marine drugs

MDPI

Review

Enhancing CO_2 Fixation in Microalgal Systems: Mechanistic Insights and Bioreactor Strategies

Zhongliang Sun *, Chenmei Bo, Shuonan Cao and Liqin Sun *

College of Life Sciences, Yantai University, Yantai 264005, China
* Correspondence: zlsun@ytu.edu.cn (Z.S.); sliqin2005@163.com (L.S.)

Abstract: Microalgae are small, single-celled, or simple multicellular organisms that contain Chlorophyll a, allowing them to efficiently convert CO_2 and water into organic matter through photosynthesis. They are valuable in producing a range of products such as biofuels, food, pharmaceuticals, and cosmetics, making them economically and environmentally significant. Currently, CO_2 is delivered to microalgae cultivation systems mainly through aeration with CO_2-enriched gases. However, this method demonstrates limited CO_2 absorption efficiency (13–20%), which reduces carbon utilization effectiveness and significantly increases carbon-source expenditure. To overcome these challenges, innovative CO_2 supplementation technologies have been introduced, raising CO_2 utilization rates to over 50%, accelerating microalgae growth, and reducing cultivation costs. This review first categorizes CO_2 supplementation technologies used in photobioreactor systems, focusing on different mechanisms for enhancing CO_2 mass transfer. It then evaluates the effectiveness of these technologies and explores their potential for scaling up. Among these strategies, membrane-based CO_2 delivery systems and the incorporation of CO_2 absorption enhancers have shown the highest efficiency in boosting CO_2 mass transfer and microalgae productivity. Future efforts should focus on integrating these methods into large-scale photobioreactor systems to optimize cost-effective, sustainable production.

Keywords: microalgae; photobioreactor systems; CO_2 supplementation techniques; large-scale deployment

Academic Editors: Cecilia Faraloni and Eleftherios Touloupakis

Received: 13 February 2025
Revised: 3 March 2025
Accepted: 5 March 2025
Published: 7 March 2025

Citation: Sun, Z.; Bo, C.; Cao, S.; Sun, L. Enhancing CO_2 Fixation in Microalgal Systems: Mechanistic Insights and Bioreactor Strategies. *Mar. Drugs* **2025**, *23*, 113. https://doi.org/10.3390/md23030113

1. Introduction

Microalgae have the capability to fix CO_2 through photosynthesis, producing a range of valuable chemicals. Certain species, such as *Botryococcus braunii*, can generate hydrocarbons that constitute 15% to 75% of their dry weight. Other species accumulate glycogen or glycerol, and many have lipid contents exceeding 30% of their dry weight [1,2]. The pyrolysis of microalgal biomass can produce biofuels with an average calorific value of up to 33 MJ/kg [3]. Moreover, microalgae can be cultivated in seawater, alkaline water, or semi-alkaline water, which helps avoid competition with crops for arable land and freshwater. They can also utilize nitrogen-rich wastewater, making them a valuable resource in areas with limited freshwater and degraded land [4,5]. Thus, microalgae present a promising future source of energy and chemicals.

The carbon content in microalgal cells represents about half of their dry weight. During growth, microalgae fix CO_2 into their cellular components through photosynthesis, necessitating a continuous supply of carbon sources in the cultivation medium [6]. In the medium, inorganic carbon exists in three forms: HCO_3^-, CO_3^{2-}, and free CO_2. The concentration and ratio of these forms depend on the total inorganic carbon concentration

and pH [7]. For most economically valuable microalgal species, the optimal growth pH ranges from 6 to 8, during which the primary forms of inorganic carbon in the medium are free CO_2 and HCO_3^-. In contrast, alkaliphilic species, such as *Spirulina*, thrive at a pH of around 9.0, where the dominant carbon species are HCO_3^- and CO_3^{2-}. When sodium bicarbonate ($NaHCO_3$) is used, the medium's pH increases due to the dissociation of HCO_3^- and CO_2 consumption. This process can convert more than half of the $NaHCO_3$ into Na_2CO_3, which is not usable by the microalgae and complicates medium recycling due to the elevated pH [8]. Using CO_2 directly as a carbon source is more effective for microalgae, as it avoids the issue of rising pH and helps maintain an optimal cultivation environment, allowing for extended or repeated use of the medium.

Microalgal cultivation methods are generally divided into open and closed systems [9]. Both typically involve bubbling CO_2-enriched gas into the cultivation medium, but this method is inefficient due to low CO_2 absorption rates (13–20%), leading to high carbon-source costs [10,11]. Since carbon accounts for approximately half of the dry weight of microalgal cells, an increase of 1 g/L in cell concentration requires the assimilation of around 2 g/L of CO_2. However, the solubility of pure CO_2 in water is relatively low, at only 1.45 g/L at 25 °C, and even lower when CO_2 is sourced from air, with a solubility of just 0.58 mg/L under the same conditions. This limited solubility poses a significant challenge for maintaining sufficient inorganic carbon availability in the cultivation medium. Therefore, enhancing the concentration of inorganic carbon species and improving CO_2 absorption efficiency are critical for supporting rapid microalgal growth and minimizing cultivation costs.

Recent advancements have led to the development of innovative CO_2 supplementation technologies designed to meet the rapid growth requirements of microalgae while reducing cultivation costs. Examples include in situ CO_2 supplementation devices in raceway ponds, which increase gas–liquid contact time and surface area, and methods that improve CO_2 absorption and conversion using immobilized carbonic anhydrase [7,12,13]. This review categorizes these CO_2 supplementation technologies based on their mechanisms for enhancing CO_2 mass transfer, assesses their effectiveness, and explores the potential for scaling up these technologies. By systematically addressing these objectives, this review aims to offer a comprehensive understanding of CO_2 management in microalgal cultivation and highlight innovative strategies to overcome the limitations of conventional carbon-supplementation methods.

2. Methodologies and Devices for Enhancing CO_2 Mass Transfer in Microalgal Systems

2.1. CO_2 Mass-Transfer Process

Under photoautotrophic growth conditions, microalgae use inorganic carbon sources to synthesize organic compounds and convert light energy into chemical energy. Microalgae can absorb both CO_2 and HCO_3^- but cannot utilize CO_3^{2-} [14]. CO_2 enters the cells through diffusion and is used directly, while HCO_3^-, being a polar and negatively charged ion, requires active transport across the cell membrane, a process that consumes energy [15]. Consequently, the absorption of HCO_3^- is slower compared to CO_2, though some algae species that thrive in high pH environments, such as *Spirulina*, exhibit better HCO_3^- absorption.

During CO_2 transfer in the cultivation medium, it can react with OH^- and CO_3^{2-}, though these reactions have minimal impact on CO_2 transfer efficiency [16]. In microalgal culture media, CO_2 transfer is a multi-step process involving several stages: from the gas phase to the gas film, diffusion within the gas film, transfer from the gas film to the liquid film, diffusion through the liquid film, movement from the liquid film into the liquid phase,

diffusion within the liquid phase, transfer from the liquid phase to the liquid film at the cell-wall surface, and finally, cellular absorption. According to the two-membrane theory, the primary resistance to CO_2 transfer occurs within the liquid film, which serves as the main barrier limiting the efficiency of gas–liquid mass transfer [17,18]. The mass-transfer rate is proportional to the driving force and the area available for mass transfer. The rate of CO_2 transfer can be expressed as:

$$N_{CO_2} = K_{L,a}(C^*_{CO_2,L} - C_{CO_2,L}) \quad (1)$$

where $K_{L,a}$ is the overall liquid volumetric mass-transfer coefficient for the absorption of CO_2, dependent on factors like phase contact area and the intensity of gas–liquid mixing. $C^*_{CO_2,L}$ represents the CO_2 concentration in the liquid phase at equilibrium with the gas-phase concentration.

2.2. In Situ CO_2 Supplementation

To enhance CO_2 absorption in algal medium, several in situ CO_2 supplementation devices have been developed. Kumar et al. improved CO_2 mass transfer by using hollow-fiber membranes, which provide a significantly larger interphase contact area compared to traditional bubbling methods, resulting in a mass-transfer coefficient approximately ten times greater (Figure 1A) [19]. This technology enhances CO_2 absorption efficiency and facilitates CO_2 recycling, thereby reducing cultivation costs [20]. Ketheesan et al. introduced a novel airlift raceway-pond design where CO_2 is injected into an ascending channel, increasing CO_2 and liquid contact time and achieving a 50% absorption rate [21]. Our research team implemented an in situ CO_2 supplementation trap device in an open raceway pond for *Spirulina platensis* cultivation (Figure 1B) [22]. This device, featuring a trap container, partition, and gas distributor, effectively extended the gas–liquid contact time from 3 s to 8 s and enhanced CO_2 utilization efficiency to over 90% (Table 1). Chen et al. utilized a leak-proof cover over the cultivation layer, which collected CO_2 and created a large gas–liquid exchange area (Figure 1C), though challenges included limited gas–liquid exchange surface area, the accumulation of oxygen and nitrogen, and reduced light transmittance [23]. Our research team also developed a submerged cover-type CO_2 supplementation device installed at the bottom of open ponds. Transparent glass covers above the aeration points and below the liquid surface allowed bubbles to have extended contact time with the liquid, reducing CO_2 escape and improving CO_2 absorption efficiency [24].

In closed photobioreactors, Bergmann et al. enhanced bubble residence time by modifying flat-panel reactors to multiple chambers, achieving over 80% CO_2 absorption (Figure 2A) [25]. Huang et al. further enhanced gas–liquid mixing in flat-panel reactors by incorporating disturbance columns or inclined baffles (Figure 2B). This modification increased mixing intensity by up to 52%, significantly boosting the CO_2 mass-transfer coefficient [26]. The tubular photobioreactor, currently the most widely used closed photobioreactor, has evolved through multiple generations into a structure comprising light absorption units, gas–liquid exchange units, and circulation pumps. CO_2 can be introduced into the gas–liquid exchange unit or before the culture liquid enters the light absorption unit, or at a specific position within the light absorption unit. Under the action of the circulation pump, the gas moves with the liquid and is gradually absorbed, resulting in high CO_2 absorption rates (Figure 2C) [27].

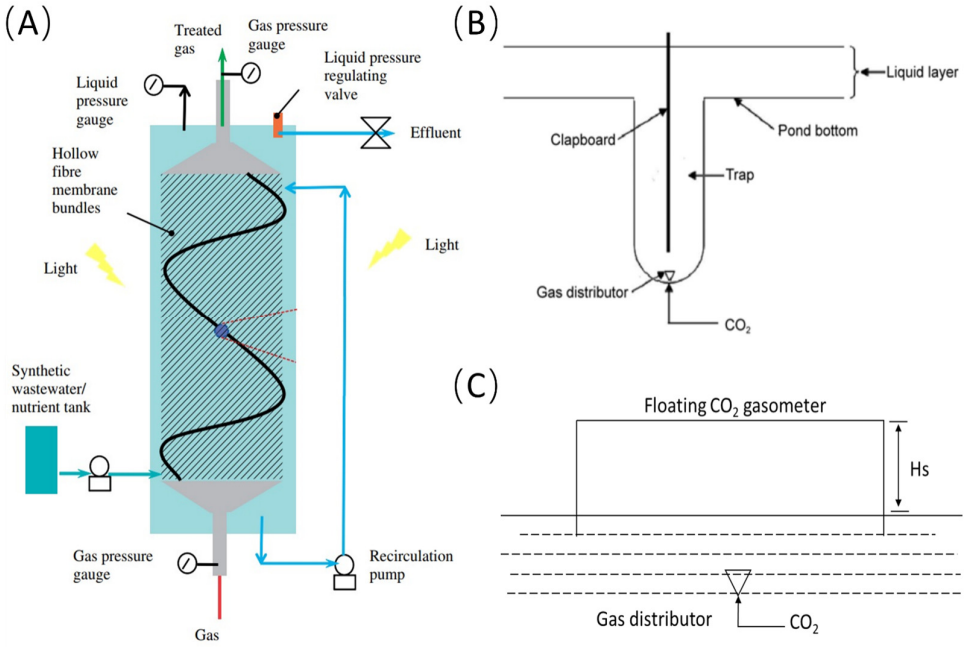

Figure 1. In situ carbon-supplementation devices in microalgae cultivation systems; (**A**) hollow fiber membrane module; (**B**) trap-type carbon-supplementation device; (**C**) leak-proof cover device.

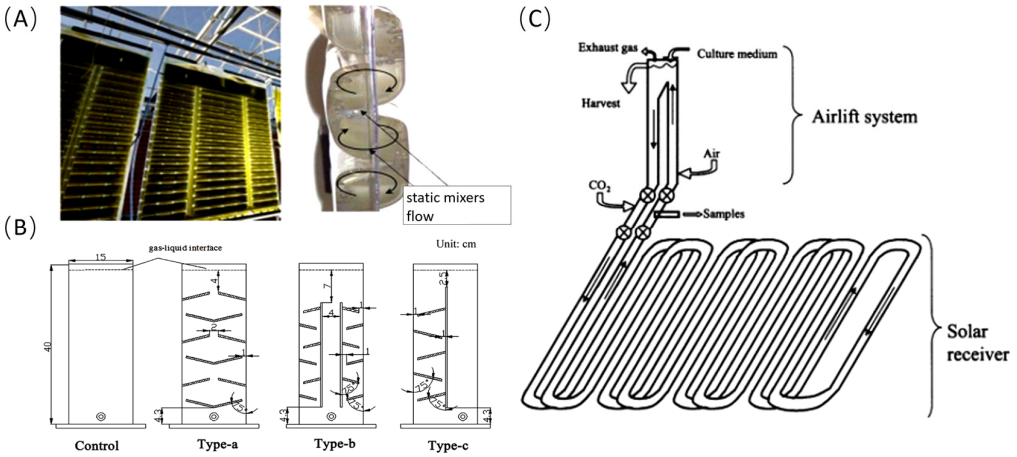

Figure 2. Closed photobioreactor and structural modifications for enhanced CO_2 mass transfer; (**A**) multi-chamber structure of flexible flat-panel reactor; (**B**) different types of baffle structures in flat-panel reactors; (**C**) tubular reactor and CO_2 supplementation positions.

Table 1. Effect of methodologies and devices on microalgae growth and CO_2 utilization efficiency.

Microalgal Culture System	Methodologies or Devices	Species	Biomass	CO_2 Utilization Efficiency	Mechanisms	Reference
Photobioreactor	Hollow fiber membrane	*Spirulina platensis*	2131 mg/ L ↑	85% ↑	Increase the interfacial contact area available for gas transfer	[19,20]
Raceway pond	An ascending channel	*Scenedesmus* sp.	0.16 ± 0.03 g/(L·d) ↑	50% ↑	Increase mixing intensity	[21]
Raceway pond	CO_2 supplementation trap device	*Spirulina platensis*	3.45–6.04 g/(m^2·d) ↑	90% ↑	Prolong gas–liquid contact time	[22]
Open pond	Leak-proof cover	*Cyanobacterium* sp.	2.5 g/L ↑	80% ↑	Create a large gas–liquid exchange area	[23]

Table 1. *Cont.*

Microalgal Culture System	Methodologies or Devices	Species	Biomass	CO_2 Utilization Efficiency	Mechanisms	Reference
Open pond	Submerged cover-type	*Spirulina platensis*	13.3 g/(m^2·d) ↑	92% ↑	Prolong gas–liquid contact time	[24]
Photobioreactor	Multiple chambers	*Nannochloropsis salina*	0.12 g/(L·d) ↑	80% ↑	Enhance bubble residence time	[25]
Flat-plate PBRs	Inclined baffles	*Chlorella pyrenoidosa.*	1.3 g/ L	No data	Increase mixing intensity	[26]
Raceway pond	Vertical absorption tower	*Chlorella pyrenoidosa.*	20 g/(m^2·d) ↑	83% ↑	Prolong gas–liquid contact time	[28]
Open pond	Absorption tank	*Spirulina platensis*	6–12 g/(m^2·d)	>50%	Increase mixing intensity	[4,29]

↑ indicates that the indicators of the experimental group have improved compared with the control group.

2.3. Ex Situ CO_2 Supplementation

In addition to in situ carbon-supplementation devices in photobioreactor systems, some ex situ carbon-supplementation devices have also been developed. Putt et al. set up a vertical absorption tower outside the raceway pond, with a dynamic pump driving the culture medium to circulate between the vertical absorption tower and the raceway pond, achieving a CO_2 absorption rate of 83% [28]. Trench-type carbon supplementation involves excavating a deep trench adjacent to the cultivation pond, allowing the culture medium to flow through it, with aeration pipes installed at the trench bottom to supply CO_2 [30]. In practical applications, these carbon-supplementation trenches are often designed in a funnel or conical shape to enhance flow dynamics. However, this method disrupts the conventional spatial configuration of open ponds, and over time, CO_2 can accumulate at the trench bottom, creating a mass-transfer dead zone and reducing the system's overall effectiveness. Increasing the aeration rate can mitigate the formation of dead zones, but it inevitably shortens bubble residence time, leading to greater CO_2 escape into the atmosphere. During large-scale *Spirulina* cultivation, the culture liquid, after being enriched with CO_2 in a carbon-supplementation tank, is returned to the cultivation pond for photosynthetic production [4,29]. The development and application of these carbon-supplementation technologies have increased the annual production of *Spirulina* by 20%, reduced annual sodium bicarbonate usage by 66%, and lowered carbon-source costs by 58% [31] (Figure 3).

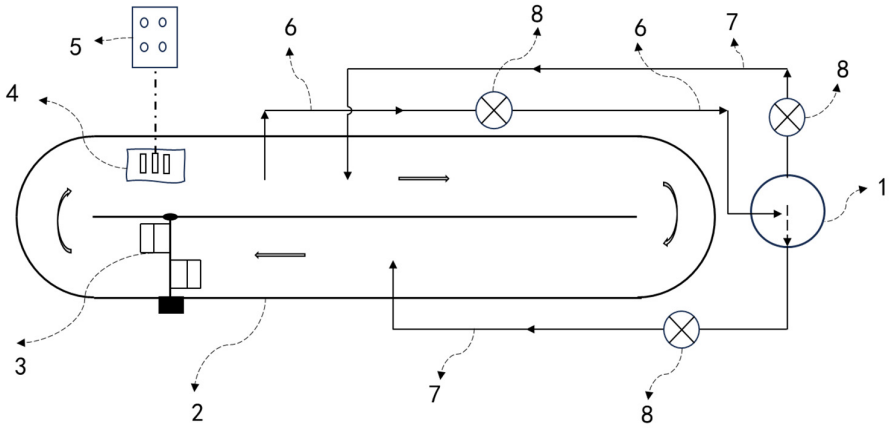

Figure 3. Microalgae cultivation system equipped with ex situ CO_2 supplementation devices. (1) vertical column CO_2 absorption tower, or CO_2 absorption tank, (2) open raceway pond, (3) paddle wheel, (4) pH/O_2 electrode, (5) control system, (6) medium outlet pipe, (7) medium inlet pipe, (8) circulation pump.

In summary, CO_2 supplementation strategies that extend gas–liquid contact time or enhance gas–liquid mixing intensity often come at the cost of increased energy consumption.

For instance, the incorporation of CO_2 supplementation trenches in raceway ponds prolongs bubble residence time; however, maintaining the same liquid flow rate results in an 80% increase in the energy consumption of the paddlewheel system [21]. In contrast, membrane-based technologies enhance CO_2 mass-transfer efficiency without significantly increasing energy demand. This approach diffuses CO_2 into the liquid medium through a nonporous hollow-fiber membrane, eliminating macroscopic bubbles. It achieves three times the CO_2 mass-transfer efficiency of conventional sparging while avoiding the shear forces associated with micro- and nano-bubbles that can damage microalgal cells.

3. Strategies for Enhancing CO_2 Mass Transfer Using Chemical Solvents

According to the two-membrane theory and the CO_2 mass-transfer model in the culture medium (Equation (1)), the primary resistance to CO_2 transfer occurs at the liquid membrane interface. In addition to increasing the overall volumetric mass-transfer coefficient by enhancing gas–liquid mixing intensity, increasing the gas–liquid contact specific area, and extending the gas–liquid contact time, the efficiency of CO_2 mass transfer in the culture medium can also be improved by introducing chemical reactions and altering the physical properties of the culture medium [32,33].

3.1. Novel Enhancing Mechanism of Introducing Chemical Reaction

During CO_2 transfer in the culture medium, it reacts with OH^- and CO_3^{2-} present in the medium. However, it has been indicated that these chemical reactions have minimal impact on CO_2 transfer and absorption, primarily because the optimal pH for most microalgae is close to neutral, resulting in low concentrations of OH^- and CO_3^{2-} in the medium under such conditions. If a high-concentration alkaline solution is thoroughly contacted with CO_2-containing gas in an ex situ absorption tower (Figure 3), the resulting carbon-rich solution can be used as a carbon source for microalgae cultivation, ensuring high CO_2 absorption rates and meeting the substantial carbon-source demand of microalgae. Zhu et al. used NaOH and Na_2CO_3 solutions as absorbents, and after convective mass transfer with CO_2-containing gas, the primary inorganic carbon species in the resulting CO_2-enriched solution was HCO_3^-. This solution was used as a carbon source to achieve high-density cultivation of *Spirulina*, alkali-resistant *Oscillatoria*, and *Isochrysis galbana*, resulting in high CO_2 utilization rates in closed floating photobioreactors [34–36]. However, as previously mentioned, the continuous proliferation of microalgal cells leads to an increase in the pH of the culture medium, promoting the conversion of HCO_3^- into CO_3^{2-}. Since CO_3^{2-} is not a bioavailable form of inorganic carbon for microalgae, this results in carbon-source wastage. Moreover, the use of NaOH or Na_2CO_3 solutions as absorbents elevates the Na^+ ion concentration in the culture medium, increasing extracellular osmotic pressure and adversely affecting microalgal growth. For instance, *Chlorella*, a typical freshwater microalga, can tolerate salinity levels of only 5–10‰. In addition to inhibiting growth, elevated salinity complicates the recycling and reuse of the culture medium, further reducing the efficiency of the cultivation process [37,38].

Our research team has explored the strategy of using "ammonium hydroxide" as a nitrogen source to enhance microalgae growth and CO_2 absorption. The primary product of CO_2 absorption by ammonia water is ammonium bicarbonate, which can be directly used by microalgae cells as both a carbon and nitrogen source without introducing additional metal ions. This strategy can significantly reduce nutrient costs in microalgae cultivation [39]. By combining pH-feedback CO_2 supplementation strategies with the metabolic kinetics of microalgae cells regarding nitrogen sources, stable control of low-concentration ammonium salts in the culture medium can be achieved, avoiding the inhibition of algal-cell growth and nitrogen-source loss due to ammonia volatilization [40]. Furthermore, using a closed

gas-lift reactor for cultivating *Chlorella* sp., CO_2 utilization rates reached 87.8%. In open raceway ponds, using a simple bottom-bubbling method for CO_2 supplementation resulted in a CO_2 utilization rate of 35.58%, which is an increase of 14.46% compared to the control group. However, 1 mol of ammonia water absorbs approximately 0.3–0.8 mol of CO_2. Based on the elemental composition of microalgae cells ($CH_{1.911}O_{0.496}N_{0.196}P_{0.007}S_{0.005}$), the required C/N ratio for the culture medium is approximately 5 [41,42]. Thus, when using the "ammonium hydroxide" strategy for microalgae cultivation, a substantial amount of CO_2 must still be supplemented to meet the fast growth requirements for carbon sources.

Amines are commonly used CO_2 absorbents in carbon capture, storage, and utilization (CCUS) applications [43]. These include monoethanolamine (MEA), diethanolamine (DEA), triethanolamine (TEA), and N-methyldiethanolamine (MDEA), which react reversibly with CO_2 as follows:

$$CO_2 + RNH_2 \rightleftarrows RNHCOO^- + H^+ \quad (2)$$

$$RNHCOO^- + H_2O \rightleftarrows RNH_2 + HCO_3^- \quad (3)$$

The impact of adding amine-based CO_2 absorbents to microalgae cultivation systems on carbon-source utilization and microalgae growth have been investigated by our team and the other researchers (Table 2) [44–67]. The introduction of chemical reactions increases the CO_2 mass-transfer rate (with a chemical absorption enhancement factor $\beta > 1.0$) [32]. Additionally, the resulting carbamate ($RNHCOO^-$) acts as a "CO_2 carrier", becoming the fourth form of carbon species in the culture medium. Under near-neutral pH conditions, as microalgae cells consume CO_2 and HCO_3^-, HCO_3^- is gradually released from the carbamate, acting as a slow-release carbon reservoir. It is indicated that the addition of amine-based CO_2 absorbents can enhance the biomass productivity of *Spirulina*, and *Chlorella*. In column reactors, CO_2 utilization efficiency increased from 44.5% to 76.1% [46–50]. It is worth noting that most amine molecules are not easily metabolized by microalgae cells, allowing them to act as a repeated CO_2 capture agent in the culture medium [48]. However, Rosa et al. found that high concentrations of chemical absorbents can inhibit microalgae growth. For instance, when MEA concentrations exceed 150 mg/L, microalgae biomass productivity decreases, and cell growth and intracellular metabolic activity are suppressed [51,52]. This phenomenon may be attributed to the corrosive nature of high-concentration amine solutions. Therefore, selecting an appropriate "CO_2 carrier" requires careful consideration of its biocompatibility.

Table 2. Effect of adding chemical absorbents or immobilized enzymes on microalgae growth and CO_2 utilization efficiency.

Absorbents or Immobilized Enzymes	CO_2 Content	Species	Biomass	CO_2 Utilization Efficiency	Reference
100 mg/L MEA	10%	*Scenedesmus dimorphus*	0.293 g/(L·d) ↑	76.1% ↑	[44]
6 mmol/L THAM	100%	*Scenedesmus dimorphus*	11.57 g/(m^2·d) ↑	35.58% ↑	[45]
2 mmol/L TEA	4%	*Scenedesmus* sp.	0.664 g/(L·d) ↑	No data	[46]
1.64 mol/L EDA + 0.41 mmol/L K_2CO_3	0.04%	*Spirulina* sp. LEB18	0.174 g/(L·d) ↑	No data	[47]
12% N-heptane	15%	*Chlorella* sp.	0.084 g/(L·d) ↑	64.7% ↑	[48]
100–150 mg/L MEA	50%	*Chlorella fusca LEB 111*	0.096–0.122 g/(L·d) ↓	37% ↑	[49]
1 mmol/L TMEDA	15%	*Chlorella* sp. *L166*	0.072 g/(L·d) ↑	43.29% ↑	[50]
CA–GA beads	Air	*Nannochloropsis salina*	0.040 g/(L·d) ↑	No data	[13]
Immobilized CA on ElectrospunNanofibers	15%	*Dunaliella. tertiolecta ATCC 30929*	6.8×10^5 cells/(mL·d) ↑	No data	[64]
Metal–organic frameworks	1.50%	*Scenedesmus obliquus*	0.240 g/(L·d) ↑	21.6% ↑	[65]
CA encapsulation using bamboo cellulose scaffolds	5%	*Chlorella vulgaris*	0.275 g/(L·d) ↑	No data	[67]

↑ indicates that the indicators of the experimental group have improved compared with the control group, ↓ indicates that the experimental group's indicators have improved compared with the control group.

3.2. Novel Enhancing Mechanism of Altering the Medium's Physical Properties

In industrial applications, methods for CO_2 capture from flue gases also include low-temperature methanol methods (Restisol process), ethylene glycol ether methods, propylene carbonate methods (Flour process), and N-methyl-2-pyrrolidone methods [53,54]. These compounds do not chemically react with CO_2 but enhance CO_2 solubility in the liquid phase by altering the physicochemical properties of the absorbent, such as reducing surface tension. Since CO_2 solubility in solvents follows Henry's Law, these absorbents generally have lower Henry's coefficients compared to aqueous solvents. Therefore, adding such absorbents to microalgae cultivation systems effectively increases the equilibrium concentration of CO_2 in the liquid phase, thereby promoting CO_2 absorption according to the mass-transfer model (Equation (1)) [50,55,56].

Using gas-lift photobioreactors to cultivate *Chlorella.* sp., our research indicated that the addition of four types of absorption enhancers—methanol, NHD, PC, and NMP—can significantly increase CO_2 utilization rates during cultivation, with an optimal condition improving CO_2 utilization by 71%, without significantly affecting the biochemical composition of the microalgae [55]. A method is utilized where a water-immiscible solvent is directly added to a microalgal culture, simultaneously allowing for increased CO_2 absorption by the algae while also extracting lipids from the cells in a single process, eliminating the need for separate cultivation and extraction steps; often, n-heptane is used as the water-immiscible solvent due to its ability to act as a "CO_2 sink" and readily extract lipids without significantly harming the algal cells [50].

4. Carbonic Anhydrase-Assisted CO_2 Absorption and Conversion

CO_2 absorption and conversion in microalgae culture can be broadly divided into two stages: gas–liquid mass transfer and biological conversion (Figure 4). After the gas–liquid mass transfer, the dissolved inorganic carbon sources include CO_2 and HCO_3^-. The latter is further absorbed by algal cells through two main pathways: active transport via membrane carrier proteins or conversion into CO_2 molecules under the action of extracellular carbonic anhydrase, which then rapidly diffuses into the cell [57–59]. Thus, highly active extracellular carbonic anhydrase facilitates CO_2 biological conversion, reducing the concentration of inorganic carbon sources in the liquid phase and promoting CO_2 gas–liquid mass transfer (Table 2). However, most microalgae exhibit low carbonic anhydrase activity under near-neutral conditions [60,61]. The addition of exogenous carbonic anhydrase may enhance microalgae's ability to absorb and convert CO_2, thereby influencing cell growth rates. However, the stability and durability of carbonic anhydrase in photobioreactors are currently poor [62]. In bubbling gas–liquid environments, shear forces significantly degrade enzyme activity, increasing the cost of microalgae cultivation beyond nutrient input and limiting the application of this strategy in microalgae cultivation technologies. The setup of efficient methods for enzyme immobilization makes carbonic anhydrase utilization in continuous bioreactors increasingly attractive and opens up new opportunities for the industrial use of carbonic anhydrase [12,63].

Xu et al. proposed a technique involving the addition of immobilized carbonic anhydrase microbeads to microalgae culture, which effectively addressed the issue of carbon limitation when cultivating microalgae with air CO_2 as the sole carbon source [13]. Jun et al. fixed carbonic anhydrase onto electrospun polymer nanofibers using enzyme deposition coating. This approach increased the microalgae growth rate by 134% in a bubbling reactor [64]. Yang et al. developed carbonic anhydrase-coated nylon fiber membranes and proposed a novel photobioreactor that improves CO_2 solubility and absorption rates in the microalgae solution. Testing revealed that CO_2 conversion rates increased by 62.7% with the use of this immobilized exogenous carbonic anhydrase [65]. Our team has isolated

mineralizing bacteria from soil environments that symbiotically coexist with *Chlorella* sp. These symbiotic bacteria can extensively express extracellular carbonic anhydrase. By optimizing algal–bacterial inoculation ratios and other parameters, we have developed a microalgae cultivation system that enhances CO_2 absorption through immobilized mineralizing bacteria [66]. Wang et al. utilized bamboo cellulose as a renewable porous scaffold to immobilize carbonic anhydrase through oxidation-induced aldehyde formation, followed by Schiff base linkage. The cellulose-immobilized enzyme significantly enhanced microalgal growth and biomass accumulation [67].

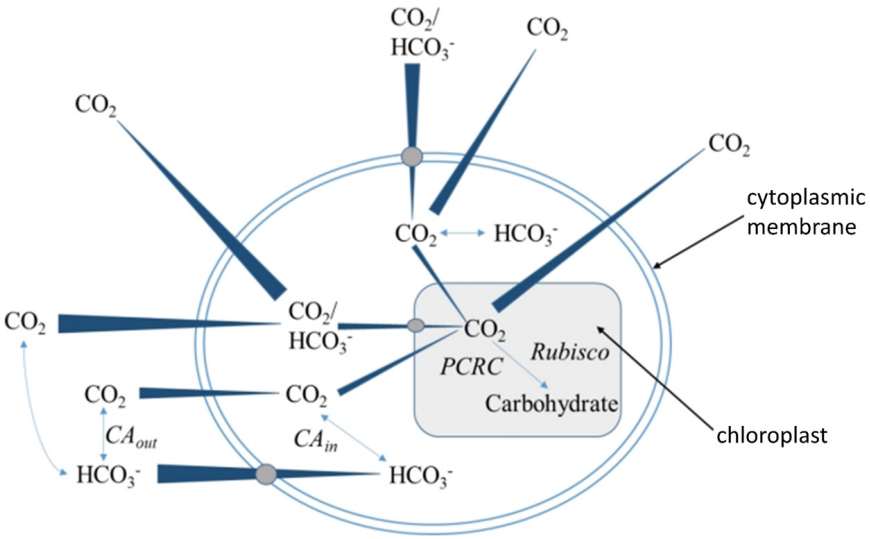

Figure 4. Diagrammatic representation of CO_2 absorption and conversion pathways in microalgae cells.

5. Challenges and Future Prospects

In the large-scale cultivation of alkaliphilic microalgae such as *Spirulina* (with an optimal pH range of 9.0–11.0), the combined use of CO_2 supplementation devices and pH-feedback CO_2 supplementation strategies can achieve carbon-source utilization rates exceeding 80%, effectively reducing cultivation costs. This is primarily due to higher CO_2 absorption rates under alkaline conditions and the higher total inorganic carbon concentration in the culture medium (maintained by adding large amounts of $NaHCO_3$), which supports rapid microalgal growth and carbon-source demands. In near-neutral (pH 6.0–8.0) cultivation systems (e.g., *Chlorella*, *Scenedesmus*, *Microcystis*, *Euglena*, and other economically significant algae), it is necessary to control the total inorganic carbon concentration in the carbon-rich culture medium from the supplementation device to prevent the loss of free CO_2 during circulation. This results in a lower total inorganic carbon concentration that only supports short-distance flow, depleting the carbon source and affecting algal biomass productivity.

For example, in a typical microalgae cultivation process with pH = 7, a liquid layer depth of 20 cm, an area productivity of 15 g/(m^2·d), and 30 °C, if the CO_2 in the culture medium is in equilibrium with the air upon leaving the supplementation point (meaning no CO_2 loss to the air), calculations show that the total inorganic carbon concentration drops to zero after 2 min of flow (although, in practice, microalgae growth is limited before reaching zero). At a flow velocity of 20 cm/s, this equates to a distance of 24 m. Thus, in fixed-scale raceway ponds, such as those with a perimeter of over 200 m in large-scale production, multiple carbon-supplementation points are needed to reduce CO_2 loss, ensure high CO_2 utilization rates, and maintain high algal-cell productivity. For a raceway pond with a single carbon-supplementation point and a perimeter of 200 m, the low concentration

of available carbon sources quickly leads to carbon-source limitation after leaving the supplementation point, impacting algal biomass productivity. Increasing the carbon-source concentration at the supplementation point forces CO_2 to escape, exacerbating losses.

Thus, CO_2 supplementation in microalgal photobioreactor systems must achieve both high CO_2 absorption rates at supplementation points and continuously increase the total inorganic carbon concentration in the culture medium. Future research should focus on multi-scale studies and the precise application of various strategies to optimize the CO_2 absorption process for microalgae.

Firstly, the kinetics of microalgae CO_2 absorption and conversion should be comprehensively studied. Utilize visual experimental methods to explore the kinetics of CO_2 dissolution and mass transfer, identify rate-limiting steps, and establish a balance between CO_2 supplementation, dissolution, transfer, and conversion processes. Precise control strategies and models should be developed. Secondly, create new and efficient microalgae cultivation systems by integrating in situ or ex situ setups with photobioreactor systems. Investigate low-energy methods for bubble nanonization, combine gas–liquid mixing with membrane technologies, and improve CO_2 mass-transfer efficiency under near-neutral pH conditions. Finally, select compounds or enzyme preparations with good biocompatibility that increase CO_2 solubility through chemical reactions or alterations in the physicochemical properties of the culture medium. This will enhance the CO_2 sink in microalgae cultivation systems, meet the rapid carbon-source demands of microalgae growth, reduce the frequency of carbon supplementation, and save energy.

6. Conclusions

This review highlights the various CO_2 supplementation strategies aimed at enhancing the efficiency of microalgae cultivation for biotechnological applications. Traditional aeration methods often result in low CO_2 absorption rates, which hinder microalgal growth and biomass productivity. To address these limitations, several innovative approaches have been developed. Direct CO_2 injection and advanced open-pond designs, such as trap-type carbon-replenishing devices and membrane-based systems, significantly enhance CO_2 mass-transfer efficiency. Modifications to closed photobioreactors further improve CO_2 utilization. Additionally, CO_2 absorption enhancer or immobilized carbonic anhydrase plays a crucial role in optimizing CO_2 absorption and promoting algal growth. Among these strategies, membrane-based CO_2 delivery systems and the incorporation of CO_2 absorption enhancers have shown the highest efficiency in boosting CO_2 mass transfer and microalgae productivity. Future efforts should focus on integrating these methods into large-scale photobioreactor systems to optimize cost-effective, sustainable production.

Author Contributions: Conceived the study, C.B., Z.S. and L.S.; analyzed the data, S.C. and Z.S.; wrote the paper, C.B. and Z.S.; revised the manuscript, L.S. and Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Natural Science Foundation of Shandong Province, grant number ZR2021MC097; National Natural Science Foundation of China, grant number 32102819.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Tang, D.Y.Y.; Khoo, K.S.; Chew, K.W.; Tao, Y.; Ho, S.-H.; Show, P.L. Potential utilization of bioproducts from microalgae for the quality enhancement of natural products. *Bioresour. Technol.* **2020**, *304*, 122997. [CrossRef] [PubMed]
2. Sathasivam, R.; Radhakrishnan, R.; Hashem, A.; Abd_Allah, E.F. Microalgae metabolites: A rich source for food and medicine. *Saudi J. Biol. Sci.* **2019**, *26*, 709–722. [CrossRef] [PubMed]
3. Ağbulut, Ü.; Sirohi, R.; Lichtfouse, E.; Chen, W.-H.; Len, C.; Show, P.L.; Le, A.T.; Nguyen, X.P.; Hoang, A.T. Microalgae bio-oil production by pyrolysis and hydrothermal liquefaction: Mechanism and characteristics. *Bioresour. Technol.* **2023**, *376*, 128860. [CrossRef] [PubMed]
4. Liu, X.Y.; Hong, Y.; He, Y.T.; Liu, Y. Growth and high-valued products accumulation characteristics of microalgae in saline-alkali leachate from Inner Mongolia. *Environ. Sci. Pollut. Res.* **2019**, *26*, 36985–36992. [CrossRef]
5. Torres-Tiji, Y.; Fields, F.J.; Mayfield, S.P. Microalgae as a future food source. *Biotechnol. Adv.* **2020**, *41*, 107536. [CrossRef]
6. Agbebi, T.V.; Ojo, E.O.; Watson, I.A. Towards optimal inorganic carbon delivery to microalgae culture. *Algal Res.* **2022**, *67*, 102841. [CrossRef]
7. Su, Z.F.; Kang, R.J.; Shi, S.Y.; Cong, W.; Cai, Z.L. An effective device for gas-liquid oxygen removal in enclosed microalgae culture. *Appl. Biochem. Biotechnol.* **2010**, *160*, 428–437. [CrossRef]
8. Su, Z.F.; Kang, R.J.; Shi, S.Y.; Cong, W.; Cai, Z.L. Study on the destabilization mixing in the flat plate photobioreactor by means of CFD. *Biomass Bioenergy* **2010**, *34*, 1879–1884. [CrossRef]
9. Vasumathi, K.K.; Premalatha, M.; Subramanian, P. Parameters influencing the design of photobioreactor for the growth of microalgae. *Renew. Sustain. Energy Rev.* **2012**, *16*, 5443–5450. [CrossRef]
10. Demirbas, A. Production economics of high-quality microalgae. *Energy Sources Part B Econ. Plan. Policy* **2017**, *12*, 395–401. [CrossRef]
11. Soares, F.R.; Martins, G.; Seo, E.S.M. An assessment of the economic aspects of CO_2 sequestration in a route for biodiesel production from microalgae. *Environ. Technol.* **2013**, *34*, 1777–1781. [CrossRef] [PubMed]
12. Russo, M.E.; Capasso, C.; Marzocchella, A.; Salatino, P. Immobilization of carbonic anhydrase for CO_2 capture and utilization. *Appl. Microbiol. Biotechnol.* **2022**, *106*, 3419–3430. [CrossRef] [PubMed]
13. Xu, X.; Kentish, S.E.; Martin, G.J.O. Direct air capture of CO_2 by microalgae with buoyant beads encapsulating carbonic anhydrase. *ACS Sustain. Chem. Eng.* **2021**, *9*, 9698–9706. [CrossRef]
14. Valdés, F.J.; Hernández, M.R.; Catalá, L.; Marcilla, A. Estimation of CO_2 stripping/CO_2 microalgae consumption ratios in a bubble column photobioreactor using the analysis of the pH profiles. Application to Nannochloropsis oculata microalgae culture. *Bioresour. Technol.* **2012**, *119*, 1–6. [CrossRef]
15. Ughetti, A.; Roncaglia, F.; Anderlini, B.; D'Eusanio, V.; Russo, A.L.; Forti, L. Integrated Carbonate-Based CO_2 Capture-Biofixation through Cyanobacteria. *Appl. Sci.* **2023**, *13*, 10779. [CrossRef]
16. Fu, J.W.; Huang, Y.; Liao, Q.; Xia, A.; Fu, Q.; Zhu, X. Photo-bioreactor design for microalgae: A review from the aspect of CO_2 transfer and conversion. *Bioresour. Technol.* **2019**, *292*, 121947. [CrossRef]
17. Ndiaye, M.; Gadoin, E.; Gentric, C. CO_2 gas-liquid mass transfer and K_L,a estimation: Numerical investigation in the context of airlift photobioreactor scale-up. *Chem. Eng. Res. Des.* **2018**, *133*, 90–102. [CrossRef]
18. Thobie, C.; Gadoin, E.; Blel, W.; Pruvost, J.; Gentric, C. Global characterization of hydrodynamics and gas-liquid mass transfer in a thin-gap bubble column intended for microalgae cultivation. *Chem. Eng. Process.* **2017**, *122*, 76–89. [CrossRef]
19. Kumar, A.; Yuan, X.; Sahu, A.K.; Dewulf, J.; Ergas, S.J.; Van Langenhove, H. A hollow fiber membrane photo-bioreactor for CO_2 sequestration from combustion gas coupled with wastewater treatment: A process engineering approach. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 387–394. [CrossRef]
20. Ferreira, B.S.; Fernandes, H.L.; Reis, A.; Mateus, M. Microporous hollow fibres for carbon dioxide absorption: Mass transfer model fitting and the supplying of carbon dioxide to microalgal cultures. *J. Chem. Technol. Biotechnol.* **1998**, *71*, 61–70. [CrossRef]
21. Ketheesan, B.; Nirmalakhandan, N. Development of a new airlift-driven raceway reactor for algal cultivation. *Appl. Energy* **2011**, *88*, 3370–3376. [CrossRef]
22. Bao, Y.L.; Liu, M.; Wu, X.; Cong, W.; Ning, Z.X. In situ carbon supplementation in large-scale cultivations of *Spirulina platensis* in open raceway pond. *Biotechnol. Bioprocess Eng.* **2012**, *17*, 93–99. [CrossRef]
23. Chen, Z.S.; Li, T.; Yang, B.J.; Jin, X.J.; Wu, H.L.; Wu, J.Y.; Lu, Y.D.; Xiang, W.Z. Isolation of a novel strain of *Cyanobacterium* sp. with good adaptation to extreme alkalinity and high polysaccharide yield. *J. Oceanol. Limnol.* **2021**, *39*, 1131–1142. [CrossRef]
24. Cong, W. Horizontal Submerged Hood Carbon Supplementation Device for Open Pond Microalgae Cultivation and Its Carbon Supplementation Method. CN201210138845.8, 30 April 2014.
25. Bergmann, P.; Ripplinger, P.; Beyer, L.; Trösch, W. Disposable Flat Panel Airlift Photobioreactors. *Chem. Ing. Tech.* **2013**, *85*, 202–205.

26. Huang, J.; Li, Y.; Wan, M.; Yan, Y.; Feng, F.; Qu, X.; Wang, J.; Shen, G.; Li, W.; Fan, J.; et al. Novel flat-plate photobioreactors for microalgae cultivation with special mixers to promote mixing along the light gradient. *Bioresour. Technol.* **2014**, *159*, 8–16. [CrossRef]
27. Razzak, S.A.; Al-Aslani, I.; Hossain, M.M. Hydrodynamics and mass transfer of CO_2 in water in a tubular photobioreactor. *Eng. Life Sci.* **2016**, *16*, 355–363. [CrossRef]
28. Putt, R.; Singh, M.; Chinnasamy, S.; Das, K.C. An efficient system for carbonation of high-rate algae pond water to enhance CO_2 mass transfer. *Bioresour. Technol.* **2011**, *102*, 3240–3245. [CrossRef]
29. Chen, J.; Wang, Y.; Benemann, J.R.; Zhang, X.; Hu, H.; Qin, S. Microalgal industry in China: Challenges and prospects. *J. Appl. Phycol.* **2016**, *28*, 715–725. [CrossRef]
30. Yan, C.H.; Wang, Z.; Wu, X.; Wen, S.M.; Yu, J.; Cong, W. Outdoor cultivation of *Chlorella* sp. in an improved thin-film flat-plate photobioreactor in desertification areas. *J. Biosci. Bioeng.* **2020**, *129*, 619–623. [CrossRef]
31. Gao, F.Z.; Ge, B.S.; Xiang, W.Z.; Qin, S. Development of microalgal industries in the past 60 years due to biotechnological research in China: A review. *Sci. Sin. Vitae* **2021**, *51*, 26–39.
32. Basheer, C.; Kamran, M.; Ashraf, M.; Lee, H.K. Enhancing liquid-phase microextraction efficiency through chemical reactions. *TrAC Trends Anal. Chem.* **2019**, *118*, 426–433. [CrossRef]
33. Morales, D.O.; Regalado-Méndez, A.; Pérez-Alonso, C.; Natividad, R. Physical and reactive absorption of CO_2 in capillaries: Mass transfer, modelling, and produced chemical species. *Chem. Eng. Res. Des.* **2023**, *198*, 247–258. [CrossRef]
34. Zhu, C.B.; Chen, S.L.; Ji, Y.; Schwaneberg, U.; Chi, Z.Y. Progress toward a bicarbonate-based microalgae production system. *Trends Biotechnol.* **2022**, *40*, 180–193. [CrossRef] [PubMed]
35. Zhu, C.B.; Zhang, R.L.; Cheng, L.Y.; Chi, Z.Y. A recycling culture of *Neochloris oleoabundans* in a bicarbonate-based integrated carbon capture and algae production system with harvesting by auto-flocculation. *Biotechnol. Biofuels* **2018**, *11*, 204. [CrossRef]
36. Zhu, C.B.; Zhu, H.; Cheng, L.Y.; Chi, Z.Y. Bicarbonate-based carbon capture and algal production system on ocean with floating inflatable-membrane photobioreactor. *J. Appl. Phycol.* **2018**, *30*, 875–885. [CrossRef]
37. Yang, J.; Xu, M.; Zhang, X.Z.; Hu, Q.A.; Sommerfeld, M.; Chen, Y.S. Life-cycle analysis on biodiesel production from microalgae: Water footprint and nutrients balance. *Bioresour. Technol.* **2011**, *102*, 159–165. [CrossRef]
38. Guieysse, B.; Béchet, Q.; Shilton, A. Variability and uncertainty in water demand and water footprint assessments of fresh algae cultivation based on case studies from five climatic regions. *Bioresour. Technol.* **2013**, *128*, 317–323. [CrossRef]
39. Sun, Z.L.; Sun, L.Q.; Liu, Y.H. The potential impact of replacing nitrate with ammonium hydroxide in microalgae production on the biomass productivity and CO_2 utilization efficiency. *Algal Res.* **2022**, *67*, 102870. [CrossRef]
40. Bao, Y.L.; Wen, S.M.; Cong, W.; Wu, X.; Ning, Z.X. An Optical-Density-Based Feedback Feeding Method for Ammonium Concentration Control in *Spirulina platensis* Cultivation. *J. Microbiol. Biotechnol.* **2012**, *22*, 967–974. [CrossRef]
41. Geider, R.; La Roche, J. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* **2002**, *37*, 1–17. [CrossRef]
42. Ho, T.-Y.; Quigg, A.; Finkel, Z.V.; Milligan, A.J.; Wyman, K.; Falkowski, P.G.; Morel, F.M.M. The elemental composition of some marine phytoplankton. *J. Phycol.* **2003**, *39*, 1145–1159. [CrossRef]
43. Dziejarski, B.; Krzyżyńska, R.; Andersson, K. Current status of carbon capture, utilization, and storage technologies in the global economy: A survey of technical assessment. *Fuel* **2023**, *342*, 127776. [CrossRef]
44. Sun, Z.L.; Xue, S.Z.; Yan, C.H.; Cong, W.; Kong, D.Z. Utilisation of tris(hydroxymethyl)aminomethane as a gas carrier in microalgal cultivation to enhance CO_2 utilisation and biomass production. *RSC Adv.* **2016**, *6*, 2703–2711. [CrossRef]
45. Sun, Z.L.; Zhang, D.M.; Yan, C.H.; Cong, W.; Lu, Y.M. Promotion of microalgal biomass production and efficient use of CO_2 from flue gas by monoethanolamine. *J. Chem. Technol. Biotechnol.* **2015**, *90*, 730–738. [CrossRef]
46. Cardias, B.B.; Morais, M.G.d.; Costa, J.A.V. CO_2 conversion by the integration of biological and chemical methods: *Spirulina* sp. LEB 18 cultivation with diethanolamine and potassium carbonate addition. *Bioresour. Technol.* **2018**, *267*, 77–83. [CrossRef]
47. Yin, Q.; Mao, W.; Chen, D.; Song, C. Effect of adding tertiary amine TMEDA and space hindered amine DACH on the CO_2 chemical absorption-microalgae conversion system. *Energy* **2023**, *263*, 125726. [CrossRef]
48. Kim, G.; Choi, W.; Lee, C.-H.; Lee, K. Enhancement of dissolved inorganic carbon and carbon fixation by green alga *Scenedesmus* sp. in the presence of alkanolamine CO_2 absorbents. *Biochem. Eng. J.* **2013**, *78*, 18–23. [CrossRef]
49. Rosa, G.M.d.; Morais, M.G.d.; Costa, J.A.V. Fed-batch cultivation with CO_2 and monoethanolamine: Influence on *Chlorella fusca* LEB 111 cultivation, carbon biofixation, and biomolecules production. *Bioresour. Technol.* **2019**, *273*, 627–633. [CrossRef]
50. Bai, L.; Lu, S.J.; Qiu, S.; Li, J.; Chen, S.M. Single-step integrated technology for enhanced CO_2 biofixation and efficient lipid extraction in microalgal system including a water-immiscible solvent. *Chem. Eng. J.* **2022**, *432*, 134374. [CrossRef]
51. Rosa, G.M.d.; Moraes, L.; de Souza, M.d.R.A.Z.; Costa, J.A.V. Spirulina cultivation with a CO_2 absorbent: Influence on growth parameters and macromolecule production. *Bioresour. Technol.* **2016**, *200*, 528–534. [CrossRef]
52. Rosa, G.M.d.; Morais, M.G.d.; Costa, J.A.V. Green alga cultivation with monoethanolamine: Evaluation of CO_2 fixation and macromolecule production. *Bioresour. Technol.* **2018**, *261*, 206–212. [CrossRef] [PubMed]

53. Chang, Y.; Gao, S.; Ma, Q.; Wei, Y.; Li, G. Techno-economic analysis of carbon capture and utilization technologies and implications for China. *Renew. Sustain. Energy Rev.* **2024**, *199*, 114550. [CrossRef]
54. Madejski, P.; Chmiel, K.; Subramanian, N.; Kus, T. Methods and Techniques for CO_2 Capture: Review of Potential Solutions and Applications in Modern Energy Technologies. *Energies* **2022**, *15*, 887. [CrossRef]
55. Sun, Z.L.; Xin, M.R.; Li, P.; Sun, L.Q.; Wang, S.K. Enhancing CO_2 utilization by a physical absorption-based technique in microalgae culture. *Bioprocess Biosyst. Eng.* **2021**, *44*, 1901–1912. [CrossRef]
56. Li, J.; Zhao, X.; Chang, J.-S.; Miao, X. A Two-Stage Culture Strategy for *Scenedesmus* sp. FSP3 for CO_2 Fixation and the Simultaneous Production of Lutein under Light and Salt Stress. *Molecules* **2022**, *27*, 7497. [CrossRef]
57. Wang, Y.J.; Stessman, D.J.; Spalding, M.H. The CO_2 concentrating mechanism and photosynthetic carbon assimilation in limiting CO_2: How Chlamydomonas works against the gradient. *Plant J.* **2015**, *82*, 429–448. [CrossRef]
58. Raven, J.A.; Beardall, J.; Giordano, M. Energy costs of carbon dioxide concentrating mechanisms in aquatic organisms. *Photosynth. Res.* **2014**, *121*, 111–124. [CrossRef]
59. Yao, D.; Wu, L.; Tan, D.; Yu, Y.; Jiang, Q.; Wu, H.; Wang, Y.; Liu, Y. Enhancing CO_2 fixation by microalgae in a Photobioreactor: Molecular mechanisms with exogenous carbonic anhydrase. *Bioresour. Technol.* **2024**, *408*, 131176. [CrossRef]
60. Swarnalatha, G.V.; Hegde, N.S.; Chauhan, V.S.; Sarada, R. The effect of carbon dioxide rich environment on carbonic anhydrase activity, growth and metabolite production in indigenous freshwater microalgae. *Algal Res.* **2015**, *9*, 151–159. [CrossRef]
61. van Hille, R.; Fagan, M.; Bromfield, L.; Pott, R. A modified pH drift assay for inorganic carbon accumulation and external carbonic anhydrase activity in microalgae. *J. Appl. Phycol.* **2014**, *26*, 377–385. [CrossRef]
62. González, J.M.; Fisher, S.Z. Carbonic Anhydrases in Industrial Applications. In *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications*; Frost, S.C., McKenna, R., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 405–426.
63. Lin, J.Y.; Sri Wahyu Effendi, S.; Ng, I.S. Enhanced carbon capture and utilization (CCU) using heterologous carbonic anhydrase in *Chlamydomonas* reinhardtii for lutein and lipid production. *Bioresour. Technol.* **2022**, *351*, 127009. [CrossRef] [PubMed]
64. Jun, S.H.; Yang, J.; Jeon, H.; Kim, H.S.; Pack, S.P.; Jin, E.; Kim, J. Stabilized and immobilized carbonic anhydrase on electrospun nanofibers for enzymatic CO_2 conversion and utilization in expedited microalgal growth. *Environ. Sci. Technol.* **2020**, *54*, 1223–1231. [CrossRef] [PubMed]
65. Yang, Y.W.; Li, M.J.; Hung, T.C. Enhancing CO_2 dissolution and inorganic carbon conversion by metal–organic frameworks improves microalgal growth and carbon fixation efficiency. *Bioresour. Technol.* **2024**, *407*, 131113. [CrossRef] [PubMed]
66. Xin, M.R. Study on the Effect of Exogenous Carbonic Anhydrase in Promoting Carbon Absorption in Microalgae Cells. Master's Thesis, Yantai University, Yantai, China, 2022. [CrossRef]
67. Wang, X.; Li, M.; Liu, Z.; Shi, Z.; Yu, D.; Ge, B.; Huang, F. Carbonic anhydrase encapsulation using bamboo cellulose scaffolds for efficient CO_2 capture and conversion. *Int. J. Biol. Macromol.* **2024**, *277*, 134410. [CrossRef]