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Microalgae for CO₂ Biofixation and Biomass Production: A Critical and Comprehensive Review of Mechanisms, Systems, Challenges, and Future Perspectives

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Abstract: The rapidly growing atmospheric CO₂ concentrations have made effective and efficient carbon capture, utilization and storage (CCUS) technologies evermore imperative. Biological routes that employ microalgae have shown immense potential for CO₂ biofixation due to their inherent efficient photosynthesis and fast growth rates, wide environmental range and generation of high value biomass. In contrast to successful advancements made at lab scale, challenges pertaining to technology, energy, economics, and ecology have severely limited large scale industrial cultivation of microalgae for CO₂ mitigation. This review proposes a critical and detailed examination of microalgal CO₂ biofixation processes, cultivation technologies, industrial constraints and avenues of future work. Mechanisms of microalgal CO₂ biofixation such as the Calvin-Benson-Bassham pathway, RuBisCO activity, and carbon concentrating mechanisms (CCMs) along with different open raceway ponds, photobioreactors, and combined cultivating techniques were thoroughly investigated. The review highlights the major bottlenecks such as reduction in productivity under outdoor cultivation, high energy input for the system and harvesting as well as biological security issues associated with genetically modified strains (which cause a 'bench-to-business' transition gap). A seven-pillar conceptual framework (SCALE-UP) that incorporates biological resistance, optimized cultivation, energy efficient harvesting, efficient utilization of all the generated biomass and resources and the establishment of benchmarks to measure industrial productivity, was then introduced to overcome the above-mentioned bottlenecks. Specific research strategies have been proposed for sustainable industrial application of microalgal CO₂ biofixation techniques at industrial level and in economically feasible manner.

Citation: Mjali H. A. A. K. Microalgae for CO₂ Biofixation and Biomass Production: A Critical and Comprehensive Review of Mechanisms, Systems, Challenges, and Future Perspectives. Central Asian Journal of Medical and Natural Science 2026, 7(3), 255-270.

Received: 10th Feb 2026

Revised: 21th Mar 2026

Accepted: 20th Apr 2026

Published: 27th May 2026

Keywords: Microalgae, CO₂ biofixation, SCALE-UP framework, bench-to-business gap, biomass production.



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1. Introduction

The ever growing amount of carbon dioxide in the atmosphere, induced mainly from the burning of fossil fuels has emerged as the most urgent environmental and energy problems in the world. Climate-neutral goals have been stated as high priority at the global level (e.g., The Paris Agreement, European Green Deal) to meet with effective CCUS methods [1],[2] Conventional techniques (e.g., amine absorption, membrane separation and mineral carbonation) are technically feasible but still hampered due to the higher energy requirement and cost, and thus CCUS is still one of the most feasible strategies to reduce CO₂ emission [3], [4]

CO fixation technologies can be categorized into physical, chemical and biological types[5]. Physical storage technologies which are defined as deep sea storage and geological storage may have problems on the stability, space and economical issues[6]. Chemical fixing technologies utilize the adsorbents or alkaline reagents to change CO to the carbonate and bicarbonate compound. This method will consume lot of reagents and needs high costs[7],[8]. In contrast, biological fixing technology is to convert CO into organic matters through photosynthesis and has the merit of preserving the balance between carbon-oxygen in the atmosphere. Among the biological systems, microalgae can be considered as potential candidate due to their rapid growth rate, high photosynthetic activity, broad tolerance of environmental conditions, and the capacity to be cultivated on saline water or waste water without occupying productive land [9], [10].

The microalgae are able to assimilate carbon using solar energy via photosynthesis such as the Calvin-Benson cycle for carbon fixation [5] The CO fixation efficiency of microalgae may be significantly higher than that of terrestrial plants and 1.83 g of CO is theoretic mass that could be fixed per gram of biomass formed[10],[11]. Apart from the CO fixation, the biomasses of microalgae which is composed of valuable lipids, proteins, polysaccharides could also be further utilized as source for bio-fuel, waste-water treatment, pharmaceuticals, food and biochemical industries [12] Indeed, several investigations reported these multifunctional roles of microalgae. Yahya et al. Presented microalgae in contribution to circular economy practices in sustainable coal-fired power plants system[13].and Dasan et al. Improved the CO₂ fixation of *Chlorella vulgaris* by the optimized culture condition [14]. In addition, Premaratne et al. Investigated carbon capture of *Desmodesmus* sp. Under nitrogen limitation followed by biomass cultivation and utilization to biofuel production [15]. while Ding et al. Reported the potential of native microalgae in abatement of industrial carbon emissions along with treatment of Palm Oil Mill Effluents (POME) [16].

Although more studies and reviews on microalgal CO fixation were published in recent years, previous reviews usually only highlighted the individual effect of single factor such as cultivation strategy, photobioreactor operation or wastewater remediation on microalgal CO₂ biofixation [17],[18]. The "system" barrier to move microalgal technology from laboratory investigation to commercial production received relatively less attention.

This review believes the primary limitation of microalgal CO₂ biofixation is not in the biological ability of microalgae themselves, but rather a "bench-to-business transition gap" including scalability, energy input, system integration, economic viability, and ecological resilience. This review gives a comprehensive critical review on the microalgal CO₂ biofixation and biomass production in an integrated "system" point of view, with a focus not only on the isolated productivity-based assessment, but also in proposing a novel 7- pillar conceptual framework named "SCALE-UP" for analyzing the transition of laboratory study to industrial application. Seven factors of the "SCALE-UP" are covered in the review, namely (i) mechanism of CO₂ fixation, (ii) cultivation system, (iii) biomass processing and biorefinery system, (iv) bottlenecks in current technology and economics and (v) engineering solutions and policy recommendation for industrial scale application.

2. Materials and Methods

2.1 Search Strategy and Data Sources

A critical review has been performed by reviewing literature sourced from three science databases: Scopus, Web of Science and Google Scholar. The literature searches are typically in the 2010-2025 range; however, to provide an accurate representation of current microalgal CO₂ biofixation and to supply a current overview literature review, the focus is more on 2018. Boolean searches of the engineering and biological literature surrounding microalgal CO₂ biofixation using the keywords 'microalgae AND CO₂ fixation', 'RuBisCO AND carbon concentrating mechanism' and

'photobioreactor AND biomass productivity.' Following literature searches and review of [72] all received peer-reviewed papers were scrutinized.

2.2 Inclusion and Quality Criteria

All literature received was screened on the basis of the scientific accuracy of its contents and the presence and relevance of quantitative data to the extent of this review. Specifically the evidence provided related to biomass productivity, energy balance and rate of CO₂ capture was considered. Unsound statistical analysis, overgeneralization of lab data into the real world' were avoided in convention papers and abstracts. The limitation of results of the overview was considered, taking account of possible book bias toward positive findings, section 7.2.

2.3 Framework Development (SCALE-UP)

The information obtained through the literature review is present within the derivation of the SCALE-UP framework presented within section 5, serving as a conceptual framework that aims to fill the gap between laboratory science and microalgal CO₂ biofixation scale-up to have global impact.

3. Results and Discussion

3.1 Biology and Diversity of Microalgae

3.1.1 Taxonomic Overview and Phylogenetic Diversity

Microalgae represent an extremely diverse polyphyletic group of photoautotrophic organisms spread across diverse phyla and include numerous environmentally and industrially significant eukaryotic phyla of algae such as *Chlorophyta*, *Cyanophyta*, *Bacillariophyta* and *Eustigmatophyta*[19]. *Chlorophyta* itself forms the best studied group of *eukaryotic phyla*, comprising *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Scenedesmus obliquus* [20]. Evolutionary significant in the context of oxygenation of Earth's atmosphere and special CO₂-concentrating structures (*carboxysomes*) found in them (*Cyanobacteria* are prokaryotes) [21]. Though the diversity of microalgae is fairly established, only a fraction of existent biodiversity is characterized and even lesser fraction has been exploited industrially. This undiscovered biodiversity is one of the most promising areas for finding strains with improved CO₂ fixation efficiency and resistance to various stress factors [22].

3.1.2 Key Growth Factors Governing CO₂ Fixation and Productivity

Photosynthesis in microalgae couples light dependent energy transduction with inorganic carbon uptake for biomass production. However the efficiency of productivity is often dictated by environmental factors and limited by light [23] [23]. While limiting light availability affects energy availability, high light conditions can cause photoinhibition due to accumulation of ROS, leading to disruption of photosynthetic complexes [24]. Hence, controlling light intensity, exposure time and spectral composition is necessary for efficient productivity [25]. Because of the low catalytic rate of RuBisCO under ambient CO₂ levels, microalgae have evolved carbon concentrating mechanisms (CCMs), which enhance the CO₂ concentration at the active site of the enzyme [26]. The role of carboxylsomes in this function in cyanobacteria is the main mechanism, while the eukaryotes form pyrenoids within chloroplasts. These are involved in active transport of inorganic carbon, increasing the efficiency of carbon fixation and lowering photorespiration [27] [28]. In addition to uptake of carbon CCMs help maintain the intracellular homeostasis, through a balanced flow of carbon within the cell and controlling the metabolism under changes in the environment [29]

3.2 Mechanisms of CO₂ Sequestration

3.2.1 The Calvin-Benson-Bassham (CBB) Cycle

The main pathway used by microalgae to fix CO₂ and convert inorganic carbon to triose phosphates is the Calvin-Benson-Bassham (CBB) cycle, consisting of a series of

enzyme catalyzed reactions [30]. The cycle has three functional stages, carboxylation, reduction, and regeneration, whereby the first stage involves carboxylation, where RuBisCO fixes CO₂ with RuBP yielding 3-phosphoglycerate [31]. Despite being highly conserved, the performance of the CBB cycle is limited by the catalytic capacities of the RuBisCO. Microalgae have some workarounds for this limitation by concentrating CO₂ through carbon concentrating mechanisms that enhance localized CO₂ concentrations, and in turn, overall carbon assimilation rates. The optimization of this pathway through metabolic engineering will have direct impact on biomass production and lipid yield[32],[33].

3.2.2 RuBisCO: Catalytic Properties, Limitations, and Engineering Perspectives

The key enzyme of the process of carbon assimilation by microalgae is RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), which is responsible for the first reaction of the carbon assimilation by fixing CO₂ into ribulose-1,5-bisphosphate. This is an essential step of the metabolism, but a low turnover enzyme and the presence of two substrate specificities, CO and O, make it so that some of the assimilated carbon will be lost through the oxygenase activity and the photorespiration. Initially, the evolutionary constraints of RuBisCO were believed to be linked to trade-offs between enzyme kinetics and specificity. However, a growing body of evidence indicates that the evolutionary constraint is not strict and naturally occurring algal lineages with more efficient RuBisCO kinetics exist. This is why engineered strategies so far have aimed to optimize the system and not the enzyme itself, focusing on improved carbon-concentrating mechanisms, optimized microcompartment structure (e.g. Pyrenoids) and synthetic biology (e.g., expression of an alternative carbon fixation pathway). Integration with computational models and the process digitalization represent the more recent, most promising research directions for optimizing overall performance in industrial settings[34],[35]

3.2.3 Carbon-Concentrating Mechanisms (CCMs)

Carbon concentrating mechanisms are the key element responsible for increasing efficiency of microalgal CO fixation, by elevating the local concentration of inorganic carbon around RuBisCO. These mechanisms are highly regulated by environment conditions such as light intensity, as well as carbon concentration, which strongly points out on close coupling between carbon metabolism and photosynthetic energy capture[36]. They are designed to improve efficiency of carbon uptake, and also simultaneously protect photosynthetic apparatus against photoinhibition at high light intensity. Through these dual roles, CCMs maintain stability of metabolism under various environment condition and ensure that carbon is directed to growth and synthesis of storage compounds, such as lipids and carbohydrates [37].

3.3 Microalgal Cultivation Systems for CO₂ Capture

3.3.1 Open Raceway Ponds (ORPs)

In general, Open Raceway ponds (ORP) consists of shallow oval-shaped raceways in which the culture is mixed and recirculated by a paddle wheel. Some apparent advantages are the low cost of installation, operating costs, and scale-up potential, but it has many disadvantages such as lower biomass productivity relative to closed PBRs, potential for contamination, culture crash, significant water evaporation rate, and poor control of cultivation[38].

3.3.2 Closed Photobioreactors (PBRs)

Photobioreactors are enclosed cultivation systems. Due to effective control of culture conditions, the biomass productivity can be much higher than that obtained by open systems. In fact, average concentration of PBRs is estimated to be around 8kg/m, or 8 times the biomass concentration of open raceway ponds. To achieve that level of biomass productivity, photobioreactors consumes considerably larger amounts of energy input;

one of them is to provide artificial light, regulate the temperature and circulate the culture [39].

3.3.3 Hybrid Systems and Integration with Industrial CO₂ Sources

Hybrid systems (combination of open and closed cultivation systems) perform reasonably well in terms of biomass concentration and areal production. The rate of energy consumed is moderate, which makes it possible to have both a high productivity of PBR and saving the energy consumed in open pond. Though hybrid systems seek to integrate the benefit from both open and closed systems, they might give ambiguous results depending on how they are designed and operated [40].

Table 1. Comparative analysis of microalgal cultivation systems for CO₂ capture.

Dimension	Open Raceway Ponds (ORPs)	Closed Photobioreactors (PBRs)	Hybrid Systems	Lab-to-Scale Interpretation	Ref.
Biomass Density	Low	High	Intermediate	Higher biomass in PBRs significantly improves productivity but increases cost and harvesting complexity	[38]
Energy Input	Low	High	Moderate	Increased energy demand in PBRs limits large-scale sustainability despite higher efficiency	[39]
Operational Control	Low	High	Moderate	Limited control in ORPs leads to variability and reduced process stability at scale	[40]
Contamination Risk	High	Low	Variable	Open systems are more vulnerable to contamination, affecting long-term industrial reliability	[39]
Economic Scalability	High scalability, low cost	Limited scalability, high cost	Balanced	Trade-off exists between cost efficiency (ORPs) and productivity (PBRs) at industrial scale	[38,39]

In table 1: Comparison between the above shows that a particular cultivation system could not fulfill the demand of high productivity, low energy input, work-ability and cost-effective in a scale-up manner.

3.4 Biomass Production and Downstream Applications

Among the main technical barriers encountered in industrial CO₂ biofixation, the large-scale production of microalgal biomass is one of the biggest ones. Despite yielding satisfactory results at laboratory-scale, high biomass production under controlled environment has proven to be more complicated at the industrial level. In large scale photobioreactors light attenuation, hydrodynamics and mass transfer limitation may significantly decrease the photosynthetic performance and biomass productivity [41].

Light intensity is the most significant factor that influences the photosynthesis and biomass accumulation of microalgae[42] and the optimal light irradiance used for microalgae may vary across strains from 26 up to 400 mol photons m⁻² s⁻¹[43]. As cells become very dense, self-shading occurs, and consequently the cells themselves inhibit light access to other cells, and this reduces the overall photosynthesis. High light intensity can induce photoinhibition and damage the cellular activity and thus biomass yield. ATP and NADPH are produced through photosynthesis of microalgae, and are utilized for fixation of CO₂ and biosynthesis of valuable compounds[44]. Thus industrial microalgal strains

should be efficient in photosynthesis and in light use and also show tolerance to photoinhibition. Light-dark cycles play also a significant role in biomass accumulation; proper optimization of light-dark periods can increase the photosynthesis and the metabolic rate [45]. Nutrient provision is a critical factor for microalgal growth and metabolism. Nutrient uptake and use depend to a great extent on the pH value of culture media [46]. Most microalgae grow optimally between the range of 6 and 10 for pH value; in this pH range nutrient availability is quite higher and the metabolic system of microalgae is also quite stable.

In summary of these technical aspects, the principal factors influencing biomass productivity and large scale microalgae cultivation performance are presented in Table 2.

Table 2. Key Technical Factors Influencing Biomass productivity and Scale-Up Performance.

Factor	Core Principle	Key Focus	Target / Outcome	Ref
Biomass Scalability	Bridging laboratory and field-scale performance gaps	Scale-up efficiency	Improved consistency between lab and outdoor productivity	[41,42,47]
Radiance Optimization	Reducing self-shading in dense cultures	Light utilization	Improved photosynthetic efficiency	[43]
Inhibition Control	Reducing photoinhibition effects	Stress tolerance	Stable growth under high light conditions	[24,25]
Metabolic Efficiency	Enhancing carbon fixation efficiency	Energy conversion	Increased biomass productivity	[44]
Light-Dark Regulation	Optimizing illumination cycles	Temporal control	Improved growth performance	[45]
pH and Nutrient Stability	Maintaining optimal culture conditions	Culture stability	Stable metabolic activity	[46]

Table 2 is a summarizes the principal technical factors affecting biomass productivity and industrial scale-up performance in microalgae cultivation system.

4. Challenges: Anatomy of the Bench-To-Industrial Gap

One main thesis of this review is that there is a pervasive, multi-dimensional gap between lab potential and commercial application in the field of microalgal CO₂ capture. In this section we quantify the four principal dimensions of this gap with discrete quality ratings.

4.1 The Productivity Gap: Laboratory vs. Outdoor Reality

One of the most prevalent barriers to industrial application of microalgal CO₂ biofixation is the large discrepancy in productivity between laboratory controlled and large scale outdoor cultivation. Whilst many laboratory investigations report very high biomass productivities and photosynthetic efficiencies with optimized conditions, this does not generally translate to large-scale industrial situations. Long term outdoor

cultivation (>1,000 m²) have shown average biomass productivities typically of around only 6 g-DWm⁻²d⁻¹ which is primarily attributed to the poor control over environmental conditions that is afforded to outdoor systems; the performance and biomass accumulation is greatly influenced by variability in incident light, temperature, contamination, and nutrients[47].

Immaturity of the industrial process is further illustrated by the typical scale of current operations; even the largest microalgal production systems are typically no larger than 36-44ha, with costs estimated to reach up to \$315 kg⁻¹ in some situations [48]. Additionally, to achieve industrially significant carbon mitigation, immense scale cultivation systems would need to be deployed. For example, treatment of wastewater from 1 million people would require 600-1,200 ha while replacing 5-10% of transportation fuel demands with algal biodiesel alone would require > 5,000 ha. [49].

These factors highlight that simply employing improved strains, or enhancing optimization of laboratory conditions is insufficient to overcome industrial productivity shortcomings, and large-scale improvements can only come through enhancements in photobioreactor design, light delivery systems, hydrodynamics and integration capable of maintaining robust photosynthetic productivity in an industrial setting.

4.2 The Energy Balance Gap

Microalgal carbon capture technology cost currently varies between 702 and 1600 USD per tonne of CO₂ captured, a figure far greater than established chemical carbon capture techniques. The cost is strongly influenced by the system being utilized for growth and the specific intended end use for the biomass (biofuel, high value bioproducts, etc.). Even with improved technologies, the cost is predicted to not fall below around 225 USD/t in the immediate future [50] The NER for biodiesel from open pond systems varies from 0.20 in the worst-case scenarios to 1.08 in the best. Horizontal tubular photobioreactors have an unfavorable energy balance (NER < 1) with demands for pumping being around 2,500 W m² as compared to 4 W m² in open ponds, a substantial difference; biomass costs are also predicted to be ~0.54 \$/kg, whereas they could be anywhere from 3.5 to 10 \$/kg in closed photobioreactors. At present, compared to the range of 50-156 EUR per tonne, amine-based CO₂ capture methods do not look to be particularly competitive, though this is an incomplete comparison; a wide range of valuable co-products may also be generated through microalgae (astaxanthin, proteins, omega 3). When income generated from these is factored in, the net cost of CO₂ capture may become zero and indeed negative [51].

4.3 Harvesting and Downstream Processing

Harvesting remains the most critical 'engineering bottleneck' within the anatomy of the bench-to-business gap. Harvesting is frequently a rapid high-speed centrifugation process in the laboratory but the large energy cost of this approach (0.7-1.3 KWhm) can be uneconomical for an operation above 100ha. The company is confronted by a "Flocculation Paradox"-although chemical coagulants represent a high capacity, low energy (< 0.1 KWhm) option, they invariably produce metal residuals (Al or Fe) which may not meet product quality standards and which limit the biomass to applications in low-value markets. Recent research focuses on 'Smart Harvesting' approaches, e.g. Electro-coagulation and Bio-flocculation, aimed at achieving high yields while meeting strict product quality standards for food-grade products[52].

4.4 Genetic Engineering: The Regulatory Ecology Gap

Genetically engineered microalgae provide a viable platform for production of expensive secondary metabolites like carotenoids and terpenoids with pharmaceutical and industrial applications [53] A variety of highly powerful genetic engineering tools like CRISPR/Cas9 and transformation approaches such as electroporation and Agrobacterium-mediated delivery have achieved remarkable success in improving metabolic production in tightly controlled laboratory systems [54].

However, a tremendous regulatory and environmental chasm lies between laboratory and outdoor industrial application. Laboratory conditions could not completely recreate biotic interactions unpredictable environmental fluctuations, or long-term genetic drift associated with outdoor cultivation environments[55]. Strains that achieve efficient productivity in lab settings are often more likely to fail due to unstable metabolic production and unpredictability when grown outdoors or semi-outdoors. There are also numerous bio-safety implications that could be presented through potential "genetic escape" of engineered organisms into the environment. Developing safe outdoor cultivation of genetically engineered microalgae may necessitate approaches based on ecological engineering, as well as AI predictive modeling [56].

Table 3. Gap Analysis of Genetically Engineered Microalgae from Laboratory Research to Outdoor Applications.

Gap Dimension	Evidence Level	Magnitude	Primary Driver	Mitigation Priority
Metabolic Stability	High	Efficiency loss in outdoor systems	Environmental fluctuations and genetic drift	■■■■■ Critical
Biosafety & Containment	Very High	Risk of genetic escape	Lack of robust containment strategies	■■■■■ Critical
Regulatory Alignment	High	Lack of standardized frameworks	Dependence on lab-scale data	■■■■ High
Biotic Interactions	Moderate	Unpredictable performance	Complex ecosystem dynamics	■■■■ High
Predictive Modeling	Moderate–High	Lab-to-field mismatch	Limited AI/ecological models	■■■ Medium

Ref. [53–56]. The dimensions present in Table 3 are derived from synthesis of reported challenges in the literature regarding the transition of genetically engineered microalgae from laboratory to outdoor environments

5. The Scale-Up Framework: A Roadmap for Industrial Translation

Extending the constraints mentioned in above sections, this work presents SCALE-UP as a conceptual framework to translate laboratory scale microalgae CO₂ biofixation studies into an industrially and climatologically relevant scale. The framework integrates seven interlinked components that addresses the main constraints regarding the productivity, scalability, energy input, environment stability and economics and instead of treating these constraints separately, the SCALE-UP framework is developed to transform the laboratory scale limitations into industrial goals, as outlined in Table 4.

Table 4. SCALE-UP Framework for Industrial Microalgae CO₂ Biofixation Optimization.

Pillar	Core Principle	Key Focus	Target / Outcome	Ref.
Synergetic Cultivation	Integration of ORPs and PBRs	Hybrid cultivation systems	Balanced productivity and cost efficiency	[38,39,40]
Carbon Concentrating	Optimization of CCMs	Cellular associated carbon fixation	Improved carbon assimilation efficiency	[26,29,34,35]
Adaptive Resilience	Stress-adapted strain development	Outdoor stability	Improved environmental robustness	[47,58]

Light Management	Optimized light distribution	light	Photosynthetic efficiency	Reduced photoinhibition effects	[24,25,34]
Energy-Neutral Harvest	Low-energy harvesting methods		Downstream processing	Reduced energy demand	[52]
Utility & Circularity	Wastewater and flue gas integration	and	Resource recycling	Improved sustainability	[41,42,59,60]
Productivity Benchmarking	Realistic productivity estimation		Lab-to-field translation	Improved scale-up realism	[47, [57]

Note: The SCALE-UP framework is a conceptual synthesis derived from the literature and is intended as a strategic guideline for improving the industrial translation of microalgal CO₂ biofixation.

5.1 Adaptive Laboratory Evolution: The Biological Resilience Pillar

This section details the operational role of ALE in bridging the bench-to-business gap based on the following methodology [58] :

1. **Non-Transgenic Strategy:** ALE is a potential non-transgenic method to enhance genetic engineering approaches such as CRISPR/Cas9. Microalgal strains are susceptible to accumulating spontaneous mutations with the right traits following the long-term application of an appropriate selection stress, CO₂, high temperature, low pH, and flue gas components, among others. Thus, an appropriate phenotype with better traits can be selected, and foreign gene elements need not be introduced to avoid possible GMO regulatory restrictions
2. **Empirical Proof-of-Concept:** In a study published in 2023, Su et al. developed *Phaeodactylum tricornutum* under low pH stress and obtained improved strains with average growth increases of 110.4%, 46.1%, and 27.5% at pH 5.5, 6.0, and 6.5, respectively, compared to the wild-type original strain.

5.2 Wastewater Integration: The Circular Economy Enabler

To overcome the difference between lab-scale achievements and real-world implementation, this framework defines a multidimensional scale-up model through 4 operational modules.

1. **Resource Synergy Stage (Feedstock Integration):** This is the stage in which municipal, agricultural, and industrial wastewater are utilized as the main culture medium. In contrast to controlled lab scale, in this stage, the variability of nutrients in actual waste effluent is considered; the combination of N, P, and organic carbon among various waste sources is made such that biomass is produced at the same time while effluent discharge can meet the standard, which could effectively reduce the use of expensive synthesized compounds [59].
2. **Techno-Economic Carbon Optimization:** One of the major constraints to the industrial application is the high cost of producing and delivering concentrated CO₂. The system has applied a combined method of supplying CO₂ in a gaseous state (1% v/v) together with the supply of NaHCO₃ (0.5 g/L) to circumvent the issue. The latter approach has been shown to cut down the costs of carbon supply from \$1.37/kg to \$0.86/kg and enhances FAME production by roughly 80%. Similar optimizations are needed to facilitate the application of microalgae cultivation at an industrial scale in a profitable way [60].
3. **Environmental validation (LCA):** The overall environmental benefit of the process can be assessed using LCA. Here, the indirect emissions produced during cultivation and nutrient supply should be considered; e.g., about 1.2 kg of CO₂ is emitted during the production of 1 kg of NH₃. It means that industrial-scale implementation would

be feasible only if the CO₂ sequestration achieved is higher than the carbon footprint generated by the whole system [61]

4. **Biotic and operational resilience:** As a move from sterile conditions in a laboratory setting to practical and non-sterile operation conditions in large-scale outdoor culture, microalgae require more resistance to contamination. Strategies to enhance the contamination resistance through co-culturing with other microorganisms have been discussed. Moreover, the harvesting of microalgal cells, which have an average size of 2-20 μm and take about 30% of production cost, should still be the biggest challenge for profitable industrial application [62].

5.3 Renewable Energy Integration: The Carbon Negativity Threshold

In addition to the circularity of processes, promote the economic sustainability, increase the efficiency of CO₂ biofixation, and accelerate the carbon-negative process system by three pillars [63].

1. **The Anoxic Stabilization for Biohydrogen:** The limiting factors of the high-yield of biohydrogen on a large scale are the sensitivity of oxygen inhibiting hydrogenase activity. To solve this problem, this framework proposes using the microalgae-bacteria consortia to consume the surplus oxygen and form a relatively stable anaerobic environment [64],[65]. In the course of the consortia interaction, CO₂ biofixation process could continuously produce bioenergy without any additional chemical treatments.
2. **Bioelectrochemical Scalability (MFCs):** The research introduces microalgae in the MFC system to achieve the economic potential with photobiocathodes of microalgae, resulting in the decreased need of external mechanical aeration [66],[67] This system can realize high removal efficiency of COD, such as 90.5% and meanwhile consume CO₂ to generate bio-electricity.
3. **The Integration of Energy and Resource Recovery:** At last, it integrates resource recovery process to increase the added value of biomass in the third stage by photoelectrogenic systems and enhance electron transfer pathways [68],[69]. CO₂ biofixation could be integrated with the production of useful biochemicals and energy streams.

5.4 Artificial Intelligence and Digital Optimization: The Emerging Enabler

Recently, artificial intelligence started to make a great contribution to microalgae technology development; this transition would take microalgae technology from laboratory research into industrial production. The previous traditional method of trial and error approach will be substituted by the ability of analyzing abundant amount of data from AI and predicting optimum culture conditions such as light, temperature, nutrients[70]. Furthermore, machine learning and artificial neural networks have successfully applied and increased the productivity of microalgae, the prediction ability has been greatly promoted compared to other methods [71]. Application of AI to bioreactor can offer a real-time, on-line, and automatic control strategy, minimize the human involvement in operator as well as ensuring the stability and operability of industrial production processes [72]. Because of the features, it is recognized that AI can benefit multiple components in the SCALE-UP framework concurrently, specifically, light controlling system, energy saving and productivity evaluation of the harvest process. Though, tremendous work has been done; there is still a considerable gap between the applications in lab and those in actual large scale industry, it will be more critical to develop AI eco-prediction model to predict changing conditions in the real environment for the future research [56].

6. Future Prospects and Specific Research Priorities

As a result of the multidimensional gaps evaluation Section 4 and the SCALE-UP framework a number of future research directions to be a realistic medium term or long

term prospect and practical in terms of industry deployment. These are listed below according to their potential impact and time frame for industry use.

6.1 Short-term Priorities

1. **Outdoor productivity report standardization.** The most important challenge and research is a reporting standard on outdoor productivity of microalgal CO₂ biofixation. An outdoor report on the productiveness of microalgal growth should cover defined elements such as boundary of the equipment, feed stream concentration of CO₂, length of light path in the culture medium, level of electrical energy use for agitation and time-averaging interval. Such research will make intercomparison and data consistency in reported work become more feasible.
2. **Real-time process control driven by AI.** Some impressive gains in the productiveness and steadiness operation were already reported for both PBR and raceway systems through the integration of artificial intelligence technology and real-time monitoring via IoT sensor network, but still they are on the preliminary stage which needed to be systematic tested under wide ranges of climate and working conditions.
3. **Comparison of harvesting methods.** We propose a direct comparison of different main microalgal harvesting methods, including centrifugation, flocculation, DA F, biofilm-based harvesting methods. The cultivation and operational environment have to be consistent under testing so that meaningful data on productivity, cost, and power consumption for different methods will be obtained.

6.2 Medium-Term Priorities

1. **Pyrenoid and carboxysome transplantation into industrial strains:** Engineered higher-plant CCMs with cyanobacterial carboxysome or microalgal pyrenoid factors have proven promise in mannequin organisms. A precedence have to be systematic transplantation into high-productivity industrial lines with validation of outside overall performance.
2. **Regional climate-specific strain selection:** systematic prospecting of extremophilic and climate-adapted microalgal lines from geothermal, hypersaline and high-altitude environments blended with ALE below regionally applicable stress conditions.
3. **Techno-economic model standardization:** Development of open-access, community-validated TEA models with standardized boundary prerequisites and obvious uncertainty quantification.

6.3 Long-Term Priorities

1. **Regulatory pathway development:** Engagement with regulatory our bodies in the EU, USA, and different international locations to increase risk-proportionate frameworks for outside cultivation of non-GMO expanded strains, doubtlessly consisting of contained ALE-derived variants.
2. **BECCS integration modeling:** Quantitative evaluation of the local weather mitigation manageable of microalgal-BECCS at regional and international scales, with specific modeling of land use, water use, and nutrient provide constraints to make certain net-negative carbon emissions.

7. Limitations of This Review

In spite of our attempts to present an objective and detailed review on microalgal CO₂ biofixation, some limitations of the present review are worth acknowledging:

1. heterogeneity in reported data

A significant limitation of this review is the enormous heterogeneity in the reported experimental conditions, the reporting methods and the boundaries of the system among

the literature. The vast range of models and assumptions used in theoretical analysis of the costs and environmental impacts would make quantitative comparisons among studies very difficult[47]. This highlights the urgent need for a standardization report system as defined above in 6.1.

2. publication bias toward positive result

Only few commercial microalgae production facilities were implemented worldwide despite decades of research and investment; it reveals a discrepancy between research prediction and industrial practice [47],[48]. Studies with failure in cultivation, crashes in the cultures or with negative energy balance are often not reported, which could lead to overestimate the current TRL of microalgal CO₂ biofixation.

3. lack of industrial long-term data

Productivity data reported for decades from outdoor raceway pond are highly variable [47] which represents the lack of standardize long-term industrial data. It makes it difficult to perfectly validate the productivity standards and cost estimations proposed in this review and the specific benchmarks proposed by SCALE-UP [47],[48],[49]

4. techno-economic uncertainty

Various techno-economic analysis that are cited in this review were performed with different assumptions on energy costs, carbon credit prices, market prices of the algal biomass and geographical location. The cost of CO₂ fixation ranged from USD 225 to USD 1600 per ton of CO₂ fixed, reflecting a significant uncertainty that limit comparison between studies [50].

8. Conclusion

Microalgae provide a promising biological alternative to convert CO₂ to biomass at the environmental scale due to their high photosynthetic rate and fast growth coupled with their biomass utilization as useful resources. Despite substantial progress achieved at the laboratory level, scale-up in industrial application is still hindered by the gap existing from bench to industrial scale regarding productivity decrease, high energy input, the limits of microalgae harvesting, and the stability and reliability in outdoor cultivation.

This review demonstrates that it is not the biological capacity of microalgal systems to fix CO₂ that limits microalgal carbon fixation, but rather the ability to maintain stable, cost-effective, efficient, and scalable performance at the industrial scale. Therefore, to overcome these constraints, an adaptive roadmap combining effective biological approaches, highly effective cultivation methods, energy-saving harvesting processes, wastewater utilization, and realistic productivity standards was proposed, termed the SCALE-UP framework.

Future development for microalgal CO₂ biofixation requires integrated studies bridging engineering, biology, techno-economic evaluation, and environmental sustainability.

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