## REVIEW

**Microbial Cell Factories** 



# Microalgae: a multifaceted catalyst for sustainable solutions in renewable energy, food security, and environmental management

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### Abstract

This review comprehensively examines the various applications of microalgae, focusing on their significant potential in producing biodiesel and hydrogen, serving as sustainable food sources, and their efficacy in treating both municipal and food-related wastewater. While previous studies have mainly focused on specific applications of microalgae, such as biofuel production or wastewater treatment, this review covers these applications comprehensively. It examines the potential for microalgae to be applied in various industrial sectors such as energy, food security, and environmental management. By bridging these different application areas, this review differs from previous studies in providing an integrated and multifaceted view of the industrial applications of microalgae. Since it is essential to increase the productivity of the process to utilize microalgae for various industrial applications, research trends in different microalgae cultivation processes, including the culture system (e.g., open ponds, closed ponds) or environmental conditions (e.g., pH, temperature, light intensity) to improve the productivity of biomass and valuable substances was firstly analyzed. In addition, microalgae cultivation technologies that can maximize the biomass and valuable substances productivity while limiting the potential for contamination that can occur when utilizing these systems have been described to maximize CO<sub>2</sub> reduction. In conclusion, this review has provided a detailed analysis of current research findings and technological innovations, highlighting the important role of microalgae in addressing global challenges related to energy, food supply, and waste management. It has also provided valuable insights into future research directions and potential commercial applications in several bio-related industries, and illustrated how important continued exploration and development in this area is to realize the full potential of microalgae.

Keywords Microalgae, Culture system, Bioreactors, Biofuel production, Wastewater treatment, Sustainable food sources, Biohydrogen production, Environmental sustainability

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#### Introduction

An unprecedented surge in greenhouse gas (GHG) emissions from rapid industrial activity seriously threatens the global environment and future human life, with 40.9 billion tons of GHG emissions identified as of 2023 [1]. The six primary GHGs that cause the greenhouse effect are CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>0, HFCs, PFCs, and SF<sub>6</sub>. Still, the most significant GHG generated by human activities is  $CO_2$ , produced by fossil fuel combustion.  $CO_2$  emissions, the primary GHG, are constantly increasing and already exceeded 36.8 billion tons in 2023; hence, reducing CO<sub>2</sub> emissions is a top priority for human survival [1, 2]. In response to these challenges, much research is underway worldwide to develop carbon capture and storage (CCS) and carbon capture, utilization, and storage (CCUS) technologies, including chemical or biological processes. However, despite the promising potential of CCS strategies, critical drawbacks such as safe storage, siting, and high upfront costs have prevented widespread utilization and adoption worldwide. As a result, there is growing interest in CCU technologies that provide both environmental protection and economic benefits by reducing CO<sub>2</sub> while converting it into industrially applicable or human-beneficial products [3-5]. In particular, the use of photosynthetic microalgae for the biological conversion of CO<sub>2</sub> has gained attention due to their rapid growth, efficient CO<sub>2</sub> uptake, wastewater treatment capabilities, and the absence of associated ethical concerns [6-8]. Existing studies have shown that more than 1.7 kg of  $CO_2$  is required to produce 1 kg (dry weight basis) of microalgal biomass through phototrophic cultivation, which indicates how effective microalgae biomass production can be in the CO<sub>2</sub> reduction process compared to other terrestrial plants [9]. Indeed, a wide range of industrial uses, including biofuels, cosmetics, nutraceuticals, medications, agricultural fertilizers, and animal feed, is possible with microalgal biomass and internal extracts. For instance, dietary supplements containing vegetable omega-3 fatty acids derived from Chlorella, Spirulina, and Schizocitrium are currently very well-liked. Phycocyanin from Spirulina has been shown to inhibit the growth of leukemia factors, while  $\beta$ -glucan from Chlorella has the function of an immunostimulant. Furthermore, because of its potent antioxidant properties, astaxanthin derived from Haematococcus pluvialis is employed as a cosmetic ingredient and has recently been utilized in health foods and medications. The biomass left after utilizing astaxanthin is used as feed for aquaculture to induce astaxanthin synthesis in salmon and shrimp [10, 11]. Additionally, microalgae are rich in carotenoid pigments and proteins such as lutein and beta-carotene, and can therefore be used to raise larvae in fish farms or as food for bivalves, in addition to serving as additives in broiler feed [12]. Chlorella culture solutions are commercially available as an organic biofertilizer for cultivating fruits and vegetables such as strawberries and lettuce. In several countries, microalgae are also actively used to treat residual phosphorus, nitrate, heavy metals, and other contaminants in secondary wastewater, with a high efficiency in removing well-documented aquatic pollutants [13, 14]. Microalgae biomass has great promise as a biological resource that can help with two of humanity's most urgent issues: industrial development and mitigating the climate crisis, as evidenced by its application in various industries. In this context, photosynthetic microorganisms offer a uniquely promising means to simultaneously address both of these challenges [15]. However, scaling up microalgae cultures to achieve current CO<sub>2</sub> reduction requirements poses many challenges due to inherent limitations associated with microalgae physiology [16, 17]. Even if microalgae produce valuable substances, depending on the species, their growth rates are slow in commercial-scale cultures, which can significantly reduce process productivity and  $CO_2$  removal efficiency. Therefore, to maximize  $CO_2$ removal efficiency, it is necessary to develop effective technologies that can maximize the productivity of biomass containing useful substances per unit area as well as a large culture area [18]. To overcome the limitations of microalgae cultivation, a variety of specialized methods are being used, such as developing photosynthetic photobioreactors that are easy to scale up or dramatically improving process technologies such as biomineralization and semi-continuous culture to increase productivity. There are two approaches to carrying out the biomineralization process. The first is to increase the efficiency of photosynthesis through the double scattering effect of calcium carbonate (CaCO<sub>3</sub>) produced on the cell surface, thereby maximizing the productivity of biomass and valuable substances and reducing CO<sub>2</sub>. The second is to control contamination by crystallizing contaminants that are prone to occur during the culture of environmentally sensitive species, such as *H. pluvialis*, thereby increasing the productivity of axenic biomass, including high amounts of astaxanthin [3, 4, 19, 20].

As the demand and need for renewable energy have increased in recent years, researchers have become increasingly interested in discovering and developing new energy sources that can completely replace fossil fuels [21]. The term "biomass" originally referred to the total number of living organisms in a given space. Today, it has expanded to refer to all living organisms that can be utilized as a general energy source. The discovery that ethanol, methanol, and biodiesel can be produced from biomass has led to many technological advances, and various studies have been conducted to increase their productivity. Furthermore, the amount of biomass produced on Earth each year is about the same as total oil reserves, so it has the advantage of being less likely to be depleted [22, 23]. However, obtaining biomass energy from plants or animals has the potential to promote the ecosystem degradation or threaten food security. Microalgae, on the other hand, are well suited for biodiesel production because they are sustainably cultured in the ocean and contain large amounts of lipids (up to 70%); thus, the production of biomass energy from microalgae could put an end to various environmental concerns [3, 24]. A typical biomass production process involves selecting microalgae species with high lipid content, culturing them in large quantities in the sea, and then drying them to extract lipids from the algal biomass. Subsequently, a catalyst is added to these vegetable lipids to produce a mixture, which is then reacted with alcohol to obtain alkyl esters and glycerin. The alkyls obtained from this process can be utilized as biodiesel [25]. Optimizing the culture environment to maximize biomass productivity is crucial in stably producing microalgae for biodiesel extraction. To achieve this, the pH must be kept constant, light and temperature must be controlled, and photosynthetic efficiency must be increased by supplying an appropriate amount of  $CO_2$  and nutrients [17, 26]. In addition, because existing methods using catalysts and alcohol require a large amount of chemical input and have a relatively slow reaction time, the development of technology to optimize the process of converting lipids into biodiesel is also necessary. In conclusion, if each technology for more efficient conversion of biodiesel is optimized, commercial use of biodiesel from microalgae can be expected to become a reality in the near future [16, 27].

Along with biodiesel, biohydrogen is a renewable energy source that can be produced from microalgae and provides more energy per unit mass, which means that hydrogen has a higher energy density and can do more work with the same amount of fuel. Because hydrogen is the smallest and most abundant element, and the combustion product of hydrogen fuel is primarily water, it is a clean energy source that can reduce  $CO_2$ , CO,  $NO_x$ ,  $SO_x$ , and other pollutants produced by conventional hydrocarbon fuels such as gasoline and diesel, and reduce the impact on climate change. It also has very low ignition energy, which makes it easy to burn, enabling efficient energy conversion in combustion engines or fuel cells, making hydrogen a very effective fuel. However, hydrogen storage and transportation require special requirements, such as high-pressure tanks and low-temperature liquefaction, and hydrogen production is still primarily done using fossil fuels, which emit  $CO_2$  in the process [5, 28, 29]. Therefore, there is still a need for new methods of hydrogen production using microalgae and for robust process technologies to increase productivity.

In this review, various technologies for optimizing microalgae culture systems were introduced and the expected environmental and industrial benefits of this system were also explained in detail (Fig. 1).

### **Outdoor cultivation for biomass production** Outdoor cultivation parameters

Several studies have focused on using  $CO_2$  and light to grow microalgae to reduce atmospheric  $CO_2$  levels, in addition to exploring outdoor cultivation technology to maximize the production of biomass, a high-value-added substance [30]. The culture parameters include bioreactor type/form, gas supply method (e.g., the composition and concentration of a gas and flow rate), pH, temperature, light intensity, pollution control, and nutrients, among others (Fig. 2).

### Comparative analysis of microalgae cultivation systems: advantages and limitations of open ponds and photobioreactors

Open pond systems, which typically consist of shallow tracks (0.15 to 0.45 m deep) constructed with ponds lined with concrete, clay, or plastic, are more efficient than closed systems regarding production capacity and cost energy. Open systems are exposed to the external environment and utilize 100% natural light to induce photosynthesis, making them easy to design, build, and operate. However, because this method is in direct contact with natural environments, culture parameters cannot be controlled and the conditions are heavily influenced by climatic conditions such as the weather [31]. *H*. *pluvialis*, which produces valuable substances with high market value such as astaxanthin, is quite challenging to control in open ponds because it is sensitive to light and temperature and is particularly susceptible to contamination. Additionally, there have been many instances of culture failure due to typhoons and rainy seasons, and culture efficiency is often negatively impacted by high evaporation rates. Moreover, natural pollutants can be introduced through uncontrollable phenomena such as wind, rain, snow, and air. This method is also notoriously difficult to scale up and is therefore only suitable for countries with large land areas, which poses an important limitation [32, 33].

A complex closed-system method for cultivating microalgae, photobioreactors (PBRs) significantly improve the efficiency and control of microalgal growth. It is possible to grow species in these systems, but it would be difficult, if not impossible, to culture in open systems since they are designed to sustain photobiological processes under precisely controlled circumstances. The capacity of PBRs to reduce  $CO_2$  loss, stop water from evaporating, support larger cell densities, and drastically lower the risk of contamination are some of its main advantages. However, when compared to open pond systems, construction and operating costs are significantly



**Fig. 1** Diverse applications of microalgae as sustainable and environmentally friendly resources. Microalgae are emerging as a sustainable solution for biodiesel, wastewater treatment, future food sources, food waste treatment and hydrogen production. Biodiesel production from microalgae helps to reduce  $CO_2$  emissions and provide a sustainable source of energy. In wastewater treatment, microalgae have the ability to remove pollutants and reduce  $CO_2$ . Microalgae is a promising alternative for future food source as it could potentially use as a high-protein food and a variety of nutrients. In food waste treatment, microalgae can efficiently use organic wastes like banana peels and convert it into valuable products. Microalgae can also be used to absorb solar energy and produce hydrogen in an environmentally friendly way. Thus, microalgae can be applied in a variety of industries for sustainable and environmentally friendly techniques

higher, as important design considerations for PBRs include ensuring rapid mass transfer of  $CO_2$  and  $O_2$ , accommodating a variety of microalgae species, managing strong foaming conditions, and minimizing areas not exposed to light [34–36]. Achieving sufficient light penetration through an optimized surface area-to-volume ratio is also crucial, as it directly influences algal productivity. Interestingly, the adaptability of most microalgae species to varying light intensities suggests that lower light conditions do not necessarily equate to reduced biomass yield. Furthermore, closed PBR systems offer superior control over environmental parameters compared to open ponds, leading to higher biomass productivity. This control extends to pollution management, where the closed nature of PBRs presents fewer challenges related to the introduction of exogenous contaminants. Recent advancements have demonstrated the feasibility of using CO<sub>2</sub>-enriched flue gas from LNG-fired power plants to enhance microalgae cultivation in PBRs, thus providing a sustainable means for high-value product generation [37–39].

In other words, if large-scale production of biomass is the goal, an open pond system may be more suitable, but if the production of high-value materials is to be maximized, a closed system is effective. Since each approach has clear advantages and disadvantages, open-closed hybrid systems are also being investigated, which combine the benefits of both systems to balance cost and productivity. By starting the initial culture in a closed system to protect contamination-sensitive high-value substances and stabilize the culture and then using a low-cost open system for the mass production phase, it becomes possible to combine the precise control of a closed system with the mass production capabilities of an open system. These two-stage hybrid systems are seen as a promising option for maximizing biomass and valuable material



**Fig. 2** Parameters necessary to maximize the biomass and valuable substances productivity in the microalgae outdoor culture systems. There are many ways to produce biomass effectively. For example, there are ways to utilize culture systems suited to each environment, such as open ponds, raceway ponds, and closed systems, or to control contaminants that cause significant losses in biomass and valuable substances productivity. In addition, regulating environmental conditions like pH, temperature, and light intensity can maximize biomass and valuable substances productivities. These factors can also increase by improving the reactors used in each culture system

production while reducing costs and are increasingly being used in experimental research and commercial applications [40].

### Scaling up microalgae production: innovations in photosynthetic vinyl bioreactor technologies and their commercial implications

Large-scale photosynthetic vinyl bioreactors-a recent development in PBR technology-have allowed for a notable increase in microalgae cultivation capacity while maintaining the same spatial footprint as conventional photobioreactors. Superior light penetration provided by these innovative systems increases photosynthetic efficiency and biomass productivity per unit area. Their design optimizes growing conditions and helps to mitigate  $CO_2$  more effectively by facilitating smoother mixing processes [39, 41]. The primary benefit of these systems is their ability to consolidate the number of bioreactors needed for the large-scale cultivation of photosynthetic organisms, which greatly streamlines production processes. These efficiencies minimize spatial efficiency and shorten the time needed for reactor construction, which results in significant cost savings, including decreased lease costs. The significant increase in productivity of microalgae cultivation processes through these optimized transparent photosynthetic bioreactors directly benefits the market potential of microalgae-derived products such as pharmaceuticals, nutraceuticals, cosmetics, and animal feed while also contributing to the reduction of  $CO_2$  emissions associated with the cultivation process itself [42, 43]. On the other hand, maintaining optimal outdoor culture parameters—such as pH, light intensity, and temperature—is essential to optimizing the efficiency of these large-scale cultivation systems.

Innovative technologies such as vertical film bubble column PBRs equipped with stone spargers have improved microalgae cultivation by optimizing cell growth conditions. Moreover, common issues such as cell aggregation, reduced mixing efficiency, and potential contamination have been addressed using V-shaped film PBRs to maintain optimal culture conditions [44]. However, due to recent limitations associated with the scaling up of V-shaped film PBRs, baffles have been incorporated inside existing square-shaped reactors to increase mixing efficiency and other issues have been solved by improving the sparger system. Many researchers have successfully cultivated microalgae and produced useful substances using film PBRs and exhaust gas supplied by power plants such as the Korea District Heating Corporation and coalfired power plants [45].

#### Optimizing microalgae cultivation: innovations and challenges in tubular photobioreactor design and operation

Tubular PBRs represent a significant advancement in microalgae cultivation, offering a sophisticated platform for efficiently capturing solar radiation over large areas [46]. These systems are strategically engineered to optimize light exposure and biomass productivity through a variety of configurations, such as vertical, horizontal, and helical orientations. The selection of construction materials, usually glass or transparent plastics, in addition to the strategic arrangement of tubes, whether in parallel formations or coiled designs, plays a pivotal role in enhancing light absorption and facilitating microorganism growth. The performance parameters of these PBRs, especially the culture's flow rate and gas exchange mechanisms, are crucial for ensuring ideal growth conditions [5, 47]. Conversely, horizontal and helical designs prioritize higher light energy conversion rates and the effective management of gas exchange, albeit with some drawbacks associated with high energy consumption and the potential for photobleaching. Maintenance of these reactors is also critical, and innovative cleaning methods and the integration of temperature control and degassing systems are being developed to mitigate weather damage and maintain internal cleanliness. These developments increase the PBRs' operational lifetime, productivity, and efficiency, which benefits the biomass production [5, 45, 47, 48].

To summarize, tubular PBRs are exceptional instances of integrating biological and engineering principles to improve the productivity of microalgae biomass and useful substances. Their diverse configurations, coupled with advanced gas supply and cleaning technologies, make them uniquely promising alternatives for sustainable biomass production in both academic research and commercial applications.

# Advancements in biomass production: the role and optimization of flat-panel photobioreactors in microalgae cultivation

Flat-panel PBRs are known to be the most efficient design for maximizing light utilization and, thus, biomass productivity yields among the various PBR designs. Flat-panel PBRs are made of durable and cost-effective materials such as glass, plastic, and stainless steel. The thickness of the reactor is also designed with precise dimensions to provide a more uniform light distribution throughout the culture and maximize biomass productivity [49–51]. Several studies show the use of flat-panel PBRs for microalgae growth such as the green alga *Chlorella vulgaris* and the diatom *Phaeodactylum tricornutum* actually increased biomass production [52, 53]. One technique that has been applied to flat-panel PBRs is stirring or mixing. This is typically accomplished by

introducing air into the interior using nanoporous or perforated tubes or mechanical means such as motors. This agitation is crucial for preventing the accumulation of dissolved oxygen, which can inhibit photosynthetic efficiency if left unchecked. Despite these many advantages, there are crucial limitations to panel design. Particularly, scaling up flat-panel systems requires additional compartments and makes it difficult to control the culture temperature up to the expanded space, resulting in serious problems with biomass productivity due to changes in the culture environment. To overcome these limitations, various research efforts are underway, including integrating advanced cooling systems and developing new materials and differentiated engineering techniques to reduce energy consumption during mixing [5, 54]. Furthermore, incorporating innovative technologies that can monitor and modify culture preferences in real-time holds promise for efficiently cultivating different microalgal species with different cultural requirements.

In summary, flat-panel PBRs offer a sustainable and efficient method for biomass production. This method stems from the reactor's components and minimal thickness, allowing a large amount of light to be uniformly irradiated inside, maximizing photosynthetic efficiency. It is important to develop flat-panel PBRs to overcome the limitations of conventional culture systems for applications ranging from biomass to biofuel production or carbon fixation.

### Optimizing light availability and energy efficiency in microalgae cultivation: Challenges and innovations in stirred tank photobioreactors

Glass lab-scale stirred tank PBRs have emerged as a versatile choice for microalgae production, supporting a wide range of growing tactics including autotrophic, mixotrophic, and heterotrophic modes. Constructed from materials such as glass or stainless steel, these reactors are designed to optimize light availability through internal or external illumination devices. These reactors are characterized by their small diameter, which is pivotal for achieving sufficient photon flux densities, thereby minimizing light limitation within the microalgae suspension. This design consideration is crucial for increasing the photosynthetic efficiency of microalgae cultures [55]. Stirred tank PBRs are known for their operational flexibility, accommodating different batch modes and ensuring uniform nutrient distribution. This is primarily due to their basic design and mechanical agitation methods, such as impellers or magnetic stirring, which allow for efficient aeration, mixing, and overall system homogeneity. These characteristics are critical for boosting heat and mass transfer within the reactor and ensuring optimal growth conditions [55, 56].

However, some issues and limitations are associated with the usage of stirred tank PBR. Despite the efficiency of nutrient distribution and the potential for large-scale biomass production, biomass productivity is typically only in the range of 30–50 mg  $L^{-1}d^{-1}$ . In large-scale microalgae production, these reactors struggle to reach adequate light uptake levels and also suffer from energy requirements for internal illumination and cell damage due to excessive agitation [57]. Addressing these challenges will require changes in agitation methods that increase energy efficiency and reduce the biomass productivity of microalgae, as well as the development of new lighting technologies that increase light utilization to provide optimal light. This may include developing more efficient lighting systems or exploring novel reactor designs that allow for better light distribution without requiring high energy inputs or excessive mechanical agitation (Table 1).

# Strategies for effective contamination mitigation in microalgae cultivation systems

Culture-associated costs increase with culture size, and therefore, contamination can lead to considerable losses in large-scale microalgae culture systems, thus highlighting the crucial importance of contamination control. In cross-contamination situations, microalgal strains with high adaptability and nutrient uptake maximize resource utilization by competing with less adaptable strains [19, 58]. Therefore, contamination of microalgal bioreactors can be devastating, as fast-growing strains can quickly dominate the entire system. Various approaches have been used to control contamination, including chemical, biological, physical, and environmental methods. Among these control strategies, applying pesticides and antibiotics at specific concentrations is widely recognized as an efficient way to achieve selective and complete control of particular contaminants. However, this approach has a significant limitation in that as the culture scale increases, the injected concentrations become pretty high, resulting in a substantial economic loss. In addition, unwanted chemical residues accumulate in the microalgae culture, resulting in a considerable loss in biomass productivity. More recently, substances such as ammonium bicarbonate, natural plant-based pesticides, and antioxidant quinine, which have inhibitory properties against contaminants, have been utilized instead [4, 59, 60]. Furthermore, recent studies have reported that increasing pH and salt concentration when culturing species such as Chlorella sp. and Dualella sp. can prevent contamination in open pond systems [61, 62]. Another recent study showed that microalgal species such as N. oceanica can produce bioactive compounds that can inhibit the growth of protozoa such as E. vannus, U. marinum, and Litonopus sp. and affect their bioactivity, impairing their viability [63]. To prevent critical contamination, most microalgae culture systems utilize PTTE film (0.20 µm) filters to purify the supplied gas before it is injected into the reactor. However, despite these measures, H. pluvialis has a slow growth rate and a high sugar content in its cell walls, making it highly susceptible to contamination. A recent study proposed a CaCO<sub>3</sub> biomineralization-based decontamination strategy (CBDS) to address these challenges. The authors reported that controlling contamination with CBDS under autotrophic and mixotrophic conditions increased the average astaxanthin productivity by approximately 14-fold compared to contaminated cells. Therefore, the surface of H. pluvialis was successfully protected from bacteria and fungi through biological mineralization [4, 19]. He et al. also conducted a study to prevent protozoa contamination using NH<sub>4</sub>HCO<sub>3</sub>. Upon co-culturing amoeba, a contamination source, and P. tricornutum, a culture target cell, higher NH<sub>4</sub>HCO<sub>3</sub> concentrations were linked to more significant decreases in amoeba contamination. Additionally, the periodic supply of NH<sub>4</sub>HCO<sub>3</sub> could further control cell contamination [64].

While there are many ways to control contamination, the most important thing is to ensure that the risk of contamination is minimized from the start of the grow system design. This can be done by implementing high-efficiency filtration systems, such as HEPA filters for  $CO_2$ -containing air, to prevent the introduction of

Table 1 Comparative analysis of microalgae cultivation systems: features, advantages, and limitations

Cultivation system	Key features	Advantages	Limitations	Refer- ence
Open Ponds	Shallow tracks, natural light	Low cost, easy to design/build/operate	Susceptible to contamination, weather effects, limited control over environmental conditions	[33]
Tubular PBRs	Various configurations (vertical, horizontal, helical), transparent materials	Maximizes light exposure, efficient gas exchange, versatile design	Potential for photobleaching, high energy consumption for certain configurations	[42, 48]
Flat-Panel PBRs	Minimal thickness, high sur- face area to volume ratio	High photosynthetic efficiency, adapt- able to indoor and outdoor setups	Complexity in scaling up, temperature modula- tion challenges	[52, 53]
Stirred Tank PBRs	Glass/stainless steel, internal/ external illumination, mechani- cal agitation	Operational flexibility, uniform nutrient distribution	Light penetration challenges in larger volumes, energy demands for lighting and agitation, potential for cellular damage	[5, 42, 57]

airborne microorganisms and dust. Regular disinfection and cleaning can also prevent biofilm formation and other contaminant buildup. Furthermore, automated monitoring systems can help you detect changes in  $CO_2$  concentration, pH, temperature, and more in real-time and take immediate action to reduce the likelihood of contamination. Additionally, since not all of the  $CO_2$  supplied to the microalgae is utilized by the microalgae, implementing a system to recapture and recycle the remaining  $CO_2$  supplied can be a significant benefit in terms of maximizing  $CO_2$  reduction and ensuring that the  $CO_2$ , once purified, can continue to be utilized.

# Optimizing microalgae growth through precise pH management strategies

When the pH of the medium drops below a certain level, the activities of extracellular carbonic anhydrase (CA) and rubisco enzymes are significantly inhibited, reducing the carbon-fixing capacity of microalgae. This leads to the accumulation of CO<sub>2</sub> in the culture and further lowering of the medium's pH, resulting in a significant drop in biomass productivity [65]. Conversely, inadequate  $CO_2$ supply can also lead to a sharp increase in pH, as damage to cell membranes and decreased enzyme activity also occur under high pH conditions, reducing photosynthetic efficiency and disrupting the carbon fixation process. In addition, the availability of some inorganic nutrients may decrease, making it difficult for microalgae to absorb nutrients necessary for growth. Therefore, a buffer system is required to maintain a pH of 7-8, the ideal growth range that creates a favorable environment for maximizing biomass productivity [66-68]. Traditionally, Tris buffer, HEPES (4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid), phosphate buffer, etc., have been used, but due to their limited economic feasibility in large-scale cultivation, the buffer system by injecting  $CO_2$  bubbles into essential substances such as potassium hydroxide (KOH) has been recently utilized [3, 4].

### Critical role of temperature regulation in enhancing microalgae cultivation efficiency

During the peak summer season, temperature and light intensity rise dramatically within microalgae culture system in outdoor conditions. Temperature strongly influences microalgal growth dynamics and lipid production, with various species varying adaptability across the temperature spectrum. Microalgae typically exhibit optimal growth within a temperature range of 20–30 °C. Lower temperatures tend to restrict cellular metabolic activity, whereas increasing to species-specific ideal temperatures promotes biomass accumulation. However, exceeding this ideal temperature threshold usually causes slower growth rates [4, 20, 69, 70]. In particular, rising temperatures significantly affect the growth of *C. vulgaris*. The species achieves peak growth at 30 °C; however, increasing the temperature to 35 °C reduces the growth rate by approximately 17%, with higher temperature rises proving lethal to the cells. This effect is visually evident as cell pigmentation changes from green to brown, indicating cell death. Interestingly, reducing the temperature from 30 to 25 °C increases lipid content from 5.9 to 14.7% without negatively affecting growth rate.

In outdoor mass culture, the production of less reactive oxygen species (including LROS,  $O_2^-$  and  $H_2O_2$ ) increases with higher temperatures. This has an enormous impact on the carotene synthesis pathway of microalgae, resulting in a significant loss of valuable end products such as astaxanthin, lutein, and zeaxanthin [71]. As previously noted, large-scale outdoor cultures have difficult environmental conditions to manage, and these culture systems can quickly become contaminated if left unattended. To address these issues, a recently developed solution introduced precisely calibrated iron sulfate volumes into the growth media. This addition contributed to converting cell-harmful LROS into more reactive oxygen species (MROS), which increased the synthesis of bioactive substances [5, 19, 20, 72].

Even if culture technology minimizes the damage caused by temperature fluctuations, the first step is establishing a temperature control system within the processing facility. This can include using in-reactor cooling systems to remove excess heat, circulating the culture to maintain temperature uniformity, and recycling waste heat to maintain the temperature of the culture system, which can significantly reduce energy costs by regulating the temperature without additional energy sources.

# Importance of controlling light intensity in microalgae cultivation for optimal growth and productivity

Light is a vital element to the metabolic activity of microalgae, serving as the primary energy source for synthesizing essential molecules such as ATP and NADPH, which are critical for growth and survival. During photosynthesis, microalgae use light energy to convert CO<sub>2</sub> into chemical energy sources such as glucose while also emitting oxygen as a byproduct. The duration, wavelength, and intensity of light exposure affect photosynthetic efficiency, which is directly linked to microalgae growth. Increased light intensity promotes microalgae growth and lipid accumulation up to the light saturation point, which is a delicate balance between photorespiration and photoinhibition [73-75]. In outdoor biomass culture, strong natural light can significantly limit cell growth due to uneven light distribution within the reactor, resulting in cell death and, in some species, the release of toxic substances such as chlorellin. To address these issues, recent research has proved that biomineralization can be used to effectively harness the effects of strong light and turn them into positive effects [3, 18]. Due to the double scattering phenomenon of calcium carbonate produced on the cell surface through biomineralization, the light is evenly distributed to all cells in the culture, even under strong light. This technique is very effective compared to the previous method, which concentrated the light on a few cells and caused cell death. This homogeneous light exposure significantly increased photosynthetic efficiency, cell number, biomass and lipid productivity, and  $CO_2$  removal efficiency [3, 4, 19, 72]. Furthermore, this biomineralization process was combined with a semicontinuous process that maintains high biomass and lipid productivity even in strong light conditions [18].

In addition, shading facilities can be built in the culture facility to control the light intensity by opening and closing the shades according to the weather or culture conditions. In addition, in the evening, when light is not naturally available, artificial light irradiation consumes energy, and recently, solar cells have been utilized to store and utilize electricity when light is intense. Another alternative is to identify the permeability of the reactor material and utilize it selectively according to the microalgae species.

#### Advancements in algal biomass: from energy conversion to high-value applications Biodiesel

Biodiesel is emerging attention as a new resource to replace conventional fuels due to its environmental and economic sustainability and versatility [76]. Microalgae are indeed promising candidates for biodiesel production due to their high lipid content; in particular, certain species such as Schizochytrium sp., Botryococcusbraunii, Nannochloropsis and Chlorella sp. can produce up to 77%, 75%, 68%, and 53% of their dry weight in lipids, respectively, making them highly suitable for biodiesel production [77]. In contrast to conventional fuels, biodiesel from microalgae can reduce pollutants such as hydrocarbons, particulate matter, and carbon monoxide. In addition, conventional sources of biodiesel have the problem of destroying arable land and threatening human food production, but biodiesel production from microalgae requires only an aquatic environment, so there are fewer problems, such as loss of arable land and destruction of ecosystems. In this sense, microalgae-based biodiesel production is considered environmentally friendly [78]. The process from microalgae to biodiesel is very complicated, from cultivation to purification, however, since the field is gaining attention, many studies have been conducted on the cultivation and purification process. This process begins with introducing a catalyst into vegetable fat, triggering a chemical reaction with alcohol to produce alkyl esters and glycerin, with the former serving as the primary component of biodiesel [79]. This method of biodiesel production has environmental significance in that it solves many of the challenges faced by conventional biodiesel, as microalgae can grow in water bodies of varying conditions without damaging environmental elements [28, 80]. The rapid growth of microalgae makes them even more valuable in the future, as they provide a pathway for efficient biomass production and a scalable, sustainable biodiesel industry. Recent technological advancements have significantly improved the extraction and conversion of microalgaederived lipids, developing novel techniques that optimize lipid recovery and enzymatic processes that enhance biodiesel quality [76, 80]. These innovative technologies not only increase the efficiency of biodiesel production but also improve its economic efficiency. When the cost per unit of energy produced by microalgae-based biodiesel is compared to conventional biofuels and fossil fuels, it has emerged as an emerging and competitively priced energy alternative in the marketplace [81]. Furthermore, when evaluated through life cycle analysis, The environmental impact of microalgae-based biodiesel reveals its potential to significantly reduce greenhouse gas emissions and preserve biodiversity, further solidifying its position as an eco-friendly energy source [82]. However, current productivity falls far short of meeting diesel demand, and further research and development are needed to increase the lipid content further. This includes improving microalgae strains, enhancing PBRs efficiency, and utilizing more cost-effective carbon sources as nutrient sources [83]. Policy and regulatory frameworks are critical to facilitate the adoption and expansion of microalgae-based biodiesel [84]. For example, establishing incentive policies such as incentives for the use of renewable energy and strict carbon emission standards would accelerate the development of the microalgae biodiesel industry [85]. In conclusion, microalgae-based biodiesel represents a significant development in renewable energy as a sustainable, adaptable, and environmentally friendly alternative to conventional fuels [86]. To date, as research and development continue to address the technical, economic, and regulatory challenges of biodiesel production, microalgae-based biodiesel is gaining traction as a more sustainable and environmentally friendly resource for the future and has the potential to help meet global energy needs [86, 87].

# Microalgae in wastewater treatment: advancements, challenges, and potential for biofuel production

Rapid population growth and industrialization worldwide have led to increasing discharge of wastewater containing various organic and inorganic compounds, including nitrogen and phosphorus, in many regions [13]. Traditional wastewater treatment methods have utilized a variety of process technologies to remove contaminants.

These methods include purging bleach or chlorine gas directly into the water to form disinfectant compounds that effectively remove pathogens or reverse osmosis, which removes inorganic minerals and pesticides present in wastewater [88, 89]. Moreover, these methods can significantly reduce chemical oxygen demand (COD) while converting organic pollutants into biogas by treating wastewater anaerobically [90, 91]. However, conventional wastewater treatment methods are expensive, consume large amounts of energy, and may even pollute the environment further during the process. One of the most modern approaches to wastewater treatment involves utilizing specific biomaterials to remove contaminants. Microalgae have garnered increasing attention as an energy-efficient and widely used alternative biomaterial in modern wastewater treatment applications [74]. Microalgae are a diverse group of single-celled photosynthetic organisms that can thrive in various environments, including many wastewater types. The microalgae cell wall contains a highly complex array of functional groups, which facilitates the binding of contaminants to the cell surface through the process of biosorption [92, 93]. Microalgae are emerging as an alternative to conventional wastewater treatment methods because they effectively prevent eutrophication caused by excess nutrients in wastewater by bio-absorbing nitrogen and phosphorus from wastewater and utilizing them for growth. In addition, microalgae can purify water quality by adsorbing heavy metal ions present in wastewater on their surface or accumulating them intracellularly. They can also decompose organic matter and convert it into biomass, generating oxygen in the process, which can improve water quality [94]. In other words, microalgae can significantly reduce COD levels, N, and P sources through biomass accumulation in wastewater and improve water quality restoration by adapting to different environmental conditions such as temperature changes, pH changes, and light intensity fluctuations [95]. Chlorella sp., Botryococcus sp., Senedesmus sp., Nannochloris sp., Desmodesmus sp., and Atronema sp. are the most commonly used strains for wastewater treatment [14] (Table 2). Various strains are widely utilized in wastewater treatment because of their fast growth rate, cost-efficiency, and ability to tolerate various environmental conditions such as temperature shifts, pH fluctuations, and changes in light intensity [96, 97]. A significant advantage of using microalgae in the wastewater treatment process is their ability to produce oxygen through photosynthesis. This is essential for promoting the biodegradation of carbonaceous materials by heterotrophic bacteria. Thus, applying microalgae provides a compelling biological and environmentally friendly wastewater treatment method [101].

Furthermore, as highlighted by Matin et al., the utilization of a symbiotic system of bacteria and microalgae for wastewater treatment has the benefit of not necessitating the employment of damaging technologies to remove inorganic nitrogen and phosphorus. This method consolidates the treatment process to a single stage, thus significantly decreasing its complexity and energy needed. Nevertheless, microalgae-based wastewater treatment systems still encounter significant limitations to overcome. There is a need to develop cultivation techniques that can maximize the biomass productivity of microalgae even in large volumes of wastewater. This will allow for more efficient removal of contaminants from wastewater [102–104].

A recent study proving CO<sub>2</sub> reduction through microalgae wastewater treatment reported that Chlorella and Arthronema species can effectively reduce CO<sub>2</sub> [105]. The study used 18 S rRNA and 16 S rRNA analysis to isolate eight microalgal strains from sewage treatment plant effluent. The strains with the highest lipid content and growth rate were found to be Chlorella and Arthronema species. The authors also evaluated the effects of N, P, and C sources in wastewater on the growth rates of Chlorella and Arthronema species. This study found that biomass and lipid productivity reached a maximum when the algae were cultured in a medium containing 1 mM NaNO<sub>3</sub> as a N source, 0.04 mM KH<sub>2</sub>PO<sub>4</sub> as a P source, and 50 mM NaHCO<sub>3</sub> as a C source. Furthermore, among fatty acids, the ratios of stearic acid (C18) and palmitic acid (C16) were higher, confirming that these acids are beneficial options for biofuel producers. When taken as a whole, the research findings mentioned above verify that microalgae culture is a viable way to produce biofuel, reduce  $CO_2$  emissions, and treat wastewater [105–107].

Table 2 Strains commonly used in wastewater treatment and TN, TP, COD content of wastewater

Microalgae species	TN (mgL <sup>-1</sup> )	TP (mgL <sup>-1</sup> )	COD (mgL <sup>-1</sup> )	Culture condition	Reference
Chlorella vulgaris UTEX 265	2600.0	120.0	2000.0	12 h:12 h light/dark photoperiod Allen medium	[96]
Diplosphaera sp. MM1	284.75±7.13	$77.94 \pm 3.05$	5562.64±153.78	24 h light BG-11 medium	[97]
Chlorella vulgaris	$0.93 \pm 0.01$	$6.01 \pm 0.05$		BBM-medium	[98]
Scenedesmus spp.	$662.4 \pm 39$	120±12	$153.5 \pm 9$	KEP I medium w. swine urine	[99]
Scenedesmus sp.	9.93±1.87	30.25±3.28	3000.15±28.15	16 h:8 h light/dark Basal Media	[100]

Another study demonstrated that *Chlorella vulgaris*, a eukaryotic microalgal species widespread in natural and artificial freshwater and soil environments, has promising potential for wastewater treatment [108, 109]. Ronald et al. demonstrated that *C. vulgaris* efficiently reduces the amounts of nitrogen, carbon, and phosphorus in wastewater by consuming  $CO_2$  produced by phosphoric acid bacteria in wastewater [109]. Based on these findings, wastewater may be a feasible supply of nutrients for synthesizing microalgal biomass, offering an environmentally friendly way to manage waste and produce biofuel in agriculture [109, 110].

Various technological approaches are needed to maximize the efficiency of future wastewater treatment with microalgae. First, it is necessary to introduce an optimal PBR system that can uniformly provide the light required for photosynthesis and efficiently control the circulation of nutrients in the wastewater to promote microalgae growth. In addition, genetic engineering approaches can also be considered to enhance the wastewater treatment capabilities of microalgae. Genetic modifications that improve the nitrogen and phosphorus uptake or heavy metal adsorption capacity of certain microalgae species can maximize wastewater treatment efficiency. These technological approaches could also play a role in largescale commercialization. Finally, combining microalgae and microorganisms through mixed culture systems is another practical approach to wastewater treatment. Microorganisms break down organic matter, and microalgae utilize the byproducts to purify wastewater, forming a complementary system that can further increase the efficiency of wastewater treatment.

Thus, using microalgae in wastewater treatment is the most environmentally friendly and promising approach to solve the problems of toxic sludge, high operating costs, and waste by-products that are problematic in other wastewater treatment processes [111]. As the water demand is expected to increase by about 80% from 2000 to 2050, using microalgae-based wastewater treatment technology that converts pollutants into valuable biomass and biofuel is expected to increase further [112].

# Potential and utilization plan of microalgae to replace conventional food

According to United Nations (2017) estimates, the world's population is expected to reach 9.7 billion by 2050, suggesting that the existing food system will face significant challenges in meeting the growing demand for food, especially protein [113]. The depletion of arable land and overfishing of the oceans exacerbate the shortage of food resources [114]. This emphasizes the urgent need for sustainable solutions to accommodate population growth without degrading natural resources, as highlighted by Draaisma et al. [115]. Due to their many desirable qualities for large-scale, sustainable production, microalgae have emerged as a prospective food resource in response to these difficulties [116] (Table 3). Microalgae can be grown on non-arable land with non-potable water sources such as saline water and show significant biomass productivity per unit area [127]. Even though algae

Table 3 Microalgae used in food production and the active substances they produce

Microalgae species	Active Substances	Applications	Characteristics of Substance	Refer-
				ence
Chlorella sorokiniana	Lutein, astaxanthin chlorophyl II, proteins, lipids	Nutritional Supplements	High lutein contents, poly unsaturated fatty acid (PUFA),	[117]
Chlorella vulgaris	β-carotene, minerals, vitamin, pro- teins and polysaccharides	Nutritional Supplements	High lutein contents, Antioxidant, alleviation of hyperlipidemia and hyperglycemia	[118]
Spirulina platensis	β-carotene, protein, lipids, iron, calcium	Nutritional Supplements	High protein contents, healthy alternatives such as vegetarian foods	[119]
Haematococcus pluvialis	Astaxanthin, lipids, proteins and carbohydrates	Nutraceuticals, Aquacul- ture and Broiler Industry	High astaxanthin contents, higher lipid content under nutrient depletion, antioxidants, anti- inflammatory and anti-aging properties	[120]
Muriellopsis sp.	Lipids, vitamins, toxins, enzymes	Food Supplements, nutraceuticals	High lutein concentrations, PUFAs,	[121]
lsochrysis galbana	Lipids, chlorophyll, carotenoids	Food Supplements, nutraceuticals	Hight docosahexaenoic acid (DHA) contents	[122]
Spirulina	Amino acids, lipids, carotenoids, proteins	Animal feed, livestock supplements, food supplements	Contain all essential amino acids, Enhancing animal growth and fertility	[123]
Fistulifera solaris	Lipids, protein, vitamin, mineral	Nutraceuticals	High-eicosapentaenoic acid (EPA) contents. high- est productivity of PUFA in microalgae.	[124]
Phaeodactylum tricornutum	Lipids, protein, nucleic acids, chlorophyll	Feedstock/oil	High oil content, high-EPA content	[125]
Crypthecodinium cohnii,	Lipids, proteins and carbohydrate	Food Supplements	High content of EPA and DHA	[126]

have been used for thousands of years, several obstacles have prevented them from becoming essential crops in agriculture. Significant productivity challenges include the need for specialized cultivation systems, scalability issues, and optimization of growing conditions to achieve high yields [116, 128]. Furthermore, algae's unique flavor and texture distinguish it from conventional crops, and a general lack of awareness or misunderstanding of its nutritional benefits creates social acceptance issues [129]. Recently, however, various microalgae species, including C. vulgaris, Auxenocholella protothecoides, Dunaliella bardawil, Chlamydomonas reinhardtii, Euglena gracilis, and Arthrospira platensis, have received FDA approval for consumption as edible algae worldwide. Additionally, extensive research is underway to utilize microalgae as an alternative food source [130].

Recent studies have shown that many microalgae species, such as Arthrospira sp. (71%), Synechococcus sp. (63%), Aphanizomenon flosaquae (62%), Galdieria sulphuraria (62%), Chlorella sp. (58%), Dunaliella salina (57%), and others, contain excellent protein content, which is significantly higher than the levels found in animal sources such as eggs and milk and plant sources such as rice and soybeans [131, 132]. Microalgae are high in lipids, including essential fatty acids like linoleic and a-linolenic acid, which are known to improve human health, in addition to protein [133]. Notably, some microalga species, notably Euglena gracilis, are rich in omega-3 fatty acids, including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are known for their beneficial properties in the body, such as the brain and eyes. The most common way humans get DHA and EPA is by consuming omega-3-rich fish from the ocean. However, recent concerns about consuming omega-3 fatty acids directly from fish due to water pollution have led to microalgae being proposed as a new sustainable alternative. Because microalgae-derived omega-3s are produced while removing CO<sub>2</sub>, they address environmental concerns and can serve as a cleaner and safer source of essential fatty acids without the contaminants commonly found in fish [134, 135]. Tanaka et al. observed that Fistulifera solaris, a marine microalga, is recognized for its noteworthy production of EPA. In particular, the authors found that higher nitrogen concentrations in the culture medium increase the biomass productivity of *F*. solaris. This suggests that higher nitrogen concentrations enhance the production of polyunsaturated fatty acids (PUFAs), especially EPA, and allow EPA content to accumulate to levels that can make up as much as 35% of total fatty acids. Furthermore, Crypthecodinium cohnii has a high DHA concentration, making up as much as 65% of the total PUFA in its lipid profile [124].

Strong carotenoid antioxidants like lutein and zeaxanthin are essential for maintaining eye health because they guard against oxidative stress and age-related macular degeneration (AMD). Carotenoids significantly function in light absorption, energy release, and photosynthetic apparatus protection. They also reduce inflammatory reactions and provide antioxidant protection against diseases like early aging and some types of cancer. Zeaxanthin and lutein, which are both present in the retina and lens, protect the evesight through their antioxidant properties [136]. Degenerative disorders including Alzheimer's disease (AD) and AMD may therefore be prevented or lessened by properly incorporating these substances into the diet [137]. Green leafy vegetables and fruits are the main sources of lutein. However, obtaining active ingredients such as lutein from flower petals poses significant challenges due to the nature of the plant, which only thrives during certain seasons. Currently, with the increasing demand for lutein, the utilization of microalgae has gained attention as a viable and practical alternative to meet the demand for this bioactive compound [138]. Several genera of microalgae, such as Tetraselmis, Scenedesmus, Chlorella, Parachlorella, and Cocco*myxa*, have been the subject of in-depth research to date regarding the possibilities of lutein synthesis [139]. Ren et al. found that microalgal species exhibit different variations in lutein production depending on growth medium components, such as adding sodium acetate to the culture medium enhanced lutein concentration of Chlorella sp [140]. A recent study also reported that regulating the light intensity and CO<sub>2</sub> supply in Parachlorella sp. culture could increase lutein production [141].

In addition to lutein, zeaxanthin, a component of the macular membrane that supports vision and is known for its anti-inflammatory and antioxidant effects, is a primary active substance that contributes to the health of the eyes and skin [142]. A previous study found that the amount of zeaxanthin produced by *Chlorella ellipsoidea* was nine times higher than that of orange peppers, which are also high in zeaxanthin [143].

Astaxanthin, a secondary carotenoid pigment along with zeaxanthin, is highly valued worldwide due to its superior antioxidant capacity compared to other carotenoids [4]. Astaxanthin has been found to play an essential role in preventing diabetes by reducing oxidative stress and the toxicity of glucose in the blood, protecting pancreatic beta cells, and restoring lymphocyte function to reverse insulin resistance [4, 144]. In addition, in patients already suffering from diabetes, astaxanthin can strongly inhibit the development of various diabetesrelated complications by significantly lowering oxidative stress. Astaxanthin has also been found to prevent high blood pressure, boost the immune system, and improve ulcer symptoms. Astaxanthin can also prevent bacterial inflammation, provides preventive and therapeutic benefits for fatty liver disease, exhibits anti-cancer properties, and protects against stroke and Alzheimer's disease [145, 146]. In addition, the compound has been shown to alleviate menopausal symptoms in women, as well as improve male infertility and sexual function [4, 145].

In conclusion, microalgae can be incorporated into functional foods such as food supplements and protein bars because they can provide high-quality nutrients such as plant-based proteins, omega-3, zeaxanthin, and astaxanthin and help promote health. Various microalgae-based protein bars, smoothie powders, and other products are already available on the market, contributing to meeting nutritional needs. In addition, microalgae biomass with high protein content, such as Spirulina and Chlorella, can be used as a direct meat or dairy substitute, making them suitable as a protein source for vegans and vegetarians [147]. An additional benefit is that microalgae require less water and land than conventional meat and grain production, which can reduce natural resource consumption and reduce GHG emissions [148]. For example, microalgae can be efficiently produced in hydroponic or photobioreactor systems, which can also be used as a circular economy model to treat industrial wastewater while simultaneously cultivating microalgae for food. This minimizes the environmental impact of the food production process and promotes the recycling of resources.

# Microalgae in sustainable waste management: transforming food waste into valuable resources

Microalgae not only play an important role in food production but also contribute significantly to the decomposition and management of food waste, including uneaten food and residues from cooking activities. The astonishing quantity of food residues generated annually, which vary in composition depending on where they originate, can account for as much as 50% of the total volume of waste. This comprises solid garbage, household waste, municipal waste, and wastewater from various industries [149–151]. One potential game-changer for this problem is the biorefinery strategy, which involves cultivating microalgae using food waste as a source of nutrients. This sustainable technology, which utilizes pre-treated wastewater from the food industry for microalgae production, will make a substantial positive difference in both the energy and nutraceutical industries [152, 153]. In particular, recent studies have used banana peel hydrolysate as a culture medium and purified the substances produced by C. sorokiniana through appropriate pretreatment and hydrolysis processes. It showed that C. sorokiniana grown in media containing banana peel waste produced higher lipids than the control group [149, 154]. This shows how organic banana peel waste may be used as an affordable and environmentally beneficial substrate for microalgae growth. Additionally, cultured on vegetable waste culture medium, Scenedesmus sp. and Asterarcys sp. showed better growth rates and lipid production than the control group [155] (Table 4). In conclusion, since food waste can be effectively removed by utilizing it as a growth medium for microalgae, the utilization of microalgae in food waste treatment systems could significantly impact on the environmental energy and nutraceutical industries [149, 155–161].

### Hydrogen production using microalgae

Harnessing microalgae for biohydrogen production offers a renewable and eco-friendly approach to energy generation [162]. The integration of both fermentation and photolysis processes enables the exploitation of the inherent physiological capabilities of microalgae, producing hydrogen through diverse mechanisms (Table 5).

#### Direct photolysis: harnessing sunlight

Direct photolysis relies on the natural photosynthetic processes of microalgae. Utilizing sunlight, microalgae catalyze the breakdown of water molecules in photosystem II (PSII), leading to the liberation of oxygen and

 Table 4
 Microalgae for food waste disposal and associated food waste types

Microalgae species	Food waste type	Lipid content	<b>Biomass Production</b>	Reference
Chlorella sorokiniana	Banana peel	22.83%	1.72gL <sup>-1</sup>	[153]
Scenedesmus sp.	Vegetable waste	231.8±17.9 <b>—</b> 243.5±25 mgL <sup>-1</sup>	$956 \pm 70 - 964 \pm 70 $ mgL <sup>-1</sup>	[155]
Chlorella vulgaris UTEX 265	Brewery waste	18%	3.5gL <sup>-1</sup>	[156]
Chlamydomonas reinhardtii	Starch waste water	_	1.45gL <sup>-1</sup>	[157]
Rhodotorula glutinis	Fruit and vegetable waste	64.9 ± 17.4 <del>—</del> 176.5 ± 49.5 mg/g	$4.15 \pm 0.13$ — $4.63 \pm 0.67$ gL <sup>-1</sup>	[158]
Scenedesmus. quadricauda	Fruit and vegetable waste	_	506±1.75 <b>—</b> 515±1.04mgL-1	[159]
Monoraphidium contortum	Fruit and vegetable waste	2.41 — 2.42 g kg <sup>-1</sup>	$248 \pm 1.36 - 291 \pm 0.85 mg L^{-1}$	[159]
Lagerheimia longiseta	Fruit and vegetable waste	_	380±0.90 <b>—</b> 430±1.26 mgL <sup>-1</sup>	[159]
Chlorella vulgaris	Bakery and food wastes	200 mg/g	0.9 g/g of glucose	[160]
Nannochloropsis sp.	Palm oil mill effluent	_	1.268gL <sup>-1</sup>	[161]

Table 5	Strategies 1	for en	hancing	hvc	Iroaen	proc	luction	in microa	laae: a	Summarv
			· · J						J	

Method	Description	Benefits	Reference
Direct Photolysis	Sunlight-driven water molecule breakdown by microalgae's PSII, producing hydrogen and oxygen, facilitated by chloroplast hydrogenases	<ul> <li>Achieves up to 98% purity hydrogen in certain strains.</li> <li>Direct conversion of solar energy to hydrogen.</li> </ul>	[5, 164]
Indirect Pho- tolysis (Two-Step Method)	(1) Sunlight converts CO <sub>2</sub> to organic compounds for energy stor- age; (2) Stored compounds generate hydrogen in an anaerobic, dark environment, avoiding oxygen inhibition of hydrogenase	<ul> <li>Circumvents oxygen inhibition of hydrogenase.</li> <li>Utilizes stored organic compounds for hydrogen production.</li> </ul>	[5, 165]
Co-Cultivation with Specific Bacteria	Oxygen-consuming bacteria reduce oxygen levels in microal- gal cultures, preventing hydrogenase inhibition and boosting hydrogen productivity	<ul> <li>Enhances hydrogen production by reducing oxygen levels.</li> <li>Leverages microbial interactions for bioenergy applications.</li> </ul>	[5, 166]
Ca <sup>2+</sup> Injection	Enhances microalgae's photosynthesis and protects against ROS by adding Ca <sup>2+</sup> to the medium, facilitating better hydrogen production	- Safeguards PSII activity. - Promotes substrate assimilation and hydrogen production.	[5, 38, 167]
Genetic Engineering of Microalgae	Modifies microalgae genetically to improve hydrogenase activity and photosynthetic efficiency, increasing hydrogen production capabilities.	<ul> <li>Opens new avenues for optimizing biohydro- gen production with higher productivities and efficiency.</li> </ul>	[5, 168]

protons  $(2H_2O+\text{light energy}\rightarrow 2H_2+O_2)$  [5]. These protons, along with electrons driven through the electron transport chain to photosystem I (PSI), are transformed into hydrogen gas through the activity of hydrogenase enzymes. Certain strains of green microalgae and cyanobacteria, where chloroplast hydrogenases catalyze the production of hydrogen, can achieve hydrogen production with up to 98% purity [5, 163].

#### Indirect photolysis: the two-step anaerobic method

Indirect photolysis is a unique technique for producing hydrogen from microalgae that work under particular circumstances to overcome oxygen's negative effects on vital enzymes like nitrogenase and hydrogenase [168]. The indirect photolysis process can be broadly divided into two types [169].

**Phase one: light-driven carbon fixation** In the first stage of indirect photolysis,  $CO_2$  is photosynthetically fixed into useful organic compounds by utilizing the power of sunlight. In this stage, photosynthesis occurs in microalgae, where light energy is used to change  $CO_2$  and water into oxygen, carbohydrates, and lipids. This process builds up energy-rich organic compounds inside the cell's structure to support microalgal growth and provide the framework for the next stage of hydrogen production [5, 168, 169].

**Phase two: anaerobic hydrogen generation** The second phase begins in the absence of light after the buildup of organic molecules, resulting in an anaerobic environment that is favorable for the generation of hydrogen. This change to dark conditions is essential to avoid oxygen synthesis through photosynthetic processes, which could limit the action of the enzyme hydrogenase, which produces hydrogen [169, 170]. The organic substances that were previously stored become substrates for hydrogen creation in these oxygen-depleted environments. Hydrogenase enzymes seize electrons that are liberated during the metabolic breakdown of these internal carbon sources, facilitating the conversion of protons into hydrogen gas [5, 169, 170]. Strategies like nutrient starvation are used to guarantee an anaerobic environment. This entails reducing or eliminating all necessary nutrients like sulfur from the microalgae growth medium. As a result, the rate of photosynthesis is effectively lowered, which affects oxygen production. Consequently, the overall oxygen level in the culture decreases, shifting the reaction toward respiration, which produces oxygen rather than photosynthesis. This results in a net oxygen consumption, which keeps the environment anaerobic and encourages hydrogenase activity. The indirect photolysis process characterizes a complex balance between dark, anaerobic hydrogen generation and light-driven carbon fixation. The deliberate division of these phases makes the effective use of microalgae's photosynthetic capabilities possible, solving the problem of the oxygen sensitivity of the enzymes that produce hydrogen [5, 163, 169, 170].

# Enhancements in microalgal hydrogen production: exploring innovative strategies

The ongoing search for sustainable energy solutions has propelled microalgal hydrogen production to the forefront of bioenergy research. Particularly, there has been a concerted effort to overcome the natural and technical barriers of biohydrogen production, thus significantly enhancing efficiency and productivity. First, a method was recently developed to induce hydrogen production from microalgae by managing oxygen concentration through co-cultivation technology. This innovative approach targets the oxygen sensitivity of hydrogenase enzymes—a key challenge in biohydrogen production. By introducing oxygen-consuming bacteria, the microalgal culture environment maintains a lower oxygen concentration, thus mitigating the inhibition of hydrogenase activity and increasing hydrogen productivity. This symbiotic relationship enhances hydrogen production and illustrates the potential of leveraging microbial interactions for bioenergy applications [165].

 $Ca^{2+}$  injection has been found to be an effective way to boost photosynthetic efficiency and promote hydrogen production. Deliberately adding  $Ca^{2+}$  ions to the microalgal growing medium is thus another significant development. Research has indicated that  $Ca^{2+}$  can exert a noteworthy influence on the photosynthetic machinery of microalgae, hence safeguarding PSII activity from damage caused by reactive oxygen species. Together with encouraging substrate absorption, this protective effect speeds up the production of hydrogen. Specifically, the availability of micronutrients and the generation of biohydrogen are intricately linked, with  $Ca^{2+}$  promoting both direct and indirect photolysis pathways [38, 166].

Using genetic engineering to modify microalgae strains for the production of hydrogen is another technique. One area of biohydrogen research that is still unexplored is the genetic alteration of microalgae, such as Chlamydomonas reinhardtii. Targeting particular genes linked to hydrogenase activity and photosynthetic efficiency, scientists have been able to improve the hydrogen-producing capacity of microalgae through precise genetic engineering. The purpose of these alterations is to either raise the expression of native hydrogenase enzymes or provide new routes for hydrogen synthesis in order to increase the inherent ability of microalgae to make hydrogen. Investigating genetically modified microalgae provides fresh opportunities to maximize the generation of biohydrogen, perhaps resulting in increased productivity and efficiency [5, 166, 167, 170].

Co-cultivation techniques, nutrient supplementation strategies, and genetic engineering are all being incorporated to generate significant advancements in the field of microalgal biohydrogen generation. Likewise, by highlighting microalgae's potential as a sustainable source of biohydrogen, these developments also set the stage for improving production methods. Achieving effective, scalable, and sustainable biohydrogen production systems shortly appears possible with the continued investigation of novel approaches and their practical application.

### Conclusions

Microalgae-based cultivation systems are gaining attention as a sustainable solution to various environmental problems. To address these issues, industrial-scale cultivation must be possible, and to this end, many researchers are focusing on developing cultivation process systems. In particular, technological innovation to increase economic efficiency is a critical factor for commercial production, and it is essential to establish cultivation systems that can reduce initial investment and maintenance costs. For large-scale microalgae cultivation, energy consumption for light and temperature control is necessary, so utilizing renewable energy sources or solar-based natural lighting systems may be desirable. In addition, pollution control and interspecies competition are significant factors that can reduce productivity in large-scale cultivation systems, requiring sophisticated monitoring and control technologies to address them. Finally, genetic engineering approaches to improve the characteristics of microalgae to increase productivity could play an essential role in commercial utilization.

Microalgae's versatility allows it to play an essential role as a solution to some of today's most pressing environmental and energy challenges. First, microalgae are a promising biological resource for energy yield, costeffectiveness, and sustainability for biodiesel and hydrogen production. Their high growth rate and lipid content make them efficient for biodiesel production, and their ability to produce hydrogen during photosynthesis makes them a promising future energy resource. Economic efficiency can be further enhanced by utilizing integrated biorefinery systems that use the remaining biomass after lipid extraction, which has the highest content in the biomass for hydrogen or value-added materials. Second, microalgae can grow by absorbing a variety of nutrients, including nitrogen and phosphorus, present in wastewater, replacing the significant requirements of clean water and reagents for cultivation. Third, microalgae have gained considerable attention in the food and pharmaceutical industries because of their high protein content, which can be utilized as a next-generation food source, and their ability to produce healthful substances such as lutein, zeaxanthin, and astaxanthin. Lastly, it can grow effectively by utilizing various substances in food waste as nutrients, which can curb environmental pollution caused by the excessive use of chemicals in large-scale microalgae cultivation.

This review provides a comprehensive overview of the economic and environmental benefits of microalgae applications and contributes to exploring the commercial viability of microalgae in various industries. It also discusses the latest technological developments that need to be addressed for the optimization and largescale commercialization of microalgae culture systems, thus providing an important new direction for microalgae research. However, there are still several unresolved research gaps and limitations First, large-scale microalgae production and use involves several ethical and social implications. Microalgae are being touted as a renewable resource that can contribute to GHGs, but the environmental impact of the water and energy required for large-scale cultivation must be considered. Furthermore, when utilized as a food resource, microalgae can create resource competition with conventional agriculture,

requiring careful assessment of the food security of local communities. Social inequalities may arise if technology and economic benefits are concentrated in specific regions or companies. The risk of ecosystem disruption due to off-site spillage of microalgae is also essential to consider. Second, the issues of contamination control and interspecies competition still need to be addressed, and further research is needed on how to suppress contamination and competing microorganisms that occur during microalgae cultivation. Finally, there is also the social acceptability issue of using genetically modified microalgae, which requires scientific information and transparent discussion. In the future, if advances in technology for large-scale outdoor cultivation, renewable energy sources, and genetic engineering approaches are successfully combined, microalgae will become commercially viable and affordable, facilitating their expansion into various industrial sectors.

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#### Author contributions

S. P., B. Y., J. L., and K. H. wrote and revised the main manuscript. S. P. and B. Y. provided and analyzed literature. B. Y. and K. H. conceived and designed the manuscript. S. P. and B. Y. revised the manuscript for revision. All authors read and prepared for the final submission.

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#### Data availability

No datasets were generated or analysed during the current study.

#### Declarations

**Ethics approval and consent to participate** Not applicable.

#### Consent for publication

Not applicable.

#### Conflicts of interest

There are no conflicts of interest to declare.

#### **Competing interests**

The authors declare no competing interests.

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