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Review

Optimization and Process Effect for Microalgae Carbon Dioxide Fixation Technology Applications Based on Carbon Capture: A Comprehensive Review

Gang Li [1], Wenbo Xiao [1], Tenglun Yang [1] and Tao Lyu [2,*]

[1] School of Artificial Intelligence, Beijing Technology and Business University, Beijing 100048, China; ligang@btbu.edu.cn (G.L.); crisp1110@163.com (W.X.); ytl1010@163.com (T.Y.)
[2] School of Water, Energy and Environment, Cranfield University, College Road, Cranfield, Bedfordshire MK43 0AL, UK
* Correspondence: t.lyu@cranfield.ac.uk

Abstract: Microalgae carbon dioxide (CO_2) fixation technology is among the effective ways of environmental protection and resource utilization, which can be combined with treatment of wastewater and flue gas, preparation of biofuels and other technologies, with high economic benefits. However, in industrial application, microalgae still have problems such as poor photosynthetic efficiency, high input cost and large capital investment. The technology of microalgae energy development and resource utilization needs to be further studied. Therefore, this work reviewed the mechanism of CO_2 fixation in microalgae. Improving the carbon sequestration capacity of microalgae by adjusting the parameters of their growth conditions (e.g., light, temperature, pH, nutrient elements, and CO_2 concentration) was briefly discussed. The strategies of random mutagenesis, adaptive laboratory evolution and genetic engineering were evaluated to screen microalgae with a high growth rate, strong tolerance, high CO_2 fixation efficiency and biomass. In addition, in order to better realize the industrialization of microalgae CO_2 fixation technology, the feasibility of combining flue gas and wastewater treatment and utilizing high-value-added products was analyzed. Considering the current challenges of microalgae CO_2 fixation technology, the application of microalgae CO_2 fixation technology in the above aspects is expected to establish a more optimized mechanism of microalgae carbon sequestration in the future. At the same time, it provides a solid foundation and a favorable basis for fully implementing sustainable development, steadily promoting the carbon peak and carbon neutrality, and realizing clean, green, low-carbon and efficient utilization of energy.

Keywords: microalgae technology; carbon dioxide fixation; economic benefit; emission reduction; mechanism

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1. Introduction

With the abundant use of fossil fuels, the excessive emission of greenhouse gases, especially carbon dioxide (CO_2), has brought serious ecological damage and climate change to human society, and global warming has become a major problem to be solved urgently [1–3]. The impact of greenhouse gas accumulation is long term, with the average global temperature expected to rise by 2 °C by 2100 and 4.2 °C by 2400, which will cause great economic losses and be a global threat to food and nutrition security [4]. In China, the average crop yield loss is 2.58% with a temperature increase of 1 °C [5]. The CO_2 concentration increased to 2.46 ± 0.26 ppm/y in October 2021. If no measure is taken to control its development at the earliest convenience, the CO_2 concentration may reach 667 ppm in 2100 [6]. Therefore, global efforts are being made to mitigate climate change through reducing the CO_2 emission and/or enhancing carbon sequestration, which are crucial to long-term sustainable human development goals [7]. Reducing the use of fossil fuels is the straightforward way to reduce

CO_2 emissions. Further, a strategy of carbon capture, re-utilization, and storage to reduce CO_2 emissions is the most common technology for the moment [8].

Carbon capture and storage can meet the expanding worldwide energy demand by converting the captured CO_2 into fuels or using it to enhance the recovery of oil while dramatically lowering CO_2 emissions [9,10]. At present, common methods of CO_2 fixation include physical, chemical, and biological fixation methods [11]. For instance, the physical method is to inject CO_2 into the deep sea or geological layer to temporarily bury it, which cannot ensure that CO_2 will never escape due to the constraints of the geological environment, space, and cost [12]. The chemical method uses an adsorption material to directly fix or add alkaline neutralization reagent in the form of carbonate or bicarbonate to fix CO_2, which is relatively safe and permanent, but has the disadvantages of large reagent dosage and high cost [13,14]. The biological fixation method utilizes the photosynthesis of green plants to convert CO_2 into organic matter in the plant, provide energy sources, and maintain the C–O balance of the atmosphere. Of these, the biological approach has been deemed the most economically feasible, environmentally friendly, and sustainable method to capture and store carbon. Plants, microalgae, etc., are commonly used for biological carbon sequestration. In a general way, plants reduce carbon emission by only approximately 3–6%; but when grown under ideal conditions, microalgae such as cyanobacteria and green algae could be 50-fold more effective [15].

Yahya et al. studied the potential of microalgae as biological carbon fixers to support the development of a circular economy and environmentally sustainable coal-fired power plants [16]. By optimizing the culture parameter settings, Dasan et al. accelerated the CO_2 fixation efficiency of *Chlorella vulgaris* [17]. Premaratne et al. evaluated the CO_2 storage potential of *Desmodesmus* sp. in flue gas under nitrogen element limitation conditions, and applied the resultant biomass to biofuel preparation [18]. Ding et al. found that using native microalgae species to reduce effluents in palm oil mill and industrial discharge of CO_2 is an effective strategy [19]. To sum up, microalgae are a superior candidate for carbon capture and storage, which can also be utilized in biofuel production, wastewater treatment and other industries.

Microalgae with a simple structure, a short growth cycle, wide distribution, strong adaptability, and higher photosynthetic efficiency than conventional crops are mainly composed of eukaryotic and prokaryotic microalgae [20]. Microalgae directly use solar energy to fix CO_2 and produce O_2 and secondary metabolites, which is a typical representative of carbon sequestration organisms [11]. This process is also known as photosynthesis, including the primary reaction, electron transfer, photophosphorylation, and carbon assimilation (i.e., CO_2 fixation and formation of sugars). Because all crop biomasses obtain carbon from it, as shown in Figure 1, the Calvin–Benson cycle (C_3 cycle) plays a major role in the CO_2 fixation pathway in nature. Although land plants can also absorb CO_2 and produce organic matter through photosynthesis, microalgae can be planted in seawater or wastewater, can capture CO_2 from various sources, do not compete with traditional agriculture for soil resources, and achieve CO_2 fixation 10–50 fold more efficiently [21]. Every 1 g of biomass produced by microalgae can fix 1.83 g CO_2 [22]. Microalgae biomass with a high application value, which includes lipids, proteins, and polysaccharides, can be used as raw materials for biofuel, biochemical, food, medicine, and other industries. The species and environmental conditions of microalgae affect the photosynthesis and carbon sequestration efficiency of microalgae, which is beneficial to further effective utilization of microalgae [23].

In recent years, more and more reviews have focused on the influence factors and applications of microalgae CO_2 fixation technology, but there are few studies on the comprehensive analysis and discussion of microalgae based on the mechanism of carbon sequestration. Raeesossadati et al, mainly discussed the effect of important factors such as CO_2 concentration, photobioreactors, temperature, and light intensity on microalgae CO_2 fixation [24]. You et al. reviewed the application of wastewater treatment by microalgae [25]. By reason of the foregoing, based on previous studies, the optimization prospects

and process effect of microalgae CO_2 fixation technology are comprehensively reviewed as shown in Figure 2. Firstly, the mechanism of CO_2 fixation in microalgae is reviewed, and the main factors affecting carbon sequestration and growth are analyzed. Then, how to use strategies of random mutagenesis, adaptive laboratory evolution (ALE), genetic engineering to screen microalgae species that can improve the photosynthetic efficiency and the production of by-products is discussed. Moreover, combined with the current challenges faced by microalgae carbon sequestration technology, the application of microalgae carbon sequestration technology in CO_2 emission reduction, wastewater and flue gas treatment are analyzed. In the future, it is expected to establish a more optimized mechanism of microalgae carbon sequestration, which provides the reference for large-scale industrial application of CO_2 fixation in microalgae and the environmentally friendly economy in China.

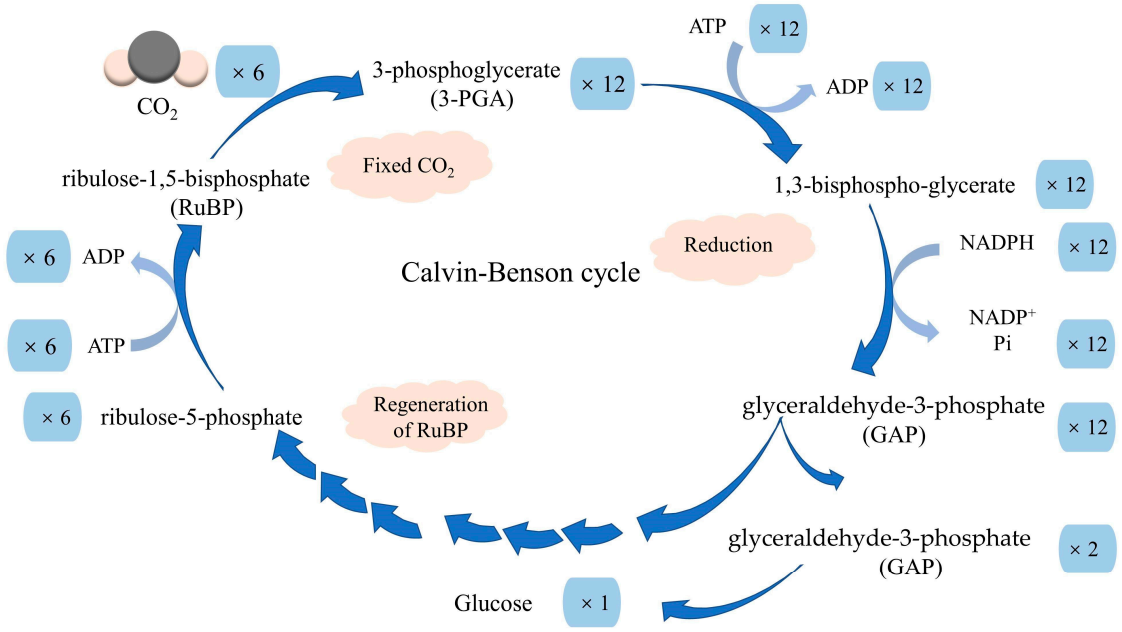


Figure 1. C_3 cycle.

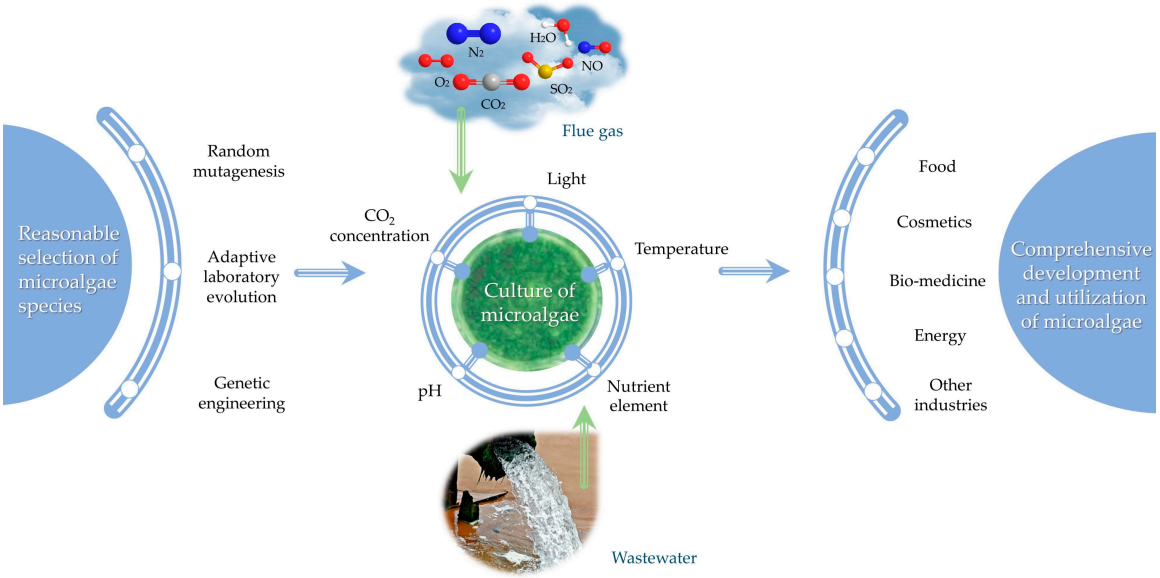


Figure 2. The technical flow chart of microalgae carbon sequestration.

2. Mechanism of Carbon Dioxide Fixation Technology by Microalgae

2.1. The Photosynthetic Carbon Metabolism Pathway

In essence, the photosynthesis of microalgae is a mechanism that allows them to utilize solar energy to exchange materials and energy with their surroundings and turn CO_2 into glucose [26]. It can be expressed by Equation (1):

$$CO_2 + H_2O \rightarrow (CH_2O)_n + O_2 \quad (1)$$

Photosynthesis is also divided into the photoreaction stage of converting the light energy into the active chemical energy and the dark reaction of converting the active chemical energy into the stable chemical energy. The dark reaction requires the strong reductant (NADPH) and the energy (ATP) generated by the light reaction to immobilize and reduce the CO_2 to sugar, as shown in Equations (2) and (3). The light energy is converted into chemical energy to resolve NADPH into $NADP^+$ through the photosystem of PSII and PSI. The pH gradient was produced on the thylakoid membrane and was applied to synthesize ATP [27].

$$H_2O + ADP + P_i + NADP^+ \overset{\text{light}}{\rightarrow} O_2 + ATP + NADPH + H^+ \quad (2)$$

$$CO_2 + ATP + NADPH + H^+ \rightarrow (CH_2O) + ADP + P_i + NADP^+ \quad (3)$$

Photosynthetic carbon metabolism in microalgae is mainly dependent on the C_3 cycle [28]. By employing ATP as an energy source, lowering the energy level, depleting NADPH, carbon enters the C_3 cycle as CO_2 and exits as sugar. This is what we see in Equation (4)—the C_3 cycle is simplified to carboxylation, reduction and regeneration of ribulose-1,5-bisphosphate (RuBP). RuBP entering the carboxysome is bound to CO_2 by the ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) enzyme, which subsequently converts to a 2 molecules 3-phosphoglycerate (3-PGA) and diffuses from the carboxysome to the cytoplasm through the pores of the hexamer shell protein. Equation (5) shows the reduction phase, where 3-PGA in the cytoplasm forms glyceraldehyde-3-phosphate (GAP) catalyzed by the enzyme. The regeneration of RuBP ensures the continuous operation of the carbon sequestration cycle.

$$3RuBP + 3CO_2 \rightarrow GAP + 3RuBP \quad (4)$$

$$PGA + ATP + NADPH + H^+ \rightarrow GAP + ADP + NADP^+ + P_i \quad (5)$$

The rubisco has the dual functions of oxygenation and carboxylation, specifically which the choice of function is influenced by CO_2 and O_2 concentrations. Due to the relatively high concentration of O_2 in the atmosphere, it is conducive to the work of oxygenase and promotes photorespiration, which leads to the reduction in photosynthesis production. Therefore, microalgae have developed CO_2-concentrating mechanism (CCM) to maximize photosynthetic efficiency under low CO_2 concentrations or inorganic carbon (Ci) conditions [29].

2.2. Carbon Dioxide Concentrating-Mechanism

When CO_2 is dissolved in fluid, it exists in four specific Ci forms: HCO_3^-, CO_3^{2-}, H_2CO_3, and dissolved CO_2. The proportion of Ci varies with pH. It's possible that different microalgae strains prefer various Ci types. For instance, *Nannochloropsis oculata can* only actively transport HCO_3^-, and *Chlamydomonas reinhardtii* be able to use simultaneously CO_2 and HCO_3^- [30]. In truth, pH 7.5–8.4 is the typical range for the culture medium used by microalgae. Approximately 90% is HCO_3^- in this culture medium, and the concentrations of CO_3^{2-} and H_2CO_3 are low [31]. In the process of acclimatizing to the concentration of Ci changes in the environment, the microalgae have formed CCM that can help to adapt to the changes in the external CO_2 concentration. CCM is based on a single cell, which can actively transport and accumulate Ci at low CO_2 levels by counteracting

the inefficiency of rubisco to boost photosynthetic efficiency. It is found in almost all eukaryotic microalgae and cyanobacteria and plays a crucial role in the fixation process of carbon [32]. In both eukaryotic and prokaryotic, the CCM of microalgae includes three main systems: (1) Ci transporter; (2) Carbonic anhydrase (CA) that is used to convert Ci to CO_2; (3) microcompartments with rubisco for the delivery of CO_2 [29]. Microalgae concentrate the Ci pumped into the cell at the photosynthetic site to form high carbon sites nearly a 1000-fold higher than the surroundings, and convert them into organic carbon through the rubisco to achieve the carbon fixation [28]. This is also regarded as the rate-limiting step of the entire cycle. Rubisco in the pyrenoids of eukaryotic algae or in the carboxysomes of cyanobacteria has a low affinity for CO_2 and requires higher concentrations of CO_2 to obtain a normal rate of reaction [33].

The simplified model of CCM is shown in Figure 3, which is mainly divided into two stages: The first stage involves obtaining Ci from the environment and delivering CO_2 and HCO_3^- to the chloroplast; in the second stage, Ci crosses the thylakoid membrane and is reduced to CO_2 by CA at higher ambient pH, which increases the concentration of CO_2 near the rubisco and ultimately enhances the photosynthetic rate [32]. HCO_3^- can enter cells through active transport, while CO_2 can enter cells through inactive diffusion in microalgae. CA regulates CO_2 and HCO_3^- to maintain proper pH in the chloroplast stroma. maintains, so CA has a noticeable effect on catalyzing CO_2 conversion [31]. In addition, the activity of rubisco, the pH of chloroplast stroma, etc., can also affect the CO_2 conversion efficiency [34]. The favored form of stacking Ci is HCO_3^- that is approximately 1000-fold less permeable to lipid membranes than the uncharged CO_2 molecules. The CO_2 absorption system can recycle or recapture leaking CO_2 in order to efficiently fix the CO_2 and prevent its escape from the cell [35].

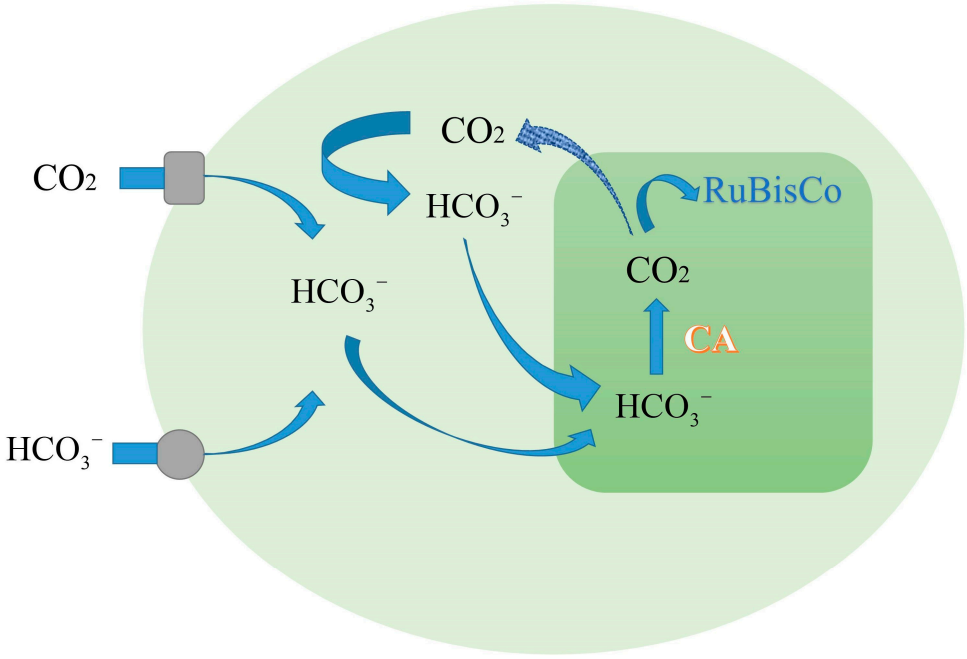


Figure 3. The simplified model of CCM.

3. Environmental Conditions for the Growth of the Microalgae

The effect of diverse environmental conditions on the growth rate of microalgae is depicted in Figure 4. The optimal range of conditions to achieve high CO_2 fixation rates and the growth efficiency also vary by microalgae species. The effects of microalgae growth conditions on carbon sequestration efficiency and biomass production are summarized below.

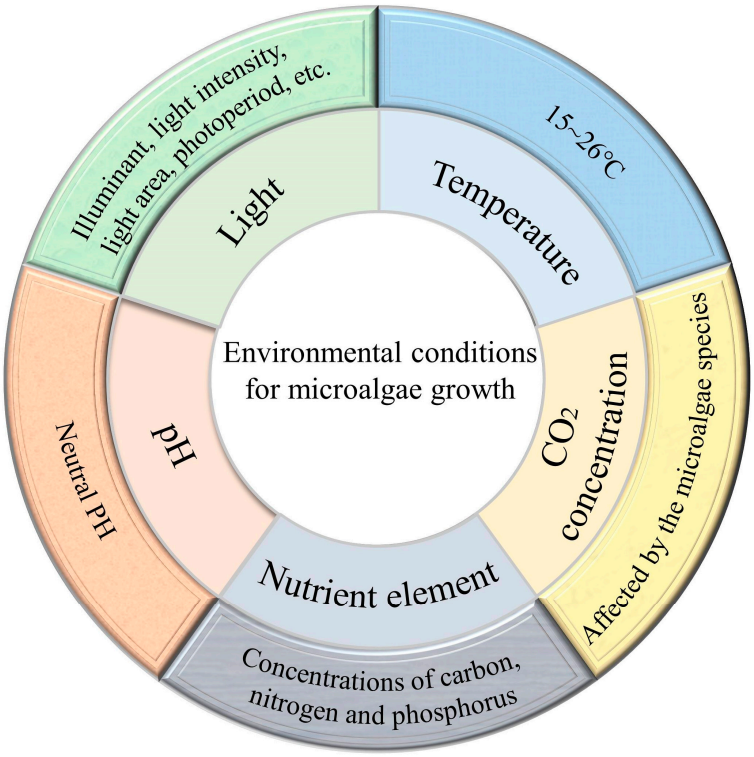


Figure 4. Different growth environment conditions affect the growth rate of microalgae.

3.1. Light

Light is the basic energy source affecting the photosynthesis dynamics of microalgae, which can affect cell growth and metabolism by controlling the light source, light intensity, interval, area, etc. [36]. Excessive light intensity may lead to photooxidation and photoinhibition, while low light levels would limit growth [37]. The majority view is that photoinhibition is the primary factor reducing algal output. Until light saturation is attained, the microalgae growth rate increases linearly with increasing light intensity at low light levels. The optimal light intensity for microalgae growth is usually 26–400 μmol photons m^{-2} s^{-1} [38]. Lipid synthesis of *tetradesmus obliquus* reached the maximum at 200 μmol m^{-2} s^{-1}, accounting for 45.31% of the dry biomass, which would decrease provided that further augmented the light intensity [39]. Additionally, according to Kim et al., microalgae production rates of *Scenedesmus* sp. was about 45% higher than at a single wavelength at 400–700 nm white light [40]. By controlling the light wavelength, photosynthesis, gene transcription, enzyme activation and cellular composition can be impacted to the point that the biomass and lipid content of microalgae are altered [41].

3.2. Temperature

Temperature is a major factor regulating the cellular, morphological, and physiological responses of microalgae, now that it impacts their photosynthesis, which leads to changes in the carbon sequestration efficiency of microalgae. Zhao et al. revealed that the most suitable temperature for the growth of the most common microalgae was between 15 and 26 °C [42]. Overall, with decreasing temperature within the appropriate range, rubisco activity reduces, the index of unsaturated fatty acid augments, the growth rate reduces, biomass is reduced, and carbon fixation efficiency also reduces significantly. Nevertheless, high temperatures frequently have irreversible damage on microalgae. Sachdeva et al. discovered that *Chlorella pyrenoidosa* M18 were able to survive at temperatures up to 47 °C, with the highest average growth rate at 37 °C. At high temperature or direct sun, this algal species had a higher lipid yield of 44.51%, compared to room temperature [43]. As shown in Table 1, the optimal growth temperature of microalgae varies by species,

and is also influenced by other environmental parameters (e.g., light intensity and CO_2 concentration) [44].

Table 1. The optimal growth temperature for different species of microalgae.

Species	Culture Medium	Optimal Growth Temperature (°C)	Average Specific Growth (d^{-1})	Ref.
Chlorella pyrenoidosa M18	BG11	37	0.70	[43]
Thermosynechococcus elongatus PKUAC-SCTE542	BG11	55	0.22	[45]
Chlorogleopsis sp.	BG11	50	0.14	[46]
Chlorella sp. MT-15	Artificial sea water	30	Approximately 0.80	[47]
Chlorella sp. MT-7	Artificial sea water	30	Approximately 0.60	
Thermosynechococcus sp. CL-1	Modified Fitzgerald	50	2.70	[48]
Nannochloropsis sp. Oculta	Modified Fitzgerald	30	1.60	

3.3. pH

By altering the activity of the cellular metabolic enzymes and the uptake and usage of ions by microalgae cells, the pH value influences the physiological metabolism of microalgae. The energy costs of HCO_3^- transfer to cytosol are decreased at pH ranges between 7.5 and 8.5, which lowers the cost of carbon fixation [49]. The depletion of Ci by microalgae cells growth leads to an increase in pH, which might alter the biochemical reaction properties of microalgae, in turn with promoting cell rupture. Changes in pH can affect CA activity, leading to affect microalgae growth. Buffers such as sodium hydroxide or calcium carbonate to adjust pH to the best level adapted to microalgae growth can further boost the fixation rate of CO_2 and biomass production [50].

Most microalgae are suitable for cultivation under neutral pH conditions [50], with exceptions, such as *Chlorococcum* could live in pH 4.0, and *Spirulina* at pH 11.0 [51]. Razzak et al. discovered that *Nannochloropsis oculata* grew well between medium pH 5.5 and 6.5 [51]. *Graesiella* sp. WBG-1 had the highest CO_2 fixation rate and lipid content at pH 8.0–9.0, which were 0.26 g/L/d and 46.28%, respectively. Although the usage rate of CO_2 increased along with the pH, the optimum pH for microalgae development did not have the highest utilization rate of CO_2 [52]. Therefore, it is essential to cultivate algae species that can grow well under high pH values in order to effectively utilize the carbon sequestration capacity of microalgae.

3.4. Nutrient Element

The building blocks of microalgae's cell synthesis include carbon, nitrogen, and phosphorus, which are also crucial nutrients for the biomass growth. To some extent, the photosynthesis of microalgae is influenced by the species, morphology, and quality of the nutrients. Carbon–nitrogen ratio (C/N) is among the important factors affecting carbon fixation efficiency, biomass accumulation and productivity of value-added components. *Spirulina platensis* had been nuclear radiated and cultured in specific cultures. When the ratio of NH_4HCO_3 and $NaNO_3$ was set at 1:4, carbon utilization efficiency of the hybrid process could reach 40.45%, But the excessively high concentration of NH_4HCO_3 will also generate toxicity for microalgae, which results in a lower biological yield [53]. Appropriately increasing the concentration of phosphorus is beneficial to microalgae development and lipid accumulation because some microalgae may utilize it to manufacture organic esters [54,55]. Microalgae cause phosphorus to convert ADP into ATP through phosphorylation, which can also precipitate phosphate through cell adsorption or regulating pH as shown in Figure 5. Because wastewater contains the above elements, growing microalgae can remove pollutants while fixing carbon, but this also faces some challenges, as detailed in a later article [30].

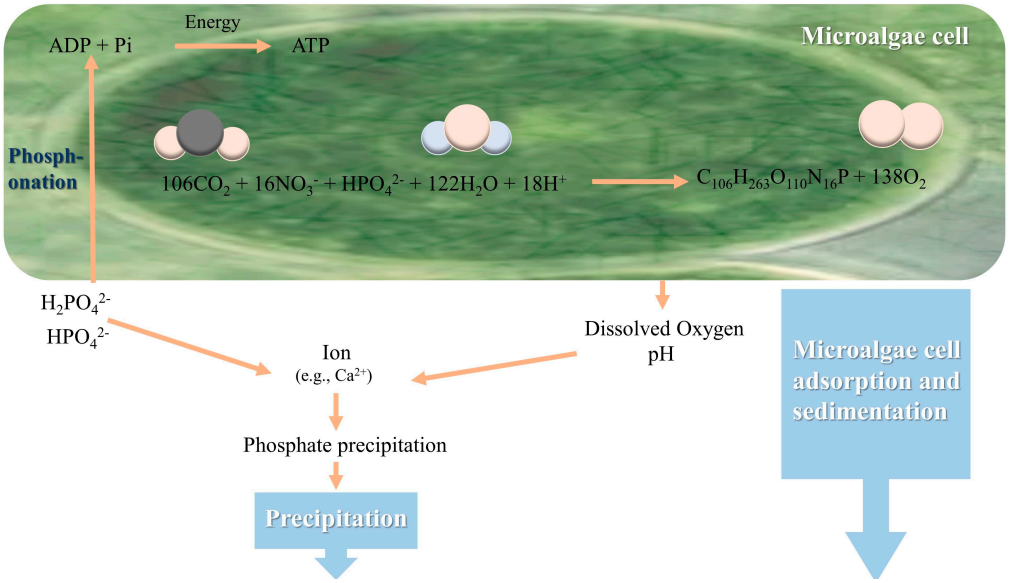


Figure 5. Mechanism of adsorption of settling phosphorus by microalgae.

3.5. Carbon Dioxide Concentration

The capability of different microalgae to tolerate CO_2 is different. Even though certain microalgae have great CO_2 tolerance and can grow across most of the CO_2 concentration range, the optimal growth concentration is determined. The statistics are shown in Table 2 below. Some microalgae can grow normally at low concentrations of CO_2, while some will only show high growth rates at high concentrations of CO_2. Either a too high or too low CO_2 concentration reduces the CO_2 fixation efficiency and the biomass yield. To some degree, increasing the CO_2 concentration can enhance the carboxylation activity of the rubisco, suppress its oxidative activity, and accelerate photosynthesis [56]. However, too high a CO_2 concentration will lower pH, which will reduce the activity of CA and impede cell growth [57].

Table 2. Growth rates of different species of microalgae at different CO_2 concentrations.

Species	CO_2 (%)	Culture Medium	Aeration Rate (vvm)	CO_2 Fixation Rate (mg/L/d)	Biomass Yield (g/L)	Biomass Productivity (mg/L/d)	Ref.
Chlorella vulgaris	2% constant	Liquid medium of 3N-BBM+V	0.4	4110	2.59	3530	[58]
	4% provided intermittently			4500	2.62	3410	
Spirulina platensis	5	Zarrouk medium	0.1	178.46	1.75	-	[43]
Dunaliella sp. ABRIINW-SH33	10	Modified Johnson medium	3	455.74	2.98	248.60	[59]
	20			317.32	2.08	173.08	
	30			279.74	1.83	152.60	
Dunaliella sp. ABRIINW-CH2	10			423.19	2.77	230.83	
	20			317.32	2.08	173.08	
	30			272.56	1.78	148.67	
Chlorella vulgaris FACHB-31	15	Modified BG11	0.02	878.40	3.35	-	[60]
Botryococcus braunii	0.03	BG11	0.1	-	0.64	-	[61]
	10				0.41		
	20				0.26		
Scenedesmus sp.	0.03	BG11	0.1	-	0.72	-	
	10				0.90		
	20				1.90		
Anabaena sp. CH1	10	Arnon medium	0.4	1010	1.16	-	[62]
Scenedesmus obliquus	5	Selenite enrichment medium	-	577.60	-	-	[63]
Chlorella protothecoides	20	BG11	-	370	1.55	190	[64]
Chlorella vulgaris P12	6.50	-	0.5	2290	9.97	1330	[56]

Table 2. *Cont.*

Species	CO_2 (%)	Culture Medium	Aeration Rate (vvm)	CO_2 Fixation Rate (mg/L/d)	Biomass Yield (g/L)	Biomass Productivity (mg/L/d)	Ref.
Chlorella sp. L38	5	BG11	-	-	0.60	-	[65]
Spirulina sp. LEB 18	10	Zarrouk medium	-	160	1.07	20	[66]
Scenedesmus obliquus (FACHB-13)	15	BG11	1	-	1.61	-	[67]
Chlorella vulgaris	15	BG11	-	120	1.83	144	[68]
Scenedesmus obliquus UTEX 393	5	Airlift photobioreactor	0.43	727.70	-	405.70	[69]
Chlorella sp. AE 10	30%	BG11	-	-	3.68	-	[70]

The maximum biomass output and the CO_2 fixation rate of *chlorella vulgaris* both increased by 57% and 56%, respectively, as the CO_2 content increased from 3% to 7% [71]. Rodas-Zuluaga et al. indicated that when growing microalgal strains at 0.03% to 20% CO_2, *Botryococcus braunii* grew well at CO_2 of 0.03%, while *Scenedesmus* sp. had the highest biomass yield at 20% CO_2 [61]. These findings all point to the influence of microalgal species on the growth and CO_2 tolerance of microalgae.

4. Strategies for Improving Photosynthetic Efficiency in Microalgae

Selection of microalgae strains with a fast growth rate, strong environmental adaptability, and high CO_2 fixation ability is the most important step to improve the effect of CO_2 fixation in microalgae. In addition to selecting microalgae with excellent traits from natural strains, random mutagenesis, ALE, and genetic engineering that enhancing the specific metabolic phenotype of microalgae and improving their environmental adaptability are also commonly used strategies, as shown in Figure 6. Moreover, the maximum productivity of target compounds and conditions for rapid growth of microalgae are often mutually exclusive, and algal productivity can be further enhanced by means such as mutagenesis and/or genetic engineering [72]. The effects of these methods on improving the growth performance of microalgae are shown in Table 3.

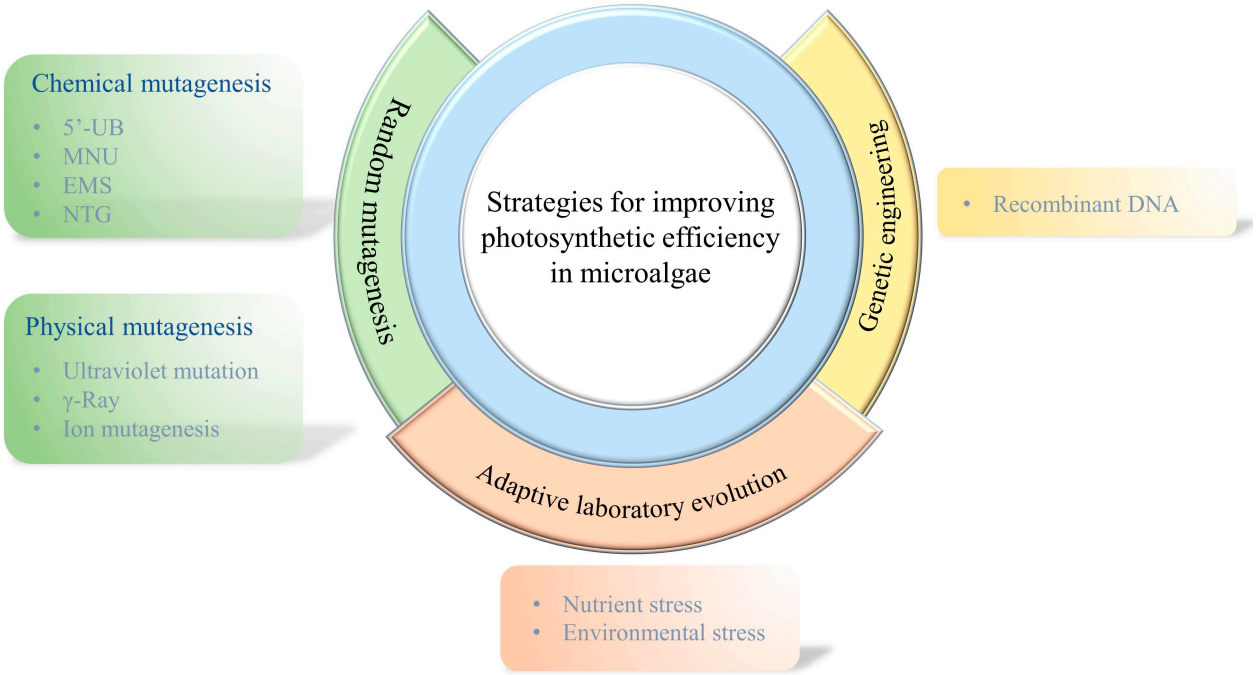


Figure 6. Strategies for improving photosynthetic efficiency in microalgae.

Table 3. Application of strategies to improve the photosynthetic efficiency of microalgae.

Strategies		Organism	Phenotype	Ref.
Random mutagenesis	Chemical mutagenesis	*Chlorococcum* sp. FFG039	The FFG039 PM exhibited 1.7-fold and 1.9-fold higher biomass and lipid productivities than those of the wild type.	[73]
		Chlorella sp.	The E100-30-60 showed that the highest biomass yield and biomass productivity were 111 and 110% higher than the wild type, respectively.	[74]
	Physical mutagenesis	*Haematococcus pluvialis*	The average specific growth rate of *Haematococcus pluvialis* mutated with 4000 Gy γ-ray irradiation was increased by 15% compared with the original strain with air aeration.	[75]
		Chlorella vulgaris	The resulting mutant resulted in a 33% increase in lipid yield.	[76]
ALE		*Chlorella* sp.	The maximal biomass concentration of AE10 was 3.68 ± 0.08 g/L in 30% CO_2, which was 2.94 fold compared to the original strain.	[70]
		Crypthecodinium cohnii ATCC 30556	The cell growth of the evolved strain (FS280) was increased by 161.87%.	[77]
Genetic engineering		*Nannochloropsis oceanica*	The growth rate of mutants was enhanced by 32%, and biomass accumulation by 46%.	[78]
		Chlamydomonas reinhardtii	The strain improved photosynthetic productivity.	[79]

4.1. Random Mutagenesis

Random mutagenesis with simplicity of operator, high reliability, and non-orientation can be applied to culture mutants with the optimal features of a high carbon fixation efficiency, a high lipid yield, and tolerated CO_2 [80]. Random mutagenesis applied to any microalgae strain can be divided into two categories—chemical mutagenesis and physical mutagenesis, using physical or chemical means to influence the strain—resulting in random alterations in the genome. Its advantage is that this technology can mutate and ameliorate various microalgae traits without requiring comprehending the complex knowledge of physiology or genetics, and the mutants produced are not genetically modified organisms [72,81].

4.1.1. Chemical Mutagenesis

Chemical mutagenesis is for the most part used as mutagens by ethyl methane sulfonate (EMS), 1-methyl-3-nitro-1-nitrosoguanidine (MNNG), N-methyl-N-nitrosourea (MNU), N-methyl-N′-nitro-N-nitrosoguanidine (NTG), 5-bromouracil (5′-UB), etc. [82]. The advantage of chemical mutagenesis is that it does not need special equipment, can produce a high frequency of point mutations, create relatively few chromosomal aberrations, but most of the mutagens are toxic or carcinogenic, easily pollute the environment [80]. Tanadul et al. showed that the strain E200-30-40, after EMS mutagenesis, produced 59% and 53% higher lipid content and productivity than the wild-type strain, respectively, which increased biomass and lipid accumulation [74]. Nojima et al. dispersed the cells of water surface-floating microalgae strains (*Botryosphaerella* sp. AVFF007 and *Chlorococcum* sp. FFG039) treated with EMS or MNNG mutagen to obtain mutant FFG039 PM, with a biomass and lipid productivity of 1.7- and 1.9-fold greater than the wild-type strains in the absence of inhibiting biofilm formation and floating capacity [73]. The total production of carotenoids and astaxanthin in the mutant G1-C1 of *Coelastrum* sp. after selection by EMS and glyphosate was approximately double that of the wild type [81]. As can be seen, chemical mutagenesis is utilized frequently due to simplicity of operation and potent mutagenicity.

4.1.2. Physical Mutagenesis

Physical mutagenesis mainly includes ultraviolet (UV) mutagenesis, γ -ray mutagenesis, and ion mutagenesis. UV mutagenesis is the most common because its control is flexible, economical, fast, and effective. Compared with chemical mutagens, it does not lead to poisoning of operators and has no secondary contamination [80]. During operation, some precautions also need to be taken to reduce the risk of UV radiation to the experimenter. The mutation effect depends on microalgae characteristics and experimental conditions, and is not applicable to all species [83]. Moha-León et al. found that using UV radiation and the herbicide quizalofop-p-ethyl to select freshwater microalgae, the chosen strains had more fatty acids and fat in them [84]. The screened mutant lipid production soared by 33% using UV irradiation of *Desmodesmus armatus* and *Chlorella vulgaris* [76]. γ-rays have more deep penetration than UV, which can penetrate cells to enhance the expression of photosynthesis enzymes (e.g., rubisco), rearrange chromosomes, screen oxygen radicals, and ultimately alter genetic traits [75]. The biomass of the γ-rays domesticated mutant *Chlorella* PY-ZU1 was increased 2.3-fold relative to the original strain [85]. Numerous studies have proved that physical mutagenesis changes the physiological and biochemical characteristics that are typical of cells. As a result, new strains with much higher lipid enrichment capacities can be chosen.

4.2. Adaptive Laboratory Evolution

ALE is a strain improvement method based on random mutation and natural selection that can be used as a tool to study evolution, which improves the phenotype, performance, and stability of microalgae, divided into intermittent and continuous culture patterns [86,87]. Additionally, it compensates for the neglect of molecular genetic mechanisms in Darwinian evolution and its development, using high-throughput DNA sequencing as a tool to effectively model the evolutionary process of selection [88]. The efficiency of ALE rests with the original strain, the initial cell density of the microalgae, and the strategy of stress [89]. Nutritional stress and environmental stress are the two types of stress. Nutritional pressure is often used to improve the completion rate of some low-cost substrates, and environmental pressure mainly includes temperature, oxidation, and organic solvent tolerance. [90]. Choosing the appropriate pressure can effectively improve the efficiency of ALE to a certain extent. Under laboratory control conditions, ALE cultures of microorganisms reach certain targets and subculture until the metabolic phenotype is stable and the evolved microalgae strains containing beneficial mutations are obtained.

In the ALE of microalgae, a single stress is typically used to better appreciate the process of tolerance [89]. Using reasonable ALE can not only improve the tolerance of microalgae to abiotic stress, but also optimize the yield of target metabolites and enhance the utilization of pollutants [86]. Controlling light factors by ALE could increase the microalgae growth rate and the accumulation of β-carotene and lutein. Thus, light stress can accelerate the removal of nitrogen and phosphate velocities in wastewater treatment [88]. At 10% and 20% CO_2, *Chlorella* sp. strains AE10 and AE20 were obtained by ALE, and had higher CO_2 tolerance than *Chlorella* sp. [70]. According to Table A1, ALE is a feasible strain enhancement tactic that can take advantage of microalgae's biotechnological potential to boost the ability of carbon fixation, lipid production, and the biomass concentration for distinct strains.

4.3. Genetic Engineering

Genetic engineering focuses on the gene expression and transcription, altering the target genome by using recombinant DNA at the molecular level. In principle, there are three pathways: (1) to provide the necessary phenotype, add heterologous genes; (2) to stop gene expression through gene disruption or gene deletion, or lower the level of existing genes' expression through RNA interference; (3) to enhance and induce expression under non-native promoters. Despite the complexity of the process, it is beneficial to boost photosynthesis, increase microalgal biomass, and increase CO_2 absorption rates [72]. The

biomass productivity of *Nannochloropsis oceanica* can be elevated by overexpression of a nuclear-encoded. At air CO_2 concentrations, the mutant growth rate increased by 32% and biomass accumulation increased by 46% [78]. Genetic engineering can decrease the uncontrollability and blindness of microalgae mutagenesis, but not all species of microalgae are suitable for genetic engineering, and the resulting transgenic organisms are still seen as potential risks to the environment and human health. The foremost limitations of genetic engineering are that it is time-consuming, costly, and requires complex equipment and experimental conditions.

5. Application of Carbon Dioxide Fixation Technology in Microalgae

5.1. In the Atmosphere

As mentioned above, microalgae cultures are considered a promising strategy to capture CO_2 from the atmosphere due to their high growth rate and CO_2 fixation capacity. In December 2022, the CO_2 levels in the atmosphere reached 418 ppm [91]. Microalgae use CCM to increase the CO_2 concentration near rubisco in order to achieve carbon fixation [92]. Tsai et al. proved that the symbiosis of microalgae with a natural medium has maximum CO_2 consumption efficiency and favorable CO_2 fixation ability [93]. Microalgae are typically cultivated in enclosed systems or open ponds, where they can absorb CO_2 from the air to supply the cell growth and eventually produce lipids, carbohydrates, and proteins [94,95]. Open ponds are often used in large-scale industrial growth systems to grow microalgae because they are cost-effective and have a higher production capacity compared to closed systems (i.e., photobioreactor). However, disadvantages include the large area, unstable culture conditions, easy pollution, and that they are limited by too few microalgae strains. A photobioreactor can achieve aseptic operation, have a relatively stable culture conditions, and prevent the evaporation of water, but its construction and operation costs are higher. Although direct air capture requires no equipment and energy, the effectiveness of carbon sequestration is relatively low and is affected by environmental factors [30,96,97]. Therefore, the common microalgae culture systems are generally open ponds and photobioreactors.

5.2. Flue Gas

Flue gas is a cheap and abundant source of CO_2, coal flue gas contains a 12–15 volume percentage (vol%) of CO_2, while natural gas contains a 4–8 volume percentage (vol%) of CO_2 [98,99]. The composition of flue gas is more complex, and will depend on the type of combustion raw materials, generally containing a large proportion of N_2 and CO_2, a little bit of H_2O and O_2, and a minor amount of nitrogen and sulfur oxides, heavy metals, dust, and other contaminants. The use of microalgae to reduce CO_2 emissions in flue gas is very promising, but the high concentration of some pollutants (e.g., SO_X and NO_X) may have severe toxic effects on microalgae cells and inhibit the growth of microalgae, as well as inhibit the ability of microalgae to sequester carbon, as shown in Figure 7 [100]. Among the four flue gas microalgae remediation strategies of microalgae, the use of microalgae to directly mitigate the flue gas pollution method has higher economic and environmental benefits because it does not use any adsorbent and can use cultured microalgae to produce secondary products [92]. The associated expenses, pollutant emissions, and energy requirements are lowered since the CO_2 concentration for algae development is comparable to the average flue gas CO_2 concentration [101]. Therefore, strain screening that can tolerate a high CO_2 content and high temperature resistance is the premise of realizing CO_2 fixation in flue gas.

The reduced growth rate at high CO_2 concentrations is often associated with acidification, leading to inactivation of key enzymes in the C_3 cycle, and homeostatic reactions enable CO_2-resistant strains to adjust their pH [102]. Yen et al. showed that *Chlorella* sp. achieved the maximum growth rate at a 10% CO_2 concentration, which shows a high CO_2 tolerance [103]. *Chlorella fusca* LEB 111 exhibited the largest daily bio-fixation amount of CO_2 in flue gas [104]. Microalgae cells can capture low levels of nitrogen oxides as sources of nitrogen. Further, there are polysaccharides, proteins, and lipids on the surface of the cell

wall of microalgae, which contain charged functional groups that can attract and combine with heavy metals. Consequently, microalgae can be employed as adsorbents to effectively remove heavy metals [103].

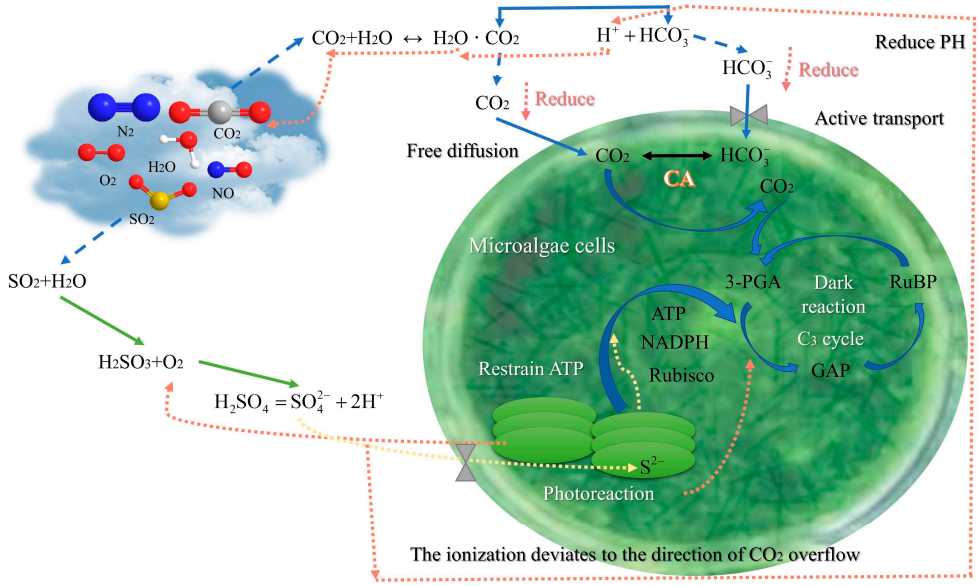


Figure 7. Mechanism of the inhibition of microalgae CO_2 fixation by SO_X.

In conclusion, the use of microalgae to capture CO_2 from flue gas, while removing the nitrogen oxides, sulfur oxides, and heavy metals in flue gas is an efficient way to reduce CO_2 emissions and air pollution. Accordingly, obtaining sufficient carbon sources contributes to the accumulation of biomass lipids and the increase in biological production, which can be applied to raw materials for biofuels or biorefinery. This approach maximizes economic profit, ensures the sustainable and ecological benign production of biomass, and is consistent with the development requirements of decreasing CO_2 emissions, which mitigates the effect of human activity on the climate. For now, most of the research on flue gas still has attached importance to laboratory research under controlled conditions, so further research and optimization are essential.

5.3. In Wastewater

The increasing urbanization and growth of the world's population have resulted in a steep rise in water consumption, making wastewater treatment among the greatest difficulties in the world. Microalgae wastewater treatment systems have a substantial economic value compared to conventional wastewater treatment technologies, which rely on expensive energy and chemicals and increase greenhouse gas emissions [105]. Wastewater contains most of the nutrients needed for microalgae growth and the trace metals necessary for photosynthesis, such as ammonias, phosphates, irons, coppers, and zincs. The combination of wastewater purification, CO_2 capture, and microalgae cultivation can reduce the cost of sewage treatment, lower the use of chemicals in the processing process, and slow down the emission of CO_2. Additionally, the resulting biomass can be further processed to produce biofuels, which alleviates the current energy crisis [98,106].

The efficiency of wastewater treatment by microalgae is relevant to wastewater type, nutritional characteristics, photobioreactor types, and culture methods [25]. The tolerance of organic pollutants in wastewater also varies among various species, as shown in Table A2. The content of nitrogen and phosphorus in domestic wastewater is low, the concentration of toxic and harmful substances in agricultural wastewater is low, and industrial wastewater usually contains a large amount of oil, heavy metals, and toxic pollutants. Song et al. showed that *Chlorella* sp. was cultivated in a system mixed with soybean wastewater and bicarbonate solution (NH_4HCO_3). At pH = 7, the low-NH_4HCO_3 concentration system had

the highest biomass yield (0.74 g/L) and the highest carbon bioconversion efficiency [107]. *Scenedesmus dimorphus* was cultured in tertiary urban sewage with different NP ratios, and when the NP ratio was 8:1 at 4% CO_2, the maximum biological fixation rate of microalgae reached 49.6 mg/L/d [108]. Hu et al. proved that *Chlorella sp*. L166 realized the efficiency of a maximum removal of TN, TP, COD, and CO_2 with an NP ratio of 5:1 in 20% soybean wastewater, introducing 5% CO_2 at a rate of 0.1 vvm [109].

Combining the capture of CO_2 from flue gas with wastewater treatment using microalgae culture to produce biofuels is a sustainable and economical wastewater treatment technology [110]. The extraction of high-value-added products in microalgae will also immensely encourage its large-scale industrialization that has a wide range of application potential.

5.4. Other Applications

Microalgae contain high-value fatty acids, pigments, and vitamins, etc. After special treatment, microalgae biomass can also be converted into biodiesel, methane, and other energy sources. Microalgae with a high oil content have the potential to produce 25-fold higher oil yields than conventional biodiesel crops such as the oil palm [111]. Astaxanthin extracted from *Haematococcus pluvialis* is often used in cosmetics as to its strong antioxidant capacity. In addition, eating astaxanthin is thought to prevent or control different diseases, and it is commonly used as a red pigment in food [112]. Polyunsaturated fatty acid has conducive effects on fetal development, preventing cardiovascular disease, and even improving cognitive function in Alzheimer's disease patients, which is used as nutritional supplements [113]. In conclusion, microalgae biomass can be applied in food, bio-medicine, cosmetics, energy, and other industries, with a high potential application value.

6. A Future Prospect for Microalgae Carbon Dioxide Fixation Technology

6.1. Selection of Seeds Rationally

Different microalgae have different carbon fixation effects, so microalgae species that have a fast growth rate, strong environmental adaptability, high photosynthetic efficiency, a high biological yield, and are easy to cultivate outdoors on a large scale can be selected in order to improve the photosynthesis efficiency of microalgae [114]. In the process of industrial application, due to the differences in actual conditions, natural strains sometimes cannot meet the production requirements. The measures of random mutagenesis, ALE, and genetic engineering are adopted to obtain more evolved microalgae strains that it has stable metabolic phenotype to improve the cumulative effect of target products [114,115]. However, random mutagenesis produces unpredictable effects, with large cost of investment and a potential for cancer in the environment and in humans. The ALE mechanism requires further exploring, and the characteristics of evolution are difficult to define. Genetic engineering is time-consuming, expensive, and limited by a deep understanding of the genetic mechanisms of microalgae [115,116]. Therefore, how to sift the appropriate species of microalgae and ameliorate it in order to obtain the commercial application of microalgae species need to be researched.

6.2. To Optimize the Culture Conditions of Microalgae

The differences in culture conditions (such as light, temperature, pH, nutrient elements, and CO_2 concentration) will change the production and CO_2 fixation efficiency of microalgae [117]. The viability of CO_2 fixation applications in microalgae is dominated by photosynthetic productivity and the biomass output of microalgae. During the growth of microalgae, carbon fixation efficiency is maximized by strictly controlling the growth conditions.

6.3. To Construct of Microalgae Carbon Dioxide Fixation Technology as the Core of the Industrial Model

By combining microalgae CO_2 fixation technology with wastewater treatment and flue gas treatment, a green and low-carbon recycling industry model with microalgae as the core is formed. Microalgae cultivation provides an effective, environmentally friendly,

and continuable solution for wastewater and flue gas treatment [95,118]. This scheme controls the emission of CO_2, fosters the recycling of nutrients in wastewater (e.g., nitrogen and phosphorus), absorbs pollutants in wastewater and flue gas, reduces the cost of CO_2 fixation, produces high-value-added products, and achieves win–win environmental and economic benefits [119,120]. However, most of the studies in the existing literature are conducted in laboratory conditions, and the follow-up studies should give priority to large-scale application to reasonably evaluate their industrial feasibility.

6.4. Comprehensive Development and Utilization of High-Value-Added Products of Microalgae

Microalgae biomass can be used extensively in a wide range of industries, which is crucial for alleviating the energy crisis, reducing environmental contamination, and promoting food security [115,121,122]. Improving the productivity of related products (such as lipids, pigments, and nutrients) is available for improving the economic benefits of microalgae CO_2 fixation technology [123,124], which contributes to impelling industrialization development of CO_2 fixation technology.

6.5. Potential for Carbon Fixation in the Microalgae

In addition to carbon sequestration in microalgae, there are some commonly used methods of carbon capture. Table 4 summarizes the advantages and limitations of various methods. Hence, microalgae CO_2 fixation technology has a wide range of application prospects as a new carbon capture technology. In the industrial field, it can be applied in the waste gas treatment of coal, chemical industry, and other industries, to convert CO_2 into useful microalgae biomass via photosynthesis [102]. Similarly, microalgae carbon sequestration technology can also be combined with wastewater treatment to purify water quality by absorbing nitrogen, phosphorus, and other nutrients in wastewater, which can be used in agriculture, industry, and other fields [125]. In the field of energy, biomass generated by microalgae CO_2 fixation technology can be used to produce biofuels, which can replace traditional petroleum energy and reduce dependence on fossil fuels, thus reducing CO_2 emissions. Microalgae biomass can also be used as a source of raw materials for medicines, cosmetics, food, and other fields [126]. There is still a long way to go to apply the achievements of carbon fixation technology from lab-scale research to large-scale application. For example, industrial carbon sequestration technology of microalgae is still faced with the problems such as low carbon sequestration efficiency, and further reduction in production costs. With the continuous progress of technology, the application scope of microalgae CO_2 fixation technology will continue to expand, the cost will continue to reduce, and the production efficiency will also continue to improve. Therefore, microalgae CO_2 fixation technology has great development potential and application prospects.

In short, microalgae carbon sequestration technology can reduce CO_2 emissions and delay global warming while providing high-value-added products to achieve CO_2 resource utilization. Therefore, compared with other carbon fixation methods, it has higher cost benefits, potential for effective carbon fixation, etc., which meets the requirements of green development and plays an important role in the realization of the goal of carbon neutrality.

Table 4. Comparison of various carbon capture methods.

Category	Method	Description	Advantages	Limitations	Ref.
Physical	Geologic injection	Separate and capture CO_2, transport it to a storage location, and inject it deep underground for long-term isolation from the atmosphere	♦ Take use of space available	♦ High costs ♦ Special geological requirements	[127]
	Oceanic injection	Injection of CO_2 into deep ocean	♦ Significant capacity to store CO_2	♦ High costs ♦ Low storage permanence ♦ Considerable ecological impacts	[128]

Table 4. *Cont.*

Category	Method	Description	Advantages	Limitations	Ref.
Chemical	Chemical absorption	Chemical absorption and desorption concept, determined by solubility of CO_2	♦ Environmentally safer ♦ Capture efficiency to 90%	♦ Inefficient CO_2 capture capacity ♦ High evaporation loss of solvent ♦ Poor thermal stability, ♦ Equipment corrosion	[26]
	Mineral carbonation	CO_2 reacts with calcium- or magnesium-bearing rocks to form magnesite or calcite	♦ CO_2 is converted to a solid substrate that can be reused as a building material or disposed of in surface facilities.	♦ Need for a significant amount of reagent	[128]
Biological	Forest planting	Absorption of CO_2 through the photosynthesis of the trees	♦ Significant social benefits ♦ Environmentally friendly	♦ Large land area requirement ♦ Low carbon fixation efficiency compared to microalgae	[30]
	Microalgae carbon fixation	Carbon sequestration by microalgal photosynthesis	♦ Environmentally friendly ♦ Economically feasible ♦ Plant in seawater or wastewater and require no a large area of soil resources ♦ CO_2 fixation more efficiently than land plants	♦ Sensitive to living conditions (e.g., pH, toxic substances, and CO_2 concentrations) ♦ Improve the cost performance of the cultivation	[21]

7. Conclusions

Due to global warming, microalgae carbon sequestration is among the most promising and sustainable methods to fix CO_2. Microalgae, with a relatively faster growth rate and higher CO_2 fixation efficiency, can also be used in wastewater treatment, flue gas treatment, biofuel production, as well as high-value-added products manufacture (e.g., proteins and lipids), which can maximize the economic benefits. Hence, it is an ideal material for biological capture of CO_2 under the background of "dual-carbon". Nevertheless, the future application of microalgae is still facing quite a few challenges. At present, many kinds of microalgae have been used for laboratory-scale testing. Limited by the technical level and production cost, the large-scale industrial biological sequestration of microalgae is still in its infancy, and no pilot-scale studies have yet been conducted.

To accelerate the realization of the carbon sequestration scale of microalgae, we should start by studying the microalgae CO_2 fixation mechanism, combine the CO_2 fixation technology of microalgae with high-value-added biomass production, promote the industrialization of microalgae CO_2 fixation technology, and establish and optimize a better mechanism, to achieve the purpose of promoting the circular economy and sustainable development. Based on this, carbon sequestration technology of microalgae needs to be innovated and improved according to the following aspects: (1) using relevant technologies to select suitable strains, (2) making the microalgae growing process more ecologically friendly by utilizing flue gas and wastewater treatment technology, and (3) to make full use of high-value-added products to achieve the greatest economic benefits. It is believed that the economically feasible and environmentally friendly industrialization model with microalgae CO_2 fixation technology as the core will be promoted and widely used in the foreseeable future.

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Appendix A

Table A1. Characteristics of the modified microalgal strains by ALE.

Initial Strains	Medium	Initial Cell Density	Cycles	Stresses	Outcome	Ref.
Chlorella sp.	BG11	OD_{750} of 0.1	31 cycles (97 days)	10% CO_2	The maximal biomass concentration of AE10 was 3.68 ± 0.08 g/L in 30% CO_2, which was 2.94 fold compared to the original strain.	[70]
Chlamydomonas reinhardtii	TAP and TAP-N	OD_{680} of 0.1	28 cycles (84 days)	Nitrogen starvation	ALE combined with nitrogen starvation substantially increased total lipid production, particularly for low-starch mutants. The endpoint strain of cc4334 under nitrogen starvation stress had the highest lipid productivity.	[129]
Schizochytrium sp. HX-308	Main culture medium contained 40 g/L glucose and 0.4 g/L yeast extract	-	40 cycles (40 days)	High oxygen	The adapted strain generated higher cell dry weight and lower lipid accumulation.	[90,130]
Schizochytrium sp. HX-308	Medium with 30 g/L NaCl at a concentration of 1% *v*/*v*	-	150 days	High salinity	The ALE150 showed a maximal cell dry weight of 134.5 g/L and a lipid yield of 80.14 g/L, representing a 32.7 and 53.31 increase over the starting strain, respectively.	[131]
Chlorella sp. L5	TAP medium with 500 mg/L phenol	0.6 g/L	31 cycles (95 days)	High concentration phenol	The upregulations of the genes according to antioxidant enzymes and carotenoids synthesis were tolerated high phenol.	[132]
Chlorella sp.	TAP medium	0.6 g/L	31 cycles (95 days)	High concentration phenol	The strain had higher phenol biodegradation rates.	[133]
Phaeodactylum tricornutum (CCMP-2561)	Artificial seawater added with f/2 medium without silica	1×10^6 cells/mL	35 cycles (nearly 252 days)	Reducing salinity	70% salinity potentiated the algae to enhance PUFAs.	[134]
Picochlorum sp. *BPE23*	Liquid growth medium	OD_{750} of 0.2	322 days	Supra-optimal temperature	At the optimal growth temperature of 38 °C, the biomass yield on light was 22.3% higher, and the maximal growth rate was 70.5% higher than the wild type.	[135]
Synechocystis sp. PCC 6803	BG11 medium supplemented with 1.5% agar	OD_{730} of 0.2	43 cycles (303 days)	3% NaCl	All ALE-generated strains except S3 and S7 had a significantly higher growth rate than the control strain	[136]

Table A1. *Cont.*

Initial Strains	Medium	Initial Cell Density	Cycles	Stresses	Outcome	Ref.
K. marxianus	YPD medium	OD_{600} of 0.8	65 days	Various temperature	The adapted *K. marxianus* strain accumulates glycerol and trehalose in response to lactose stress and ameliorate osmotolerance in *K. marxianus* cells.	[137]
Scenedesmus sp. SPP	Modified Chu13 medium	-	10 days	Salinity stress, light stress, temperature stress	The triple stress-adapted strain showed the highest lipid content.	[138]
Schizochytrium sp. CCTCC M209059	Main culture medium contained 40 g/L glucose and 0.4 g/L yeast extract	-	80 days	High temperature	The adaptive strain showed a higher growth rate and lower temperature sensitivity.	[130,139]
Nannochloroposis oculata CCMP525	The f/2 agar medium	0.32 g/L	24 cycles	High temperature	In a 2-L photobioreactor at 35 °C, biomass and lipid productivity were 1.43-fold and 2.24-fold higher, respectively, than wild type at 25 °C.	[140]
Crypthecodinium cohnii ATCC 30556	Regular fermentation medium (27 g/L glucose, 25 g/L sea salt, and 6 g/L yeast extract)	OD_{490} of 0.1	280 cycles (840 days)	Varying contents of the fermentation (30–90%) supernatant	The cell growth and DHA productivity of the evolved strain (FS280) were increased by 161.87 and 311.23%.	[77]
Chlorella sp. AE10	BG11 medium	0.04 g/L	46 cycles (138 days)	High salinity	*Chlorella* sp. S30, has the potential for CO_2 capture under 30 g/L salt and 10% CO_2 conditions.	[141]
Dunaliella salina CCAP 19/18	The f/2 medium	1×10^5 cells/ml	5 cycles (25 days)	Blue LED	The beta-carotene concentration (33.94 ± 0.52 μM) was enhanced by 19.7% compared to that observed for the non-ALE-treated wild type of D. *salina* under the B-R system (28.34 ± 0.24 μM).	[142]

Table A2. Comparison of the removal efficiency of pollutants in different wastewater types by various microalgae.

Waste Stream	Wastewater Source	Species	Reactor	Light Intensity	Ambient Temperature (°C)	Sampling Time (day)	Water Quality Index (mg/L)			Nutrient Removal Efficiency (%)			Maximum Biomass Concentration (g/L)	CO_2 Bio-Fixation (mg/L/d)	Lipid Productivity (mg/L/d)	Ref.
							COD *	TN *	TP *	COD	TN	TP				
Agricultural wastewater	Soybean processing wastewater	*Chlorella* sp. L166	Erlenmeyer flask	6000 lux	25 ± 1	18	5320	106.99	23.28	78.20	96.07	95.55	1.52	-	-	[109]
	Starch processing wastewater	*Chlorella pyrenoidosa*	Conical flask	127 μmol m^{-2} s^{-1}	25 ± 1	6–7	702.4–1026.2	240.3–382.7	22.7–40.2	65.99	83.06	96.97	1.90	-	30.15	[143]
	Swine wastewater	MBFJNU-1	Flask	-	-	12	824.53	547.78	81.72	-	90.51	91.54	0.63	-	-	[144]
Agricultural wastewater and municipal wastewater	Swine wastewater 2:2 secondary treated municipal wastewater	*Chlorella sorokiniana*	Erlenmeyer flask	126 μmol m^{-2} s^{-1}	28 ± 2	10	-	337.32	30.88	-	63.90	93.02	1.31	-	23	[145]
	Swine wastewater 1:3 secondary treated municipal wastewater	*Desmodesmus communis*	Erlenmeyer flask	126 μmol m^{-2} s^{-1}	28 ± 2	10	-	188.61	15.77	-	88.02	99.73	1.02	-	-	
	Wastewater influent after primary settling tank	*Chlorella sorokiniana pa.91*	Erlenmeyer flask	4000 lux	30	16	211.4	2.01 (NO_3^-) 0.06 (NO_2^-) 34.1 (NH_4^+)	6.1 (PO_4^{3-})	76	73 (NH_4^+) 93 (NO_3^-)	83 (PO_4^{3-})	3.21	-	-	[146]
Municipal wastewater	The primary sedimentation tank	*Chlorella vulgaris* ATCC 13482	Cylindrical glass bottles	90 ± 5 μmol m^{-2} s^{-1}	25	10	293	46.67	19.50	-	93.40	94.10	0.94	140.91	-	[147]
		Scenedesmus obliquus FACHB 417	Cylindrical glass bottles	90 ± 5 μmol m^{-2} s^{-1}	25	10	293	46.67	19.50	-	91.50	91.30	0.87	129.82	-	
	Effluent of anaerobically digested food wastewater	*Scenedesmus bijuga*	Erlenmeyer flask	80 μmol m^{-2} s^{-1}	30	28	5923	2370	47.80	-	86.60	90.50	1.49	-	15.59	[148]
	The sewage from the sewer	*Tetradesmus obliquus* PF3	Conical flask	6000 lux	25 ± 1	5	267	43	4.9	90	93.20	99	1.8	551	-	[149]
	Sterilized sewage	*Tetradesmus obliquus* PF3	Conical flask	6000 lux	25 ± 1	5	210	40	4.70	42	94.70	99	1.8	558	-	
	Targeting the tertiary treatment of wastewater	*Neochloris oleoabundans*	Erlenmeyer flask	60 μmol·m^{-2} s^{-1}	25	14	-	42	18.50	-	100	31.30	1.17	145	-	[150]
	Domestic wastewater from secondary settling tanks	*Scenedesmus obliquus*	Conical flask	14,500 lux	23 ± 2	10	72.16	12.44	1.08	-	98.90	97.60	-	-	-	[151]
	Manure wastewater	*Scenedesmus dimorphus* (FACHB-496)	Erlenmeyer flask	60–80 μmol·m^{-2} s^{-1}	26 ± 2	7	-	306.15	115.08	-	88.16	73.98	-	638.13	-	[152]
	Artificial brewery wastewater	*Scenedesmus* sp. 336	Erlenmeyer flask	6000 lux	25 ± 1	10	2100	45	7	73.66	75.96	95.71	-	-	38	[153]
		Chlorella sp. UTEX1602	Erlenmeyer flask	6000 lux	25 ± 1	10	2100	45	7	44.97	81.43	97.54	-	-	-	
	Palm oil mill effluent from an anaerobic treatment pond	*Chlorella* sp. (UKM2)	Transparent glass bottle	266 μmol m^{-2} s^{-1}	25 ± 2	15	2900	330	-	-	80.90	-	-	120.80	-	[154]
Industrial wastewater	Waste molasses	*Scenedesmus* sp. Z-4	Erlenmeyer flask	3000 lux	10	7	514,000	458	67	87.2	90.50	88.60	2.5	-	78	[155]
	Membrane-treated distillery wastewater	*Chlorella vulgaris*	Erlenmeyer flask	2000 lux	25	9	-	-	-	72.24	80	94	0.65	-	-	[156]
	Textile wastewater	Mixed microalgae (*Chlorella* Species and *Scenedesmus* sp.)	Conical flask	212.77 mol m^{-2} s^{-1}	-	13	1900	480.50	31	78.78	93.30	100	0.4	-	-	[157]
	The simulated brewery effluent	*Scenedesmus obliquus*	Erlenmeyer flask	12,000 lux	30 ± 3	9	3635	54	-	57.5	20.80	-	0.9	-	-	[158]

* COD: chemical oxygen demand; TN: total nitrogen; TP: total phosphorus.

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Li, Gang; Xiao, Wenbo; Yang, Tenglun; Lyu, Tao

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