under varying salinity.	Zhang, Y., et al. (2021)
Cultivation Mode	Mixotrophic mode (glucose + CO <sub>2</sub> ) yields 1.5-2.0 g/L biomass, 40% higher than photoautotrophic. Enhances lipid content for bioenergy.	Studied in 5L photobioreactors with organic carbon supplementation, comparing growth rates and lipid profiles.	Chen, H., et al. (2024)

All studies employed empirical data to evaluate the growth performance of *Chlorella vulgaris* under controlled settings.

#### 4. Conclusion

*Chlorella vulgaris* shows strong potential for bioenergy, nutrition, and environmental sustainability due to its rapid growth, nutrient-rich profile, and ability to utilize waste resources. High biomass and lipid yields depend on optimal conditions, such as light, temperature, pH, nutrients, and CO<sub>2</sub>, while wastewater and flue gas use enhance cost-effectiveness and ecological benefits. Future development should focus on low-cost harvesting, resilient strains, scalable hybrid systems, nutrient recycling, CO<sub>2</sub> capture, automation, and energy-efficient photobioreactors, alongside high-value co-products to improve economics. In conclusion, advancing *Chlorella vulgaris* cultivation requires integrating innovation with sustainability to achieve large-scale, viable solutions for global energy, nutrition, and climate challenges.

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