



Review

Utilization of microalgal-bacterial energy nexus improves CO₂ sequestration and remediation of wastewater pollutants for beneficial environmental services

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ABSTRACT

Carbon dioxide (CO₂) emissions from the combustion of fossil fuels and coal are primary contributors of greenhouse gases leading to global climate change and warming. The toxicity of heavy metals and metalloids in the environment threatens ecological functionality, diversity and global human life. The ability of microalgae to thrive in harsh environments such as industrial wastewater, polluted lakes, and contaminated seawaters presents new, environmentally friendly, and less expensive CO₂ remediation solutions. Numerous microalgal species grown in wastewater for industrial purposes may absorb and convert nitrogen, phosphorus, and organic matter into proteins, oil, and carbohydrates. In any multi-faceted micro-ecological system, the role of bacteria and their interactions with microalgae can be harnessed appropriately to enhance microalgae performance in either wastewater treatment or algal production systems. This algal-bacterial energy nexus review focuses on examining the processes used in the capture, storage, and biological fixation of CO₂ by various microalgal species, as well as the optimized production of microalgae in open and closed cultivation systems. Microalgal production depends on different biotic and abiotic variables to ultimately deliver a high yield of microalgal biomass.

1. Introduction

Climate change and environmental safety action with growing water pollutants globally remain the most complex challenges that present and future generations of humankind face and raise several security risks. The evidence of security risks arising from these challenges in the Global South provides forward-looking perspectives on increasing the resilience of affected individuals and communities. It is crucial to demonstrate different strategies as key elements to drive a transformation toward greater sustainability and resilience (Thorn et al., 2023). The

demand for energy from fossil fuels is continuously increasing with the growth in the world population in recent years, which has led to increased emissions of greenhouse gases (GHGs). However, using fossil fuels is an environmentally unsustainable source because the GHGs cause global warming (Sial et al., 2021). Since the late nineteenth century, increased carbon dioxide (CO₂) emissions have reached their highest level in the last thirty-five years, particularly after 2010, due to anthropogenic activities that have raised the earth's average surface temperature by 1.1 °C (Gür, 2022). One-third of total CO₂ emissions are from fossil fuel combustion in power plants. The highest value of CO₂

Abbreviations: CCS, Carbon Capture Storage; CO₂, Carbon dioxide; C, Carbon; N, Nitrogen; P, Phosphorus; K, Potassium; HMs, Heavy metals and metalloids; CCM, Carbon concentrating mechanism; GHG, Greenhouse gas; GHGs, Greenhouse Gases; HM, Heavy Metal; PBR, Photobioreactor; PBRs, Photobioreactors; RSW, Real Swine Wastewater; RAB, Rotating Algal Biofilm; HRAPs, High-rate algal ponds; ABSs, Algal-bacterial systems; AST, Activated sludge treatment.

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concentration, from 280 ppm to 420 ppm, in the last decade has led to global climate change and biological extinction. Currently, over 33.1 GT of CO₂ is emitted into the atmosphere, including a significant portion of carbon (C) associated with direct combustion (Qin et al., 2021).

The complex challenges associated with CO₂ generation and emissions (increased by 1.7% annually) make them difficult to address and undermine the adaptive capacities of affected individuals and societies. Climate change erodes agricultural production and disrupts food supplies (Nguyen et al., 2023). The Paris Agreement was ratified in 2016 to mitigate, adapt, and finance strategies to tackle GHG emissions. The agreement's goal was to limit global warming to 1.5 °C. Given the current situation, the World Meteorological Organization envisages that the global average temperature will be raised to 3–5 °C by 2100 (Gebel et al., 2022). Therefore, the strategies to deal with CO₂ emissions involve:

- Reduction of non-renewable energy sources such as the use of fossil fuels.
- Optimize and enhance low-C emission renewable energy sources, including carbon-neutral technologies.
- Utilization of carbon capture and storage as post-treatment technologies.

Recent research has described carbon dioxide capture storage (CCS) and sequestration technologies. Therefore, there is a need to achieve environmental and economic sustainability. For example, future fuel production processes must be renewable and capable of sequestering atmospheric carbon dioxide. So, shifting from fossil fuels to low-carbon fuels becomes the highest priority. The most effective ways to reduce CO₂ emissions are to improve the energy efficiency of each economic sector and to reduce the cutting of tropical and temperate forests around the world (Tang et al., 2022). These methods, however, may not be able to control CO₂ emissions due to various political and socio-economic barriers, so other more innovative and less well-defined CO₂ mitigation measures are required. The most practical of these innovations is to increase CO₂ sinks through photosynthesis, including increased carbon storage in standing tree biomass, the substitution of fossil fuels with biofuels, an increase in soil carbon sequestration, and an increase in soil primary productivity (Zahoor et al., 2022). Microalgae can be extensively used to capture CO₂ from power plants, steel, cement, oil, automobiles, and many other industries. The resulting algal biomass can be used not only for biofuel production but also for various industrial products. Besides giving environmental and economic benefits, large scale algae cultivation can create millions of jobs at different levels of society (Tarafdar et al., 2023). Much work has been done on carbon sequestration and algae biofuel production, but it still needs much research to meet the increasing energy demand. We hope that biofuel will replace fossil fuels to a larger extent and reduce atmospheric CO₂ to combat Global warming (Nguyen et al., 2023).

Renewable energy sources are considered an alternative to fossil fuels due to low C footprints (Yadav and Mondal, 2022). Biological CCS technologies have gained popularity due to their ability to convert C sources into valuable products (Daneshvar et al., 2022; Shahbaz et al., 2021). Bioethanol and biodiesel are currently available renewable energy sources from conventional crops. However, crop production and price pose a real challenge for these sources. Therefore, biofuel production from microalgae could be a potential source that does not interfere with human food (Onyeaka et al., 2021). Microalgal cultivation has advantages over crop-based production, such as high biomass production, less land cultivation requirement, and low cost per yield. CO₂, water, nutrients, and sunlight are required to grow microalgae, but water and inorganic nutrients are limiting factors in using microalgae as a biofuel source. Effluent wastewater contains nitrogen (N) and phosphorus (P) as the primary nutrients for microalgae cultivation. Hence, the same process can achieve effluent wastewater treatment and microalgae cultivation (Bolognesi et al., 2022).

In this review, we focus on utilizing the algal-bacterial energy nexus. This concept involves harnessing the synergistic relationship between algae and bacteria to improve CO₂ sequestration and other environmental and energy-related benefits. Both algae and bacteria benefit from the synergistic relationship between them (Sial et al., 2021). Organic matter is produced by algae in the process of photosynthesis, which is then consumed by bacteria. By breaking down organic matter, bacteria release nutrients and other compounds that enhance algae growth. This cyclical process enhances CO₂ sequestration and biomass production efficiency. Besides, wastewater can be treated by algae, which absorb nutrients and contaminants, and by bacteria, which break down organic substances (Jiang et al., 2021). This article reviews the processes used in CO₂ capture, storage, and biological fixation by various microalgae species. It also reviews microalgae production in open and closed cultivation systems. The growing concern for the increase of global warming effects raises the challenge of finding novel technological approaches to stabilize CO₂ emissions in the atmosphere. Biological- CO₂ mitigation, triggered through biological fixation, is considered a promising and eco-sustainable method. Due to their faster growth, microorganisms such as cyanobacteria, green algae, and some autotrophic bacteria could potentially fix CO₂ more efficiently than higher plants. Biological CO₂ mitigation intensively studied in the last few years is related to the possibility of performing carbon dioxide sequestration using microalgae, obtaining, at the same time, bioproducts of industrial interest. This paper presents the current scenario regarding microalgal CO₂ fixation, their cultivation, processing, and applications to be economically competitive with other biomitigation. The major objective was to provide sufficient information about the role of microalgal organisms in sequestering CO₂ more efficiently compared to higher plants.

2. Microalgae and its advantages

Microalgae are regarded as one of the most important photosynthetic resources on the planet. Nearly half of the global photosynthesis activity is done by microalgae. Photosynthesis is a crucial component for the survival of autotrophic microalgae, as the microalgae consume CO₂ and solar radiation to produce energy. Microalgae treatment methods using photosynthesis have recently gained much attention to lower CO₂ concentrations in the atmosphere and creating a clean environment (Munir et al., 2021). The microalgal photosynthesis process has the highest CO₂ fixation rate among the biological processes, with no further disposal requirement for trapped CO₂. After removing CO₂ from the atmosphere, microalgal photosynthesis also produces lipid-rich biomass as a source of energy. However, little research has been conducted on using the microalgal photosynthesis process to reduce CO₂ concentrations in the environment (Sharif et al., 2021). The rapid growth of microalgae in a photobioreactor (PBR) to efficiently consume CO₂ is another benefit of a microalgae-engineered treatment system. The conversion of CO₂ by microalgae into food derivatives, food additives, and biofuels helps ensure efficient CO₂ circulation in the atmosphere. Effective microalgal photosynthesis can achieve CO₂ sequestration from flue gas and biogas (Hasnain et al., 2022c).

The removal of inorganic elements in wastewater, including N and P, is critical. Microalgae have proven beneficial in eliminating inorganic contaminants by utilizing them for their growth. Therefore, microalgae can be considered for tertiary wastewater treatment (Khilji et al., 2022). Tertiary wastewater treatment is costlier than primary treatment due to the massive investment. As a result, microalgal culture growth offers a cost-effective solution for tertiary water treatment while reducing CO₂ concentration. Furthermore, microalgae treatment has proven effective in removing heavy metals (HMs) and toxic contamination from wastewater (Munir et al., 2023). So, the microalgae system provides an affordable and optimized solution for water ecosystem management. In addition, low capital investment, lower operating costs, and low energy consumption make the microalgae-engineered system a favorable option for wastewater treatment (Alami et al., 2021b).

3. Microalgal-bacterial nexus

The microalgal-bacterial nexus, which combines algae and bacteria, is an effective solution to tackle increasing atmospheric CO₂ (Viswanaathan et al., 2022). The ability of microalgae to absorb and convert CO₂ into organic biomass makes them well known for their impressive photosynthetic rates. The CO₂ fixation process can be significantly enhanced when combined with certain bacterial strains. For example, organic compounds can be decomposed by bacteria, releasing carbon dioxide as a result, which microalgae can use for carbon. Furthermore, some bacteria release growth-promoting compounds that stimulate algal proliferation, creating a symbiotic relationship. With this relationship, carbon sequestration is maximized, particularly when organic wastes are used (Smith and Thompson, 2018; Viswanaathan et al., 2022).

Algal-bacterial systems can demonstrate their full potential in wastewater treatment plants. In such treatment facilities, organic pollutants and abundant nutrients are combined to create a favorable environment for both bacterial decomposition and algal growth (Viswanaathan et al., 2022; Wang et al., 2023). By incorporating an algal-bacterial tandem system into these wastewater treatment setups, it becomes feasible to tackle dual environmental issues concurrently: ever-increasing CO₂ emissions and wastewater purification. During the breakdown of organic contaminants, bacteria release CO₂ as well as providing the microalgae with a constant source of carbon. In addition to ensuring clean water output, this intricate process reduces wastewater treatment's carbon footprint significantly (Martinez Kim, 2019; Viswanaathan et al., 2022). Despite the promise of the microalgal-bacterial nexus in environmental remediation, its large-scale deployment can be challenging. For example, the stability of such intricate systems is paramount, especially when exposed to changing environmental conditions. Researchers are also studying the optimal algae-to-bacteria ratio that will maximize CO₂ capture while decomposing waste efficiently. Choosing compatible microalgae and bacteria species is crucial to ensuring that they coexist productively and efficiently. Despite these challenges, the cost-effective and environmentally beneficial algal-bacterial system is an area of significant interest in both the academic and industrial sectors (Chen and Gupta, 2020).

4. CO₂ capture and fixation by microalgae

CO₂ capturing and fixation by microalgae is preferred for sequestration, as microalgae fix CO₂ 10 times greater than plants. Approximately 280 tons/ha/year of microalgae sequester 514 tons of CO₂ by consuming 10% solar energy (Song et al., 2019). Microalgae can absorb CO₂ from flue gas produced by industries and fix more than 66 Gt of C per year, equal to 66,000 of 500 MW-producing plants with a fast growth rate (Alami et al., 2021a). Microalgae use three different inorganic carbon assimilation pathways: (1) direct carbon dioxide assimilation via the plasmatic membrane, (2) the use of bicarbonate by inducing the enzyme carbonic anhydrase, which converts HCO₃ to CO₂, and (3) the direct transport of bicarbonate via the plasmatic membrane. PH measurements can evaluate the control of CO₂ feeding to minimize the loss of CO₂. As a result, CO₂ fixation using microalgae can reduce CO₂ emissions from power plants, which has a positive environmental impact. The efficiency of CO₂ removal or fixation in a closed cultivation system depends on (1) microalgal species, (2) CO₂ concentration, (3) photobioreactor design, and (4) operating conditions (Razzak et al., 2017). *Chlorella vulgaris* possesses a maximum CO₂ removal efficiency of 55.3% at 0.15% CO₂ in a membrane photobioreactor, and *Spirulina* sp. and *Scenedesmus obliquus* possess a maximum CO₂ removal efficiency of 27–38% and 7–13%, respectively, in a three serial tubular photobioreactor. However, their CO₂ fixation efficiencies were reduced to 7–17% and 4–9% under 12% CO₂ aeration (Cheng et al., 2006). In other words, the CO₂ removal efficiency and fixation depend on the microalgal species due to the physiological conditions of microalgae, such as the potential for cell growth and CO₂ metabolism. The CO₂ fixation rate

could be determined from the carbon content of the microalgal cell (Razzak et al., 2013). Microalgae can grow in poor-quality water, from manure to industrial waste to seawater, by using CO₂ and carbonates. Moreover, microalgae can fix up to 50% of CO₂ with SO and NO from exhaust gases and show a 40% increase in growth. Table 1 shows the CO₂ fixation potential of various microalgae (Priyadharsini et al., 2022). *Spirulina* sp. can withstand 50% less growth while increasing biomass, proteins, pigments, lipids, and carbohydrate content by 20%. Various microalgae have shown diverse tolerance limits and fixation abilities (250–1000 mg/L/d) (J. Q. Cheng et al., 2018; J. Cheng et al., 2018). Sydney et al. (2014) concluded *Dunaliella* sp. (273 mg/L/d), *Chlorella* sp. (251 mg/L/d), *Spirulina* sp. (320 mg/L/d), *Botryococcus* sp. (498 mg/L/d), and *Chlorococcum* sp. (1000 mg/L/d) for bulk production.

5. Heavy metal uptake and microalgae-based wastewater treatment

Industrial development generates approximately 14 billion liters of wastewater daily, high in nutrients and CO₂. Annual production of wastewater from municipal, agricultural, and industrial aspects is huge and contains excessive nutrients, and improper treatment may lead to environmental problems such as eutrophication of water bodies. Currently, conventional wastewater treatment technologies are mainly based on physical, chemical, and biological methods, such as activated sludge to remove organic matter and nutrients and adsorption to remove heavy metals. However, these methods have the disadvantages of a large land area, high energy consumption, and a large amount of activated sludge discharge (Srimongkol et al., 2022). Besides, the nutrients in wastewater have not been effectively recycled, resulting in a waste of resources that could be recycled. However, microalgae-based wastewater treatment technology is a promising technology that can replace conventional treatment methods. Microalgae have various characteristics, such as high photosynthetic efficiency, fast reproduction speed, and strong environmental adaptability, and can convert nutrients in wastewater into algal biomass. Therefore, it is considered an ideal biological material for comprehensively utilizing wastewater (Hashmi et al., 2023). Algal remediation is an operative technique that remediates wastewater by assimilating HMs and lethal organic contaminants, which have an extraordinary tolerance for HMs. Industrial wastewater (except agro and food wastewater) contains toxic contaminants and low content of nutrients, which suppress algal biomass.

Furthermore, deceased microalgae can remediate HMs from the water via biosorption, biodegradation, bioaccumulation, etc. (Fig. 1), but with less efficiency than living algal cells (Liu and Hong, 2021). Various heavy metals such as Cu²⁺, Zn²⁺, Ni²⁺, Fe²⁺, and many others are effectively utilized as micronutrients for microalgae. This metallic content is vital for microalgae cell metabolic activity. However, other heavy metals such as mercury, titanium, cadmium, silver, and gold are not helpful for microalgae growth and behave as toxins for metabolic activity. Microalgae are promising and effective in bioremediation due to outstanding attributes such as survival in harsh environments, ease of growth, superb binding affinity, effective area, and ecological friendliness, and dead microalgae can be used for many other purposes (Goswami et al., 2022). Numerous binding groups, such as carboxyl, thiol, hydroxyl, and acyl, are present in the algal surface and cytoplasm to endorse metal biosorption. HMs attach to these groups via ion exchange, replacing calcium, sodium, and potassium (K). Metal ions excretion from the algal cell wall also produces proline, metallothioneins, and glutathione to preclude cell damage by metals. HMs accumulate within the algal cell via bioaccumulation and inhibit photosynthesis and growth. However, ion exchange, chelation, and adsorption are effective mechanisms to overcome the toxicity of HMs by converting them into non-toxic forms (Bădescu et al., 2018; Bulgariu, 2020; Lucaci et al., 2020). Microalgae-mediated detoxification is carried out by binding HM to an internal organelle, transporting it to the vacuole, and excreting it by an efflux pump (Goswami et al., 2022) (Fig. 2).

Table 1
CO₂ fixation by different microalgae.

Microalgae	Optimal Conditions	CO ₂ concentration (%)	CO ₂ Fixation (g/L/d)	References
Anabaena sp.	At 25 °C, 7 pH, light intensity 12 h light/12 h dark, providing 3000 μE m ⁻² s ⁻¹ as maximal incident irradiance on the photo-bioreactor's surface.	10	1.1	(Li et al., 2022)
Botryococcus sp.	At 25 °C in 1-L Erlenmeyer glass flasks under 12 h light/12 h dark to simulate diurnal light with fluorescent lamps of 60 μmol photons m ⁻² s ⁻¹ light intensity	5 10	0.50 0.31	(Dutta et al., 2022)
Chlorella sp.	At 30 °C in 110 mL glass bubble columns, photobioreactors	10 10 10 5	0.26 0.23 0.71	(Priyadharsini et al., 2022)
C. sorokiniana	At 30 °C, 7.5 pH with an illumination intensity of 4000 Lux	4	0.24	(Do et al., 2022)
Chlorella vulgaris	At 30 °C in 110 mL glass bubble columns photobioreactors for 7 days. Agitation during microalgae cultivation was provided by bubbling CO ₂ -enriched air through a needle. Illumination was provided by four fluorescent lamps on one side of the photobioreactors at an irradiance level of 70 μmol m ⁻² s ⁻¹	5 0.10 1 5 2	0.26 3.56 6.31 0.15 0.42	(Dasan et al., 2020)
Dunaliella sp.	At 25C, 8 pH with 30 μE m ⁻² s ⁻¹ of light intensity under the LED fluorescent light	5 3 10 15	0.28 0.33 0.28 6.12	(Goswami et al., 2021a)
Euglena sp.	3.5 pH, 27 °C, and 480 ± 10 μmol m ⁻² s ⁻¹	10	0.07	(Kim et al., 2022)
Nannochloropsis sp.	at 30 °C, 7.5 pH under 20 μmol m ⁻² s ⁻¹ light	10	0.27	(Alami et al., 2021b)
Chlorella vulgaris	At 25 °C, 7.5 pH under 80 μmol m ⁻² s ⁻¹ light	-	0.29	(Paul et al., 2021)
Coelastrrella sp.		-	0.20	
Chlorella sorokiniana		-	0.11	
Scenedesmus sp.		-	0.21	
Spirulina sp.	At 30 °C, 7.5 pH under 200 μmol m ⁻² s ⁻¹ light	5 6 12 15 2.2 10 20 10 2.5 10	0.32 0.23 0.15 4.5 1.21 0.54 0.41 0.29 0.37 0.22	(Shen et al., 2021)
Scenedesmus sp.	At 25 ± 1 °C, 7.5 pH, illuminated with 90 μmol m ⁻² s ⁻¹ fluorescent light			(Satpati and Pal, 2021)

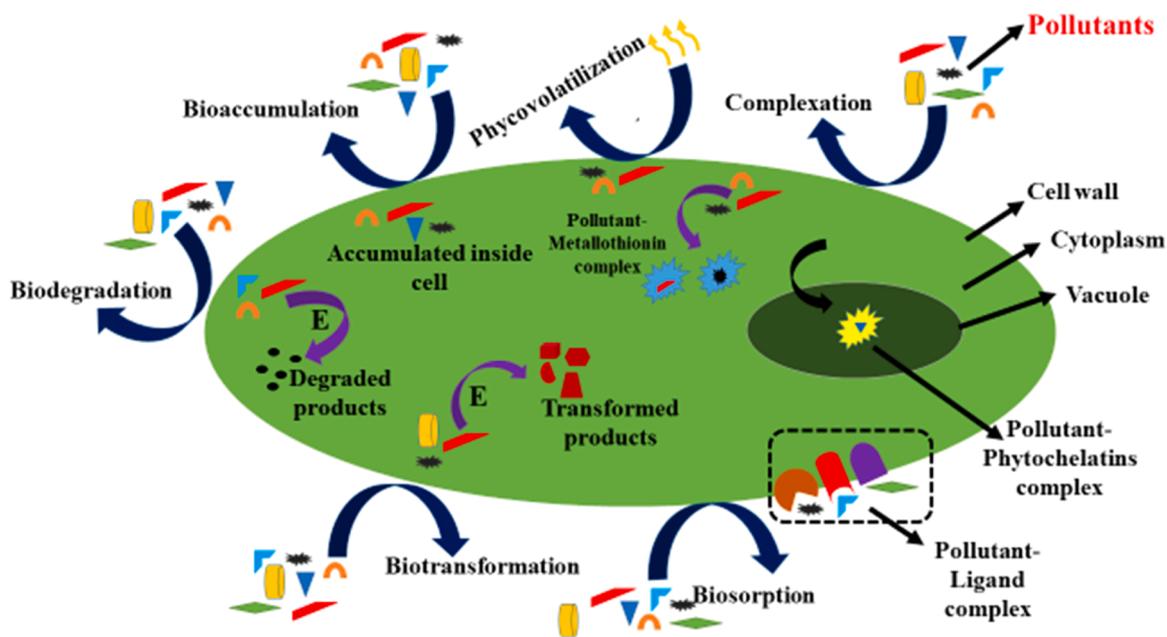


Fig. 1. Different algal mechanisms to remediate pollutants from wastewater.

Similarly, *Chlorella minutissima* removed 62% zinc, 84% manganese, 74% cadmium, and 84% copper. *Cladophora* sp. removed 99% copper and 85% zinc from oil ponds. *Cladophora* sp. was also used to remove arsenic from drinking water in India. *Oedogonium* sp. removed 46% copper, 34% nickel, 48% zinc, and 50% cobalt in acid mine drainages.

Chinnasamy et al. (2010) obtained 18 tons of biomass per hectare per year and 68% oil with 97% nutrient removal from *Botryococcus braunii* and *Chlorella* sp. using 90% carpet mill and 10% municipal wastewater. *Wang et al. (2010)* remediated 87% aluminum, 23% calcium, 100% iron, 98% magnesium, 100 manganese, and 57% zinc from municipal

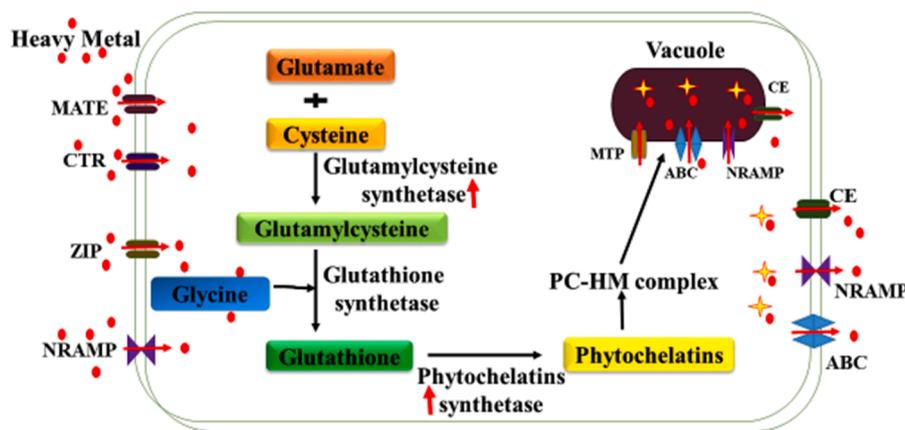


Fig. 2. Detoxification of heavy metals in microalgae via metal transporters.

wastewater by cultivating *Chlorella* sp. for 15 days. In another study, *Chlorella* sp. removed 88% BOD, 82% COD, 70% nitrogen, and 60% phosphorus in 15 days from effluent (Ho et al., 2019). Khilji et al. (2022) removed 44% chlorides, 54% chromium, 58% cadmium, and 59% lead in leather effluent after 45 days by using ZnO-NPs synthesized from *Oedogonium* sp. *C. vulgaris* removed 79% of the chromium from leather effluent in 120 h (Mirza et al., 2021). *Scenedesmus* sp. removed 99% phosphate and 87% ammonium from sludge (Chen et al., 2019). With an effective biomass gain under suitable conditions (for example, 3.9 g/L/d), *Chlorella* sp. can remediate many types of wastewater. Table 2 provides a summary of various algal species with HMs removal efficacies. Microalgae are the most cost-effective biosorbents than

bacteria and fungi due to their lower nutrient requirements and higher biosorption efficacy (15 – 85%). Usually, innate algal strains are chosen for wastewater treatment due to their greater resilience towards environmental stresses and high growth rate.

6. Heavy metal effects on the algal metabolites

Biofuel generation, along with waste management, is a very economical and eco-friendly strategy. Algal uptake of metals has triggered algal metabolites (lipids, proteins, and carbohydrates) (Hasnain et al., 2022a). *C. minutissima* abolished cadmium, copper, zinc, and manganese via intracellular accumulation with extracellular

Table 2
Metal and metalloid ion removal efficiency by microalgae in wastewater.

Wastewater	Microalgae	Optimal Conditions	Metal/metalloid	Removal efficacy (%)	Mechanism	References
Smelter Wastewater	<i>Spirulina</i> sp.	At 25–30 °C, light intensity of 1500–2000 Lux, 14 h light/10 h dark cycles, and regular air injection.	Copper	91	Biosorption	(Chojnacka et al., 2004)
Raceway pound Mine wastewater		At 3 pH over 100 h	Chromium	77		(Kiran et al., 2017)
Padina Waste	<i>P.capillacea</i>	At 35 °C, under illumination (32.7 W m ⁻²) with a photoperiod of 12 h light and 12 h dark. at 27 °C on a shaker for 120 min at 1 pH	Calcium	98	Adsorption	(Spiridon et al., 2011)
Wastewater	<i>Chlamydomonas</i> sp.	White fluorescent lamps Illumination was provided with a light intensity of 150 μmol m ⁻² s ⁻¹ and a light/dark cycle of 16:8 h. The temperature was 26 °C with 6 pH for 4 days.	Neodymium	100	Adsorption	(H. S.R.M.S.R. Mohamed et al., 2019; H.S. Mohamed et al., 2019) (Heilmann et al., 2015)
Battery wastewater	<i>Scenedesmus</i> sp.	At 7 pH	Lithium	90		(Kashyap et al., 2021)
Graphene wastewater	<i>Chloroidium</i> sp.	At 30 °C and illuminated at 25–40 μmol m ⁻² s ⁻¹		80		(Ahmad et al., 2019)
Municipal wastewater	<i>Chlorella</i> sp.	At 7 pH, 150 rpm under continuous illumination at 100 μmol m ⁻² s ⁻¹ at 26 °C for one week.	Calcium	56	Biosorption	(Wang et al., 2010)
			Magnesium	57		
			Zinc	62	Adsorption	(Manzoor et al., 2019)
			Manganese	84		
			Copper	74		
			Cadmium	84		
			Chromium	85		
Tannery effluent	<i>Scenedesmus</i> sp.	At 27 °C, continuous cool white fluorescent lamps Illumination was provided 4000 lux with a dark/light period of 16:8 h for 12 days.	Calcium	59	Biosorption	(Venkatesan and Sathivelu, 2022)
			Magnesium	29		
			Chromium	60		
Drinking water	<i>Cladophora</i> sp.	At 5 pH with 40 μmol m ⁻² s ⁻¹ , temperature 18 °C, light/dark 12:12 h.	Arsenic	100		(Ji et al., 2012)
Oil sands tailings			Copper	99		
			Cadmium	78		
			Zinc	85		
Petrochemical wastewater	Mixed culture	At 30 °C under continuous light for 15 days	Copper	94	Ion exchange	(Cechinel et al., 2018)
			Nickel	94		
			Zinc	93		
Wastewater	<i>Ulva</i> sp.	At 5 pH, the contact time was 120 min at 30 °C.	Chromium	96	Biosorption	(Ibrahim et al., 2016)
Industrial wastewater	<i>Dunaliella</i> sp.	At 2 pH, 25 °C, Continuous illumination was provided by cool white fluorescent light (2000 lux)	Cadmium	74		(Mofeed, 2017)

immobilization and triggered lipid production of up to 21% by cadmium and 94% by copper (Ahmed et al., 2022). The growth rate of *Dunaliella* sp. was increased by lead, aluminum, and cobalt, while *Nostoc* sp. was triggered by arsenic. Cobalt-enhanced proteins (34 mg/L) and lipids (10 mg/L) are present at a concentration of 0.001 mg/g in *Tetraselmis* sp. (Dammak et al., 2022). Table 3 shows the effects of HMs on algal proteins, lipids, and carbohydrates. A small quantity of HMs plays an important role in the cellular functions of microalgae, such as iron and copper in the electron transport chain, manganese in the water oxidizing center during photosynthesis, cobalt in vitamins, and zinc in carbonic anhydrase, which act as cofactors in CO₂ fixation and RNA polymerase for transcription (Dammak et al., 2022).

Many studies have shown that wastewater can not only realize the reuse of wastewater itself but can also transform and obtain a large amount of biomass, especially in producing microalgae biofuels and other applications, which has great application prospects. Treating all types of wastewater requires huge capital investment, and the win-win for producing microalgae biomass would be to reduce treatment costs while purifying wastewater.

7. Factors that influence algal cultivation in wastewater and CO₂ uptake

Microalgae cultivation and CO₂ uptake are influenced by numerous factors, some of which positively or negatively influence algal growth, such as algal strain and growth, temperature, pH, CO₂ concentration, light, nutrients, and flue gas composition.

7.1. Algal strains

The first and most dangerous step is selecting an algal species for a specific use that can be cultivated on a large scale, is native, and can survive in fluctuating ecological conditions while producing a lot of biomass. *B. braunii*, *Chlorella* sp. and *Scenedesmus* sp. are superlative entrants to mitigate CO₂ and fuel production. As different microalgae have different CO₂ sequestration mechanisms, Table 1 lists additional

algal strains and their CO₂ fixation potential. Eloka-Eboka and Inambao (2017) conducted a comparison study among four algal strains. They concluded *Dunaliella* sp. was more effective in CO₂ fixation than *C. vulgaris*, *Scenedesmus quadricauda*, and *Synechococcus* sp., while *C. vulgaris* accumulated the highest biomass. However, several researchers (e.g., Sadeghizadeh et al., 2017; Senatore et al., 2021) have shown that *Chlorella* sp. has a greater capacity to fix C (up to 2 g/L/d) because it can withstand high CO₂ concentrations (0.05 – 19%) with high photosynthesis efficacy and a better growth rate than other algal strains, it is, therefore, the best strain for CO₂ fixation.

7.2. Cultivation duration

Microalgae cultivation duration varies from 5 to 30 days, depending on the selected algal strain and cultivation conditions. Takabe et al. (2016) reported that 3 days were enough for maximum biomass (15 g/m²/d) of *Chlorophyceae* under 25 °C and 8 MJ/m²/d irradiation in effluent with CO₂ addition. Moreover, the mixed microalgae consortium in coffee industry waste was identified after 5 days of cultivation (Wong et al., 2022). However, the cultivation duration was extended to 45 days in leather industrial wastewater for efficient heavy metal removal (Khilji et al., 2022).

7.3. Nutrients

Microalgae generally need nutrients like C, N, P, and K, with additional selective requirements like iron and magnesium depending on the algal strain. The preferred form of N is ammonium (< 100 mg/L), which requires little energy to assimilate and be absorbed by microalgae (Valdivinos-García et al., 2021). *Chlorella pyrenoidosa* growth is inhibited by ammonium concentrations greater than 100 mg/L. *Scenedesmus* sp. and *Chlorella* sp. are adaptable and tolerant to different wastewaters, efficiently remove nutrients, and yield 14 g/L and 10 g/L of biomass, respectively, in food waste, which contains 600 mg/L of ammonia (Kwon et al., 2020). *Chlorella* sp. thrives in nature due to its ability to grow in both light and darkness while utilizing C, nitrate, or

Table 3
Heavy metal-triggered effects on algal metabolites.

Microalgae	Metal/metalloid	Quantity	Lipids	Carbohydrates (DW)	Protein	Reference
<i>A. coffeaeformis</i>	Copper	10 mg/L	200 mg/L	449 µg/L	350 µg/L	(Anantharaj et al., 2011)
	Cadmium	10 mg/L	170 mg/L	381 µg/L	250 µg/L	
<i>Anabaena</i> sp.	Copper	0.07 mg/L	24 mg/g	-	-	(El-Sheekh et al., 2005)
	Iron	3.7 mg/L	-	-	-	
	Lead	0.064 mg/L	-	-	-	
	Manganese	0.068 mg/L	-	-	-	
<i>C. fontana</i>	Copper	0.07 mg/L	11 mg/g	270 µg/mg	-	(Fawzy and Issa, 2016)
	Iron	3.7 mg/L	-	-	-	
	Lead	0.064 mg/L	-	-	-	
	Manganese	0.068 mg/L	-	-	-	
<i>Pavlova</i> sp.	Copper	0.05 mg/L	-	-	5 × 10 ⁶ /Cells	(Lourie et al., 2010)
	Zinc	0.65 mg/L	-	-	3 × 10 ⁶ /Cells	
<i>Chlorella</i> sp.	Iron	1 × 10 ⁻⁵ mol/L	57%	-	-	(Brar et al., 2022)
	Cobalt	10 ⁻⁹ M	-	-	0.6 µg/mg	
	Copper	10 ⁻⁹ M	-	-	0.7 µg/mg	
	Zinc	10 ⁻⁹ M	-	-	1.5 µg/mg	
<i>Spirulina</i> sp.	Lead	0.2 mg/L	-	-	100%	(Chojnacka et al., 2004)
	Copper	0.2 mg/L	-	-	90%	
	Zinc	0.2 mg/L	-	-	100%	
<i>Scenedesmus</i> sp. <i>Nannochloropsis</i> sp.	Cadmium	0.1 mM	63 mg/g	125 mg/g	60%	(Apandi et al., 2022) (Goswami et al., 2021b)
	Arsenic	3.1 mg/L	26%	-	-	
	Cadmium	0.6 mg/L	22%	-	-	
	Chromium	5.2 mg/L	26%	-	-	
	Copper	5.2 mg/L	21%	-	-	
	Cobalt	0.64 mg/L	21%	300 µg/mg	-	
	Lead	2.16 mg/L	22%	-	-	
	Nickel	10 mg/L	29%	-	-	
	Mercury	0.4 mg/L	22%	-	-	
	Zinc	17.6 mg/L	24%	310 µg/mg	-	
	Selenium	0.4 mg/L	22%	-	-	

ammonia in various pH ranges. *Scenedesmus obliquus* was grown in sugar mill effluent with a nutrient ratio of 0.1 C: 0.07 N: 0.04 P and yielded 1.3 g/L of biomass (Hernández-García et al., 2019). *Chlorella* sp. yields 100 tons/ha/year by sequestering 1500 tons C/ha/year in open culture (Koyande et al., 2019). Table 4 lists the nutrient removal efficiency of algal species in wastewater.

At a 0.2 g/L growth rate, approximately 27% lipids, 29% proteins, and 28% carbohydrates were extracted from municipal wastewater cultivated with *Chlorella* sp., with 71% COD, 82% ammonium, and 94% phosphate removal rates (Ansari et al., 2019). Krzemińska et al. (2019) extracted 45% lipids from *Auxenochlorella protothecoides*, including 35% oleic acid, 39% linoleic acid, and 9% palmitic acid. Moreover, Hasnain et al. (2022c) improved the biodiesel quality of *Oedogonium* sp., *Ulothrix* sp., *Cladophora* sp., and *Spirogyra* sp. with metabolite content (proteins, pigments, carbohydrates, and lipids) by using waste molasses as cultivation media. Table 5 shows the nutrients' utilization and lipid recovery during microalgae cultivation in wastewater.

7.4. Temperature

Temperature affects CO₂ uptake and nutrient solubility; when temperature increases, the solubility of CO₂ decreases. Moreover, in open ponds, CO₂ escapes into the air. Temperature influences the metabolic activities of microalgae by affecting the cell composition, CO₂ uptake, nutrients, and algal growth. The optimum temperature for microalgae growth is between 20 and 35 °C. Even though algal growth surges with high temperatures, photorespiration destructively affects algal production (Hasnain et al., 2022b). Fan et al. (2014) concluded that 28 °C was the optimal temperature for photosynthesis because algal growth doubles in 12 h and drops from 28 °C to 33 °C. Although algal growth varies with algal strains, *H. pluvialis* grows vigorously at low temperatures (15 °C) (Perera et al., 2021), whereas *Spirulina* sp. grows between 28 and 35 °C (Hadiyanto et al., 2021). The temperature range for *Chlorella* sp. in wastewater cultivation is 25–27 °C, but 30 °C for *Scenedesmus* sp. (Dahmen-Ben Moussa et al., 2021).

Compared to pure CO₂, a direct supply of flue gas to algal culture increased biomass by 30% due to the additional nutrients such as nitrate and sulfur. However, the growth of some microalgae is stopped by flue gas with 50 ppm SO₂; hence, flue gas with less than 50 ppm SO₂ is

recommended (Kong et al., 2021). *Nannochloris* sp. grows well at less than 100 ppm NO (Suresh and Benor, 2020). *Dunaliella* sp. can remove 96% of the NO, 15% of the CO₂, and 185 ppm of SO₂ (Viswanaathan et al., 2022), while *Tetraselmis* sp. utilized 15% of the CO₂, 186 ppm of SO₂, and 124 ppm of NO from the flue gas (Nishshanka et al., 2022). Similarly, *Chlorella* sp. removed 74% of CO₂ from flue gas produced by the oil manufacturing industry (Hariz et al., 2019).

7.5. Light properties

Light plays a vital role in biomolecule synthesis in microalgae, making light intensity, spectrum quality, and photoperiod the main concerns during cultivation. In large-scale cultivation, 1000 lux is sufficient for growth and can be increased to 10,000 lux, but overheating causes photoinhibition and hinders photosynthesis, ultimately halting algal growth (Levin et al., 2021). Red and blue light are mainly recommended, as they have active quotas to assist in photosynthesis. The biomass of *Chlorella* sp. was 0.029 g/L/d at 9 W/m. However, fluorescent lamps with 50 W are used to treat industrial waste with microalgae (Chankhong et al., 2018). The light intensity can be amplified using more lamps based on wastewater quantity. The 12 hr light to 12 hr dark ratio is normally applied during research. Algal pig and brewery waste treatment has been provided under 24-hour light (S. S. Wang et al., 2022; Y. Wang et al., 2022; S.-K. Wang et al., 2022). Nevertheless, a 14:10 hr ratio was provided for soybeans and an 11:13 hr ratio for recalcitrant processes. Different algal strains amend themselves according to light fluctuations (Büchel, 2020).

8. Synergistic effect of algal–bacterial co-cultivation in wastewater

The combination of algae and bacteria in a co-cultivation system has been shown to be highly effective in removing contaminants and promoting algal biomass growth. This is due to the synergistic cooperation between the two, which involves the exchange of carbon (refractory organics, carbon dioxide) and the promotion of beneficial metabolites. Algal-bacterial treatment systems have been widely proven to be an efficient and economical way of achieving this symbiotic association. The successful partnership could be attributed to the synergistic

Table 4
Nutrients removal from wastewater by microalgae.

Microalgae	Wastewater	Pretreatment	Cultivation period (day)	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$) light (h): dark (h)	Carbon dioxide (%)	Nitrogen (%)	Phosphorus (%)	COD (%)	Reference
<i>C. vulgaris</i>	Textile	Filtration+ autoclave	5	200 (24:0)	5	45	34	63	(Tait et al., 2019)
<i>Scenedesmus</i> sp.	Electric factory	Autoclave	4	50 (24:0)	5	47	100	-	(Apani et al., 2022)
<i>C. sorokiniana</i>	Potato processing industry	Filtration w/ (1.5 μm) membrane	21	200 (24:0)	15	97	81	85	(Rasouli et al., 2018)
	Pig manure	Filtration w/(0.22 μm) membrane	20	40 (24:0)	15	83	59	63	(de Godos et al., 2010)
<i>Chlamydomonas</i> sp.	Industrial	Filtration w/ (0.22 μm) membrane	10	125 (24:0)	5	100	34	-	(Ding et al., 2016)
<i>Auxenochlorella protothecoides</i>	Municipal	Filtration+ autoclave	5	200 (24:0)	5	60	82	89	(Zhou et al., 2012)
<i>C. mexicana</i>	Pig manure	Filtration w/(0.22 μm) membrane	20	40 (24:0)	15	61	29	-	(Abou-Shanab et al., 2013)
<i>Oscillatoria</i> sp.		Filtration w/(0.22 μm) membrane	20	40 (24:0)	15	59	69	-	(Q.Q. Cheng et al., 2018; J. Cheng et al., 2018)
<i>C. polyphyrenoideum</i>	Dairy industry	Filtration w/ (1.5 μm) membrane	21	200 (24:0)	15	90	71	-	(Muhammad et al., 2021)
<i>Euglena</i> sp.	Sewage	Filtration+ autoclave	4	120 (24:0)	5	94	65	-	(Mahapatra et al., 2013)

Table 5
Nutrients utilization and lipid content of microalgae in wastewater.

Wastewater	Microalgae	Nutrients utilization by microalgae			Biomass (mg/L)	Lipid (%)	References
		Ammonium (mg/L)	Nitrogen (mg/L)	Protein (mg/L)			
Municipal wastewater	<i>Chlorella</i> sp.	87	133	215	1180	11	(Lam et al., 2017)
Metropolitan wastewater		265	291	531	1060	10.5	(Li et al., 2011)
Centrate wastewater		126	131	56	3011	10.8	(Ren et al., 2017)
Pig wastewater	<i>C. zofingiensis</i>	-	149	157	2960	37	(Zhu et al., 2013)
		-	140	147	2861	34	
		-	138	145	2006	35	
Dairy wastewater	<i>C. vulgaris</i>	69	70	62	1871	11	(Khalaji et al., 2021)
		6	11	16	145	18	(Huo et al., 2012)
		113	172	14	1711	14	(Y.-K.Y.-K. Choi et al., 2018; K.-J. Choi et al., 2018)
Alcohol wastewater	<i>C. pyrenoidosa</i>	99	129	19	1392	10	
		170	189	47	2151	38	(Yang et al., 2015)
Coke manufacturing wastewater	<i>C. vulgaris</i>	169	884	121	1252	40	(Chen and Chang, 2018)
Brewery wastewater	<i>C. vulgaris</i>	171	91	19	2263	27	(Farooq et al., 2013)

coordination between the two kingdoms of microorganisms (Zhong et al., 2021). Bacteria, often called probiotics, break down refractory organics. They also secrete beneficial metabolites that promote bacterial growth and enhance removal efficiency. Algae, on the other hand, produce oxygen and consume CO₂, creating a closed carbon source. The extracellular organic matter produced by the algae-bacterial system allows for higher polysaccharide production, providing nutrients for the algae and bacteria in the system by transporting them to the extracellular environment. Notably, the co-cultivation of mono-algae and mono-bacteria also significantly improves microalgal biomass and removal efficiency. Moreover, bacteria in wastewater treatment systems may be profitable for enhancing algal-based remediation (Viswanaathan et al., 2022).

Algal-bacterial synergy enhances carbon capture in wastewater bioremediation and facilitates biofuel production from their biomass. This synergy holds significant potential in biorefinery operations, environmental remediation, carbon sequestration, and high-value compound synthesis. Moreover, it offers applications in controlling blooms, eliminating dyes, formulating agricultural biofertilizers, and producing bioplastics (Yong et al., 2020). Specifically, the microalgal-bacterial consortium exhibited superior efficiency in removing nitrogen, phosphorus, COD, and color in batch-scale treatment. Notably, the consortium achieved remarkable removal rates: 58.57% nitrate, 86.42% phosphate, and 91.5% COD, alongside significant chlorophyll and bacterial dry cell weight yields. Conversely, single-stage treatment (algae only) achieved a commendable 41.54% color removal. These findings highlight the cost-effective treatment potential of this method for real textile wastewater, yielding valuable biomass for biofertilizers and energy-efficient applications (Raza et al., 2022). Using glucose and sodium acetate as co-substrates in the cultivation of microalgae-bacteria consortium for enhanced sulfadiazine (SDZ) and sulfamethoxazole (SMX) removal influenced bacterial community structure greatly. Glucose demonstrated a two-fold increase in biomass production with a maximum specific growth rate compared with sodium acetate. Co-substrate supplementation enhanced the degradation of SDZ significantly up to 703 ± 18% for sodium acetate and 290 ± 22% for glucose but had almost no effect on SMX. The activities of antioxidant enzymes, including peroxidase, superoxide dismutase, and catalase, decreased with co-substrate supplementation. Chlorophyll a was associated with protection against sulfonamides, and chlorophyll b might contribute to SDZ degradation. Glucose enhanced the relative abundance of Proteobacteria, while sodium acetate significantly improved the relative abundance of Bacteroidetes (S. Wang et al., 2022; Y. Wang et al., 2022; S.-K. Wang et al., 2022).

In wastewater, normally, bacteria are present with microalgae that oxidize COD to release CO₂, which is utilized by microalgae through photosynthesis, and oxygen is produced, which triggers bacterial

growth. Hence, in algal-bacterial coordination, wastewater can be treated without oxygen supply along with CO₂ absorption from the air (Fig. 3). Moreover, bacteria convert dead microalgae into dissolved organic matter to make wastewater more nutritious for algal bacterial growth (Jimenez-Bambague et al., 2021). Table 6 shows the efficacy of nutrient removal from wastewater by an algal bacterial consortium. The improved algal biomass is then used to make valuable products like fuel (Hasnain et al., 2021), phycochar (Abideen et al., 2022), and bio-fertilizers (Jakhar et al., 2022). Fig. 3 depicts a simplified algal-bacterial system. The combination of methanophiles and microalgae oxidized methane from wastewater and prevented it from escaping into the atmosphere (Jiang et al., 2022). *Tetrademus* sp. removed 80% nitrogen, 70% phosphorus, and 98% pharmaceuticals, mainly atenolol, bisoprolol, citalopram, and diltiazem, from wastewater (Pacheco et al., 2020).

Algal-bacterial systems (ABSs) have been widely used since the mid-twentieth century to treat nutrient-rich wastewater. Research has shown that methane-oxidizing bacteria and microalgae can effectively remove methane from anaerobically treated wastewater that would otherwise be released into the atmosphere. However, the biological degradation of methanol or methane using closed algal-bacterial photobioreactors requires the supply of external oxygen or inorganic carbon, making this technique inefficient and needing further refinement (Sial et al., 2021). A novel method involves the use of algal-bacterial biofilm formation systems. These systems are easy to cultivate, relatively self-contained compared to suspended systems, and simple to harvest. However, applying this sewage treatment system on a larger scale is still far off (Qian et al., 2023). One of the challenges in using algal-bacterial biofilm systems is that light access is limited to the photic zone, just a few hundred millimeters below the water surface, which requires larger surface systems. Certain engineering-based studies are needed to overcome this limitation, such as developing moving bed biofilm reactors containing plastic biofilm conveyors to maximize the active biofilm surface area in the reactors (Deena et al., 2022).

Microalgal biofilms can treat certain wastewater and significantly recover nutrients with a remarkable biomass yield. This approach can resolve the harvesting problem for microalgae and greatly reduce the burden of conventional settling tanks in wastewater treatment processes. In addition to nutrients, various toxic metallic ions can also be eliminated by microalgae, achieving the refining properties of tertiary treatment. This method can treat different types of agro-industrial wastewater (Jagaba et al., 2022). High-rate algal ponds (HRAPs) have proven viable and cost-effective platforms for the bioremediation of secondary effluent from a WWTP. The HRAP system has achieved a nitrogen removal efficiency of 60–80% and a phosphorus removal efficiency of 60–70%. In addition, *Tetrademus dimorphus* was successfully grown in an HRAP system and was found to deplete certain types of pharmaceuticals. Removal efficiencies above 90% were demonstrated

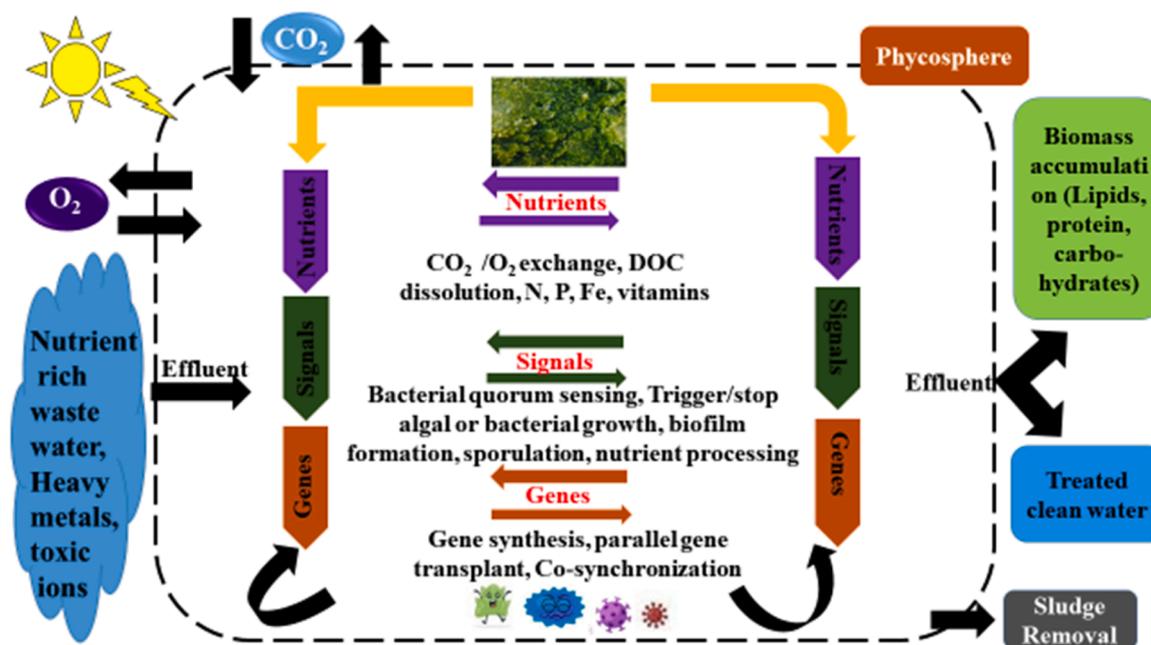


Fig. 3. Microalgal–bacterial interactions during wastewater cultivation.

Table 6
Nutrients removal from wastewater by algal–bacterial co-cultivation.

Wastewater	Microalgae	Bacteria	Nitrogen (%)	Phosphorus (%)	COD (%)	Reference
Municipal	Mixed microalgae	Proteobacteria	92	95	96	(Lee et al., 2016)
	<i>C. microporum</i>	Cyanobacteria	87	88	-	(Lee et al., 2015)
	<i>Scenedesmus</i> sp.		96	98	93	
Synthetic municipal	<i>C. vulgaris</i>	<i>Rhizobium</i> sp.	60	100	-	(Zhang et al., 2021)
		<i>Klebsiella</i> sp.	97	96	86	
	<i>N. oculata</i>	<i>Bacillus</i> sp.	60	91	-	(Daveray et al., 2019)
	<i>C. vulgaris</i>	<i>Pseudomonas</i> sp.	81	59	-	(Tait et al., 2019)
		<i>Bacillus</i> sp.	89	81	87	
Starch	<i>S. dimorphus</i>	Nitrifiers	79	100	-	(K.-J.Y.-K. Choi et al., 2018; K.-J. Choi et al., 2018)
	<i>Scenedesmus</i> sp.	Native bacteria	89	80	-	(Udaiyappan et al., 2020)
			89	81	80	
Landfill	<i>C. pyrenoidosa</i>		96	96	-	(Zhao et al., 2014)
Vinegar production	<i>Chlorella</i> sp.	<i>Beijerinckia</i> SP.	74	75	73	(Huo et al., 2020)
Winery	<i>C. sorokiniana</i>	<i>Stenotrophomonas</i> sp.	80	47	64	(Qi et al., 2018)
	<i>A. protothecoides</i>	Proteobacteria	100	-	38	(Higgins et al., 2018)
Swine lagoon	Assorted microalgae		-	94	88	(Sial et al., 2021)

for pharmaceuticals such as atenolol, atracurium, bisoprolol, bupropion, citalopram, diltiazem, and metoprolol. The HRAPs not only offer better removal rates and increase the probability of sewage treatment but also offer the potential for biofuel production with a solid yield (Ricky and Shanthakumar, 2022). Overall, using algal-bacterial sewage treatment processes offers greater efficacy, requires low energy utilization, does not require synthetic chemical substances, and results in higher microalgal biomass production, which can be used for further purposes. It could be a leading alternative technology to aeration-based methods such as activated sludge treatment (AST) (S. Wang et al., 2022; Y. Wang et al., 2022; S.-K. Wang et al., 2022).

Microalgal growth removes nitrogen, phosphorous, and carbon and recovers some resources like lipid, protein, or total algae cells. Consequently, microalgae from wastewater cultivation can be used as biodiesel, animal feed, and biofertilizer, supporting the sustainable development of agriculture and industry. However, refractory contaminants such as suspended solids and carbon source deficiency in certain types of wastewater (e.g., centrate wastewater, agricultural wastewater, etc.) seriously inhibit algal growth and other pollutants transformation efficiency, even causing the failure of microalgal remediation. Thus, improving algal growth rate and associated contaminants

transformation efficiency become critical in real-world applications (Sial et al., 2021).

9. Algal cultivation systems and potential applications

Agribusiness for algal biomass production is growing as a profitable commercial venture due to the rapid growth and effective CO₂ absorption (Piwowar and Harasym, 2020). Compared to terrestrial plants, crops, or biofuel feedstocks like soybean, microalgae have a faster growth rate, require less space, and have higher biomass productivity with a high oil content (Jalilian et al., 2020). Microalgae cultivation is ineffective due to low yield and high cultivation costs. As a result, new approaches to obtaining higher quality and quantity of biomass from various algal species have been proposed for the removal of dangerous HMs from contaminated soil and aquatic habitats (phycoremediation), carbon sequestration, and biofuel production, as well as the generation of polysaccharides, vitamins, pigments, fatty acids, and amino acids (Bordoloi et al., 2020). *Scenedesmus*, *Lyngbya*, *Spirulina*, *Chlorella*, *Anabaena*, *Chroococcus*, *Oscillatoria*, *Synechocystis*, and *Gloeocapsa* (Emparan et al., 2019) are the common microalgae species that are used for phycoremediation. Open culture systems (open ponds, tanks, and raceway

ponds) and regulated closed cultivation systems (various kinds of photobioreactors (PBRs)) are the two most used techniques for growing microalgae, the requirement for which depends on the goal of microalgal cultivation (Narala et al., 2016).

9.1. Open pond hybrid design

In an open pond, only 10–20% of CO₂ is effectively absorbed (Romagnoli et al., 2020). Identifying microalgae strains suited for large-scale ponds can be aided by a microalgae biomass kinetic model and the core design of novel stacked modular open raceway ponds (SMORPs) that maximize biomass growth in limited light conditions (Romagnoli et al., 2020). By utilizing 5-m³ outdoor open raceway ponds to treat real swine wastewater (RSW), *Chlorella vulgaris* has provided higher microalgal biomass, total fatty acid content, CO₂ fixation, an improved lipid product, and greater nutrient removal. Open raceway ponds with a 20–30 cm depth offer high area production and are thus helpful (Benner et al., 2022). The high-rate microalgae ponds (HRAPs), which use wastewater as their growth medium, comprise a shallow raceway with one or two loops and a paddlewheel to stir the microalgae cultures. In mixed ponds, a greater amount of biomass is produced at low operational costs than in PBRs because mixing promotes the growth of microalgal cells (Yew et al., 2019). A few microalgal strains, such as *Spirulina* and *Dunaliella*, were used to demonstrate the success of raceway pond culture in terms of biomass output. The most effective and ideal outdoor space to promote microalgae growth in an open system is in countries around the Mediterranean Sea coast that enjoy warmer climates (Kumar et al., 2021).

9.2. Photo-bioreactor design for CO₂ fixation

One of the popular carbon sequestration methods through biological fixation of CO₂ emitted from various industries and thermal plants includes using PBRs for microalgae-mediated CO₂ sequestration that utilize photosynthesis to produce algal biomass (Viswanaathan et al., 2022). Some common algal species used for CO₂ fixation include *Scenedesmus obliquus*, *Chlorococcum humicola*, *Chlorella vulgaris*, and *Dunaliella salina* (Kishi and Toda, 2018). For example, large amounts of algal biomass were produced from various PBRs, such as tubular PBRs, flat panel PBRs, internally illuminated PBRs, and vertical-column/airlift PBRs. Flat-plate PBRs produced higher biomass than other PBRs (Sirohi et al., 2022). Hybrid airlift PBRs, on the other hand, are also very effective since they can choose the best microalgal species and feature a mixotrophic growth mode that enables the calculation of the CO₂ fixation rate by monitoring CO₂ removal. Mixotrophic microalgal biofilm is a novel approach that has shown improved productivity and quality of biofuels (2–3 and 2–10 times better biomass and lipid output, respectively, as well as 40–60% reduced ash content). Tubular, column, and panel PBRs are the most effective outdoor cultivation systems for upcoming industrial applications (Touloupakis et al., 2022).

A novel hybrid photobioreactor design for *Scenedesmus obliquus* enhanced the surface per working volume unit, demonstrating the outstanding hydrodynamic performance of the system with great potential to scale up. It produced a maximum cell biomass of 2.8 kg/m³, with an average of 45.3 kg CO₂/m³/d CO₂ removed to produce 34.0 kg O₂/m³/d of O₂ (Deprá et al., 2019). As the growth of *Chlorococcum humicola* in an airlift photobioreactor provides greater biomass than in stirred tank photobioreactors, choosing the right photobioreactor for each species plays a significant impact on the quality of the biomass output (Powtongsook and Nootong, 2019). However, a cultivating system set up into smaller stirred tank photobioreactors in series for the photoautotrophic cultivation of *Chlorococcum humicola* has shown an approximately 2.5-fold increase in both biomass and carotenoids when compared to a single airlift photobioreactor with equivalent working volume and similar operating conditions (Wannachod et al., 2018). According to a study on the effectiveness of pollutant removal in a 1 L

PBR, after half a month of *Chlorella* sp. growth, 73% of the total organic carbon and 92% of the total nitrogen were removed from the pre-treated produced water (Das et al., 2019).

Both natural and artificial light sources have contributed to the mass production of microalgae. Nano-material light filters transmit specific wavelengths for improved yield and productivity of algal culture by 13–34% in flat panel PBRs and 70–100% in rotating algal biofilm (RAB) systems, respectively (Michael et al., 2015). Correlation between CO₂ removal rates and gas volume flux has shown that a feed of 25 dm³/h of gas by immobilized microalgae causes about 40% of CO₂ removal, while in the case of 200 dm³/h groups, the removal efficiency of CO₂ is 5.9% (Dębowski et al., 2021). In contrast, macroalgae or seaweed sequester enormous amounts of carbon (173 TgC per year) worldwide, 88% of which is sequestered in the ocean depths (Krause-Jensen and Duarte, 2016). *Dunaliella salina* uses indoor helical-tubular photobioreactors in which the maximum amount of beta-carotene produced is 4.85 µg per mg of the dry weight of microalgae at 2.5 mol/L salinity (Hashemi et al., 2020). Apart from salinity, temperature, and light intensity also play a major role in beta-carotene production (Pourkarimi et al., 2020).

9.3. Merits and demerits

Despite the benefits, microalgal production on a large industrial scale is difficult. *Scenedesmus* sp. has shown different quantities of biomass production in PBRs (1.15 kg/m³/d) and open raceway ponds (0.5 kg/m³/d) (Deprá et al., 2019). Microalgal production requires 121,000 ha of the open pond and 58,000 ha of photobioreactor area to fulfill the yearly demand for gasoline. It costs US \$1.54 for PBR and \$7.32 for open ponds to produce 1 kg of algal biomass, and US \$24.6 for PBR and \$7.64 for open ponds to produce 1 kg of oil product from algal biomass (Mona et al., 2021). Compared to an open raceway pond, the life cycle assessment estimated that an airlift photobioreactor would consume up to 3.7 times more energy and have a higher environmental impact. In an open raceway pond and airlift photobioreactor, the net CO₂-negative culture changes to a net-positive culture through CO₂ sequestration by microalgae at higher biomass productivity and lower specific energy consumption (Sarat Chandra et al., 2018). The simplicity and availability of resources for each approach shed light on the advantages and disadvantages, which impact the quantity and quality of the biomass produced. SMORPs system decreases land consumption, enhances lighting conditions, and lowers cultivation costs by using anaerobic digestion of microalgal biomass to produce biogas (Romagnoli et al., 2020). Open ponds used as small raceway ponds on an industrial scale offer low operating costs with paddle wheels for mixing and natural sunlight illumination. However, they are undesirable due to potential contamination and poor control of reaction conditions (Benner et al., 2022).

On the other hand, the controlled environment in PBRs provides numerous advantages such as large-scale biomass generation, increased photosynthetic capacity, decreased water evaporation, and reduction of CO₂ loss (Benner et al., 2022; SundarRajan et al., 2019). Closed PBRs increase productivity in both volume and area. For photoautotrophic microorganisms to create green products, closed systems are favored over open ones because they provide regulated culture conditions, optimal growth potential, useful byproducts, and lower contamination risk (Touloupakis et al., 2022). However, closed systems are also more expensive to produce, install, run, and maintain since they need special electric equipment, vigorous mixing and aeration, temperature control, and pH adjustment. A newer technology, floating PBRs use less power and are less expensive. Nevertheless, to enhance the mixing, light intensity, hydrodynamics, mass transfer capabilities, and algal growth productivity of PBRs, computational fluid dynamics modeling and process intensification techniques must be used during the design and scale-up phases (Ranganathan et al., 2022). Moreover, Table 7 describes CO₂ fixation by algae in different bioreactors.

Table 7
CO₂ fixation by microalgae in different bioreactors.

Reactor		Algal strain	CO ₂ Supply (%)	Temperature (°C)	pH	Light Conditions		CO ₂ Fixation		Reference
Type	Volume					Intensity	Photo-periods	Rate (g L ⁻¹ D ⁻¹)	Efficiency (%)	
Open pond reactors	8	<i>Spirulina platensis</i>	10	30	10	30	12:12	-	40	(Shareefdeen et al., 2023)
		<i>Chlorella</i> sp.						-	46	
Bubble column reactors	330	<i>Chlorella</i> sp.	8	-	-	Sunlight	-	-	50	(Barahoei et al., 2020)
	0.8	<i>Chlorella</i> sp.	2	26	6–7	300 mol m ⁻² s ⁻¹	24:0	-	58	
			5					-	27	
			10					-	20	
			15					-	16	
	1.6	<i>Chlorella vulgaris</i>	0.2	55	9	50 mol m ⁻² s ⁻¹		-	74	
6	<i>Spirulina</i> sp.	6	30	8.5	3200 lux	12:12	-	38	(Rajkumar et al., 2022)	
Air-lift reactors	8	<i>Dunaliella tertiolect</i>	5	25	7.2	3500 lux		0.272	-	(Iglina et al., 2022)
	8	<i>Spirulina platensis</i>	5	25	7.2	3500 lux		0.252	-	
			5	25	7.2	3500 lux		0.319	-	
	10	<i>Botryococcus brauni</i>	1	30	9			0.497	-	
Tubular reactors	10	<i>Chlorella vulgaris</i>	1	30	7.2			6.24	-	(Mohapatra et al., 2022)
	1	<i>Synechococcus</i> sp.	5	30	6.8	8000 lux		0.6	-	
	2	<i>Aphanothece nageli</i>	15	25		150 mol m ⁻² s ⁻¹		15	-	
Flat-plate reactors	4	<i>Chlorella</i> sp.	10	26		300 mol m ⁻² s ⁻¹		-	63	(Dębowski et al., 2021)
	12	<i>Spirulina platensis</i>	4	36		2900 kJ d ⁻¹		-	70	
LDOF reactor	11	<i>Chlorococcum littorale</i>	5	25	7.2	2000 mol m ⁻² s ⁻¹		200 g m ⁻² d ⁻¹	-	(Siddique et al., 2023)
	72	<i>Synechocystis aquatilis</i>	10	40		Sunlight		51 g m ⁻² d ⁻¹	-	
Other reactors	2.5	<i>Synechococcus</i> sp.	0.55			50 E m ⁻² s ⁻¹		4.44	-	(Chuka-ogwude et al., 2020)
Other reactors	1.8	<i>Scenedesmus obliquus</i>	12	30		3200 lux		-	-	(Nath et al., 2023)
	1.8	<i>Chlorella kessleri</i>	6					-	-	
	100	<i>Euglena gracilis</i>	10	27	3.5	178 lux		0.074		

10. Challenges and limitations

10.1. Algal–bacterial co-cultivation

During co-cultivation, industrial flue gas and wastewater are sources of nutrients and organic matter for cultivating a microalgae–bacteria consortium to produce biomass that can be transformed into biofuel, animal feed, and metabolites (Chia et al., 2021). The presence of both bacteria and microalgae in the wastewater in HRAPs improves the efficacy of nutrient removal (Yew et al., 2019). Prevention of bacterial biofilm is necessary as it hinders the growth of microalgae. Therefore, it is essential to regularly clean the reactor and pond (Kumar et al., 2021). The antagonistic effect among the microbes, e.g., algal metabolism, may create high oxygen levels, a raised pH, and antibacterial compounds dangerous to bacteria, particularly gram-positive bacteria. In response, pathogenic bacteria release cellulolytic enzymes that weaken and degrade particular microalgal cell walls, leading to cell death (Yadav et al., 2021). Other challenges include choosing microorganisms and optimizing growth media for co-culturing with microalgae. It is also difficult to properly pretreat biomass and extract individual lipid and fatty acid content in mixed cultures due to the heterogeneous population (Das et al., 2021). Moreover, microbial susceptibility to changing operational and environmental factors that negatively affect their growth and performance, bulking and foaming brought on by the inhibition of the nitrification process and an excess of the filamentous bacterial population, trouble in dealing with and disposing of sludge produced in bulk quantities, and ineffective microalgal harvesting systems are some of the other limitations in the algal–bacterial wastewater treatment methods (Viswanaathan et al., 2022).

In the growth medium, algae and bacteria compete for nutrients.

Such competition can affect both organisms' growth rates and overall productivity. Balancing algae and bacteria's nutrient requirements to ensure coexistence and mutual benefits can be challenging (Yong et al., 2022). Besides, maintaining a stable balance between algae and bacteria is challenging. A group outgrowing another can disrupt the synergistic relationship and lead to one species dominating the other (Schulze et al., 2006). Moreover, it is critical to note that not all bacterial species benefit from algal growth, and some can even be harmful. Identifying and promoting the growth of beneficial bacteria while suppressing harmful ones requires a deep microbial understanding.

10.2. Efficient CO₂ mixing systems

CO₂ supply in microalgal culture is necessary to overcome carbon limitation as atmospheric CO₂ availability for photosynthesis in open ponds is very low. The CO₂ supply is mainly influenced by the optimum culture pH, mixing, mass transfer coefficient, gas–liquid contact period, and sprayer type. A brief period of contact between gas and liquid due to shallow depth has identified gas transfer as a limiting factor for open photobioreactor efficiency (Costa et al., 2019). Direct and indirect methods that measure CO₂ fixation during microalgae culture involve measuring the carbon content using elemental and total organic carbon analysis by assumptive values and at the entrance and outflow of PBRs by gas chromatography or infrared sensors, respectively (Lim et al., 2021). Greater vertical mixing decreases self-shading, which is necessary to guarantee that algal cells cycle regularly between the lighter and darker phases of the culture, making the maintenance of optimum mixing costly and requiring higher energy inputs into the cultivation system (McGinn et al., 2011). With a sparger installed at the bottom, CO₂ delivered into the microalgae culture has a limited ability to transfer,

which limits both CO₂ absorption and outgassing. As a result, external gas diffusers such as hollow fiber membranes and porous materials that regulate gas flow rates are used, as are microbubbles with reduced size (< 100 μm) to improve mass transfer efficiency, CO₂-containing solvents passed through non-porous membranes to minimize CO₂ loss and transportation energy, carbonation columns, bubble sparging, and sump systems. However, the limitations of these systems include the high costs associated with the deployment of sumps, membranes, and carbonation columns, as well as the energy needs and system maintenance, such as plug biofouling mitigation and sophisticated control systems (Eustance et al., 2020; Viswanaathan et al., 2022).

10.3. HMs and other pollutants in the effluents

Various forms of wastewater, including sewage waste, landfill leachate, municipal wastewater, and anaerobic digestion effluent, can be treated by microalgae to remove contaminants (Rahman et al., 2020). Zinc, lead, mercury, cadmium, chromium, copper, and nickel are hazardous metals that should be avoided while treating industrial effluents such as pharmaceutical residues, insecticides, dyes, and metal processing. Microalgae growth in effluents produces single-cell proteins that provide animal feeds, lipids, proteins, biofuels, carotenoids, and other substances (Venugopal and Sasidharan, 2021). It can regenerate nutrients like carbon, phosphorus, and nitrogen while using less energy in waste management techniques. Microalgae prevent secondary emissions by utilizing inorganic resources for growth and bacterial assistance to make biomass. The recovered bioactive substances are also converted into bioethanol, biofertilizers, biopolymers, dietary supplements, and animal feed (Ali et al., 2021). The best algal growth was seen in revolving algal biofilm reactors, which are effective systems in wastewater treatment for total dissolved solids removal (Peng et al., 2020). Wastewaters with an excess or deficit of certain chemicals, such as low levels of N and P molecules, may negatively affect the viability of microalgae. Different microalgal species can bioaccumulate HMs and may adapt their development to the characteristics of industrial wastewater. Additionally, frequent issues with the treatment of liquid digestate include acidification, nutritional imbalance, ammonium toxicity, and turbidity (Viswanaathan et al., 2022).

10.4. Requirements for efficient microalgae harvesting techniques

The cost of harvesting, which substantially influences the practical use of microalgae as a source of biomass for fuels and chemicals, is one of the largest barriers to the commercialization of algal biofuels. Hence, it is a key component of the microalgae wastewater treatment process. The top objective for microalgae cultivation technology is creating an effective and affordable microalgal harvesting method. Nearly 30% of the overall capital expenditure for biodiesel is spent on harvesting costs alone (Mathimani and Mallick, 2018). Most harvesting techniques still include chemicals, rendering harvested biomass unsuitable for immediate food, feed, and pharmaceutical manufacturing (Kurniawan et al., 2022). Flocculation, filtration, flotation, coagulation, centrifugation, and sedimentation comprise the most used harvesting strategies (Rakesh et al., 2020). Less often used methods of harvesting include electrophoresis, ultrasonication, and electroflotation. In rare circumstances, combining two harvesting techniques can increase biomass (Saad et al., 2019). Every process, however, has its own benefits and drawbacks, including those related to time, cost, harm to biomass, the convenience of use, continuous operation, use of energy, chemicals, fouling, clogging of equipment, etc. (Viswanaathan et al., 2022). Harvesting microalgae with biocoagulants or bioflocculants is a practical and promising environmentally friendly method of producing microalgal biomass (Kurniawan et al., 2022). Low biomass output in an open system due to contamination raises the harvesting price. Therefore, mixotrophic microalgal biofilm offers high production at low harvesting costs. A compact biofilm of algal biomass produced by algal flow reduces

harvesting costs by being easily scraped off by hand or mechanically using a dry suction pump (Marella et al., 2019). Flocculation is used to simplify harvesting before being purified using membrane filtration or ultrafiltration, centrifugal sedimentation, or gravity settling (Viswanaathan et al., 2022).

10.5. Post-harvest preservation and storage

Freshly harvested algal slurry or in paste form may degrade biochemically, and all its constituent parts will eventually deteriorate, often starting with valuable substances like lipids and specialized goods like vitamins (Viswanaathan et al., 2022). After being harvested, algal biomass can be treated via thermochemical, biochemical, transesterification, and photosynthetic microbial fuel cell conversion techniques. Post-harvest separation, recycling of nanoparticles, practical application, and the generation of uncontaminated microalgal biomass remain the main barriers during microalgae cultivation (Mathimani and Mallick, 2018). In some instances, harvesting may be followed by dewatering. Dewatering involves eliminating the water content of cells to produce dried material (Saad et al., 2019). Since harvested algal biomass is subject to microbial deterioration, long-term preservation of microalgae biomass is difficult, necessitating active storage strategies to prevent biomass loss. The conventional method of drying high-moisture plant material is technically difficult and expensive for microalgae due to the high moisture content (80%) and rheology of the algal biomass (20% solids) (Wahlen et al., 2020). The fatty acid profiles and lipid-yielding efficiency may be increased, and costs associated with cell drying and related equipment could be decreased by using the appropriate wet storage conditions and stress induction techniques (Shokravi et al., 2022). It is difficult to dry the harvested wet microalgal slurry, which has an average moisture content of 80%, to produce storable dry biomass with a 5–10% moisture content (Hosseinizand et al., 2018). Although drying, such as spray drying, drum drying, and sun drying, are effective methods for manufacturing algal-based fuel, they are not yet economically feasible due to the challenges involved. The most cost-effective method is filtering, followed by sun drying (Viswanaathan et al., 2022). A possible method of preserving algal biomass for conversion in the winter when microalgae productivity drops is the wet anaerobic storage of microalgae mixed with herbaceous biomass (Wahlen et al., 2019).

11. Conclusions

Microalgae are a promising solution to tackle our growing environmental challenges, such as carbon sequestration and wastewater bioremediation. Biological algal systems for carbon capture have emerged as a critical strategy to combat climate change and achieve the goal of net-zero CO₂ emissions by 2050. Microalgae are an attractive option due to their superior photosynthetic rates and wastewater treatment. Besides, microalgae have significant potential for producing biodiesel, animal feed, and other bioproducts. Furthermore, the microalgal-bacterial nexus represents a highly synergistic and efficient CO₂ capture and pollutant removal system. Combining the innate abilities of both microorganisms increases the efficiency of the process. Microalgae, with their superior photosynthetic capacities, efficiently utilize carbon dioxide for growth, aiding carbon sequestration. Simultaneously, when paired with specific bacterial strains, nutrient uptake and pollutant breakdown are enhanced, particularly in wastewater environments. Bacteria break down complex pollutants into simpler forms, which algae can readily assimilate. Although most foundational research is still in the laboratory, promising startups like AlgaEnergy and AlgoSource are leading the way toward practical and scalable applications. However, there are challenges to up-scaling microalgae-based solutions, including ensuring cost-effectiveness. We must balance economic feasibility with environmental stewardship as the world moves towards a greener future. Collaborative efforts between industries, dedicated research,

technological innovation, and strategic investments are crucial to harnessing the transformative potential of microalgal-bacterial nexus in carbon sequestration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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